

# Chemistry and Biological Activities of Caffeic Acid Derivatives from *Salvia miltiorrhiza*

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**Abstract:** Caffeic acid (3,4-dihydroxycinnamic acid), one of the most common phenolic acids, frequently occurs in fruits, grains and dietary supplements for human consumption as simple esters with quinic acid or saccharides, and are also found in traditional Chinese herbs.

Caffeic acid derivatives occur as major water-soluble components of *Salvia miltiorrhiza*, including caffeic acid monomers and a wide variety of oligomers. This review provides up-to-date coverage of this class of phenolic acids in regard to structural classification, natural resources, chemical and biosyntheses, analytical methods and biological activities including antioxidant, anti-ischemia reperfusion, anti-thrombosis, anti-hypertension, anti-fibrosis, antiviral and antitumor properties. Special attention is paid to both structural classification and biological activities. The structural diversity and the pronounced biological activities encountered in the caffeic acid derivatives of *S. miltiorrhiza* indicate that this class of compounds is worthy of further studies that may lead to new drug discovery.

**Keywords:** Caffeic acid derivative, salvianolic acid, antioxidant, anti-ischemia reperfusion, anti-thrombosis, anti-hypertension, anti-fibrosis, antiviral, antitumor, *Salvia miltiorrhiza*.

## 1 INTRODUCTION

Caffeic acid (3,4-dihydroxycinnamic acid), one of the most common phenolic acids, frequently occurs in fruits [1], grains [2] and dietary supplements [3] for human consumption as simple esters with quinic acid or saccharides, and are also found in traditional Chinese herbs [4]. *Salvia miltiorrhiza* is a representative example.

The dried root of *Salvia miltiorrhiza* (Dan-Shen in Chinese) is one of the most popular traditional herbal medicines in some Asian countries, and has been used extensively for the treatment of coronary artery diseases, angina pectoris, myocardial infarction, cerebrovascular diseases, various types of hepatitis, chronic renal failure, dysmenorrhea, and also to improve microcirculation in human body [5]. Studies on its chemical components and biological activities have been mainly confined to the lipophilic diterpenoid tanshinones before the nineties, and several reviews on these components have been published [6]. Research interest directed toward the water-soluble components of *S. miltiorrhiza* in recent years was initially motivated by its uses as a water decoction in some Chinese prescriptions and as an injection agent for the treatment of cerebral and coronary vascular diseases [7].

Caffeic acid derivatives occur as the major water-soluble components of *Salvia miltiorrhiza*. To date, altogether twenty-five caffeic acid derivatives have been isolated from *S. miltiorrhiza* and had their structures elucidated through chemical and spectroscopic methods. This class of compounds includes caffeic acid monomers and oligomers, and the latter are also called depsides or salvianolic acids in the literatures [8]. More recently, chemical syntheses and biosyntheses of some of these phenolic acids were reported, and a wide variety of biological activities of some representatives have also been evaluated. This review focuses on the structural classification, natural resources, chemical synthesis and biosynthesis, analysis and biological activities of these caffeic acid derivatives. Special attention is paid to both structural classification and biological activities.

## 2 STRUCTURAL CLASSIFICATION

The skeletons of these caffeic acid derivatives can be classified into five categories according to the number of caffeic acid units. The group denomination adopted in this review is similar to that suggested by Lu and co-workers [9].

### 2.1 Caffeic Acid Monomers

The caffeic acid monomers as a group currently comprise four known members: caffeic acid (1) [10], 3-(3,4-dihydroxyphenyl)lactic acid (2) [11], 3-(3,4-dihydroxyphenyl)lactamide (3) [12], and isoferulic acid (4) [13] (Fig.

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1). Compounds **1** and **2** are basic building blocks of a variety of multiple condensation metabolites. Compound **2** is also called 'dan-shen-su' in Chinese, and can be considered as a hydration product of **1**.

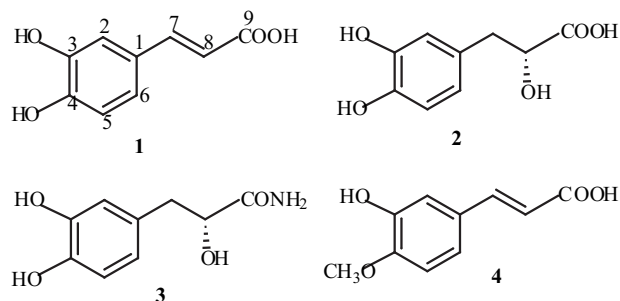


Fig. (1). Structural formulae of caffeic acid monomers.

## 2.2 Caffeic Acid Dimers

Compounds of this group can be structurally divided into two caffeic acid units and comprise seven members, i.e.

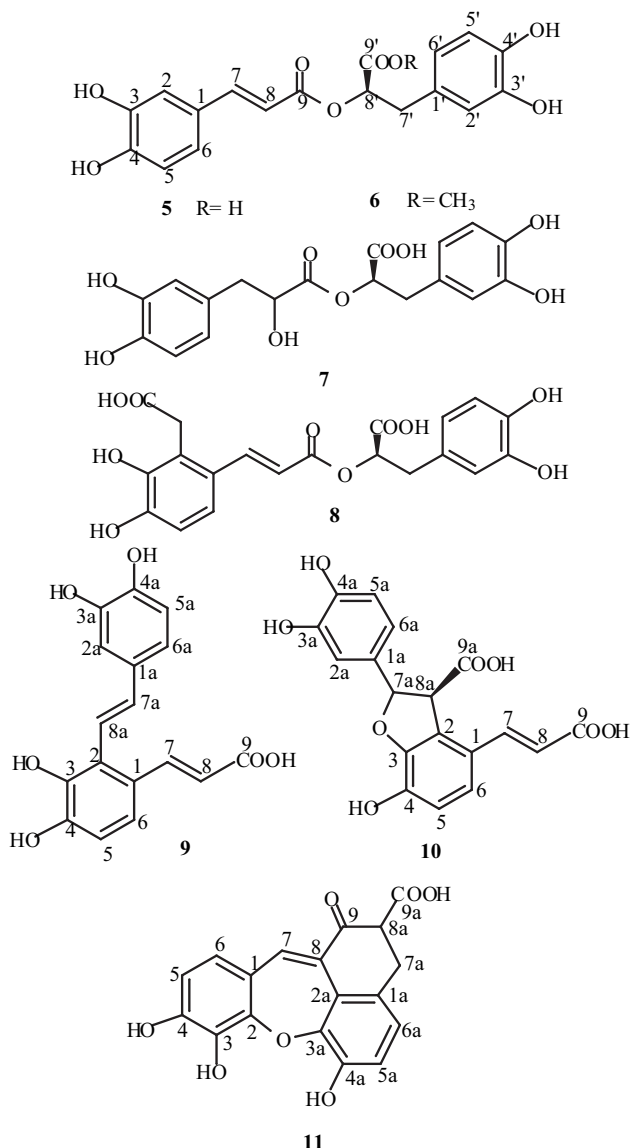


Fig. (2). Structural formulae of caffeic acid dimers.

rosmarinic acid (**5**) [14-16], methyl rosmarinate (**6**) [16], salvianic acid C (**7**) [17], salvianolic acid D (**8**) [13], salvianolic acid F (**9**) [18], prolithospermic acid (**10**, przewalskinic acid A) [19, 20] and salvianolic acid G (**11**) [21] (Fig. 2). Compound **5**, constructed from the condensation of caffeic acid (**1**) with *R*-(+)-(3,4-dihydroxyphenyl)lactic acid (**2**), is the simplest representative of this group. It was firstly isolated in 1958 and named according to the plant from which (*Rosmarinus officinalis*) it was isolated [15]. Compound **7** was isolated from the injection agent of *S. miltiorrhiza*, and had its structure elucidated through mass spectrometry [17]. Compound **7** can be considered as a hydration product at the  $\Delta^{7,8}$  double bond in **5**. Compound **8** is a 2-carboxymethyl analog of **5**, and its structure was confirmed by comparison with a synthetic product derived from isovanillin [13]. Compound **9** is an 8a-decarboxylated caffeic acid dimer possessing a trans-stilbenoid skeleton [18], and its structure has been confirmed by a total synthesis (discussed under chemical synthesis, see below). Compound **10** is a dihydrobenzofuran-type caffeic acid dimer. It was also named przewalskinic acid A, reflecting its original isolation from *S. przewalskii* [20]. Compound **11**, a caffeic acid dimer forming a tetracyclic skeleton, is the only example bearing a dibenz[*b,f*]oxepin nucleus in *S. miltiorrhiza* [21].

## 2.3 Caffeic Acid Trimers

Compounds of this group can be structurally divided into three caffeic acid units and comprise five members: salvianolic acid A (**12**) [22], lithospermic acid (**13**) [16] lithospermic acid monomethyl ester (**14**) [16], lithospermic acid dimethyl ester (**15**) [16] and salvianolic acid C (**16**) [23] (Fig. 3).

Compound **12**, bearing a trans-stilbene nucleus, is related to **9** with the 8-carboxyl group esterified by compound **2** [22]. In contrast, compounds **13-15**, characterized by the presence of a benzofuran nucleus, are related to compound **10** [16]. In compounds **13** and **14**, the 8-carboxyl groups are condensed with compound **2** and the monomethyl ester of **2**, respectively, while in compound **15**, the 8a-carboxy group further forms a methyl ester as compared to **14**. Compound **16** is an 8a-decarboxy derivative of **13**. The fact that compound **12** is converted into compound **16** in the TLC plate impregnated with 2% formic acid suggests **16** to be the cyclization product of the former. Thus compound **16** might be an artifact [23].

## 2.4 Caffeic Acid Tetramers

Compounds of this group are constructed by four caffeic acid units, and can also be considered as dimeric coupling derivatives of rosmarinic acid through different linkage modes. This group includes five members: salvianolic acid E (**17**) [13], salvianolic acid B (**18**, lithospermic acid B) [23], ethyl lithospermate B (**19**) [13], magnesium lithospermate B (**20**) [24] and ammonium-potassium lithospermate B (**21**) [24] (Fig. 4).

Compound **17** is dimerized via a C-2 — C-8a linkage [13]. In compound **18**, the two rosmarinic acid units are also linked at C-2 — C-8a, but the 3-hydroxy and C-7a from two

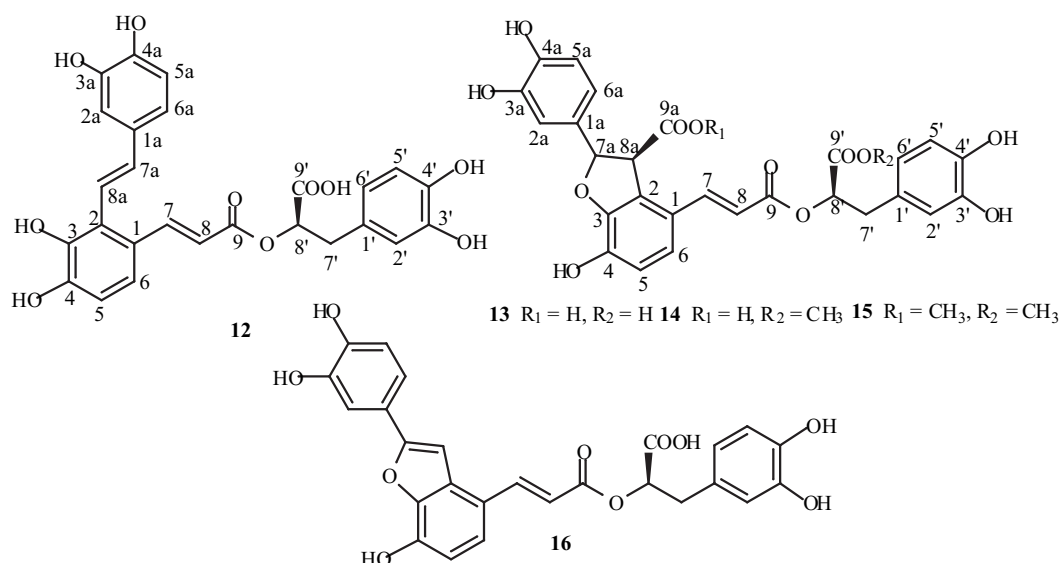


Fig. (3). Structural formulae of caffeic acid trimers.

different rosmarinic acid units are further cyclized to form a benzofuran moiety. Compound **18**, which is also known as lithospermic acid B, was isolated as the major component of *S. miltiorrhiza*, and its absolute configuration was established based on chemical degradation and circular dichroism spectral correlation [23]. Compound **19**, an ethyl ester of **18**, was proposed to be produced during the process of extraction and isolation [13]. Compounds **20** and **21**, two salts of **18**, were reported as active principles against uremia symptom [24].

### 2.5 Miscellaneous Group

This group includes protocatechuic acid (**22**) [25], protocatechuic aldehyde (**23**) [25], 5-(3-hydroxypropyl)-7-methoxy-2-(3'-methoxy-4'-hydroxyphenyl)-3-benzo[*b*] furancarbaldehyde (**24**) [26], and 1-hydroxypinoresinol-1-*O*- $\beta$ -*D*-glucoside (**25**) [27] (Fig. 5). Compounds **23** and **24** possess the hydroxybenzoic framework, in contrast to the

hydroxycinnamic skeleton found in caffeic acid. However, the former was proposed to originate from the side chain degradation of the latter [28]. Compound **24** was isolated as a novel adenosine  $A_1$  receptor ligand bearing the same benzofuran skeleton but different substitution pattern as compared to compound **10**, and its structure was substantiated by a total synthesis [26]. Compound **25** is a bisepoxylignan bearing a  $\beta$ -*D*-glucoside. It is the only phenolic glycoside isolated from *Salvia miltiorrhiza* and reported to exhibit radical scavenging activities toward peroxyxynitrite, 1,1-diphenyl-2-picrylhydrazyl and reactive oxygen species [27].

### 3 NATURAL RESOURCES

*Salvia* genus, belonging to the Labiaceae family, contains about 1000 species in the world, of which 78 are distributed in China [29]. Most caffeic acid derivatives (Table 1) reported in *S. miltiorrhiza* were also found in other

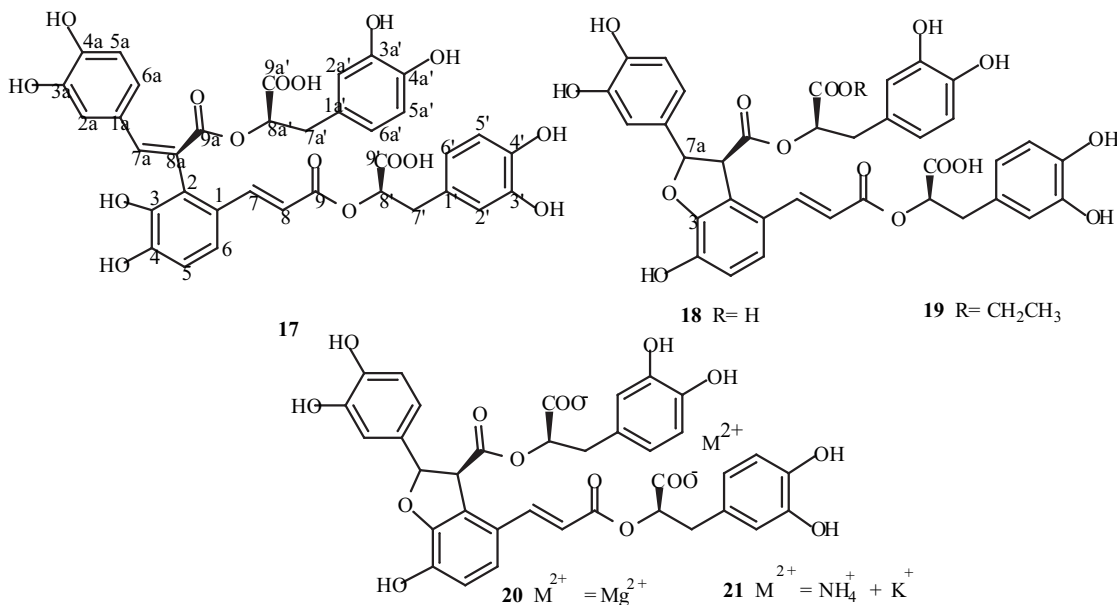
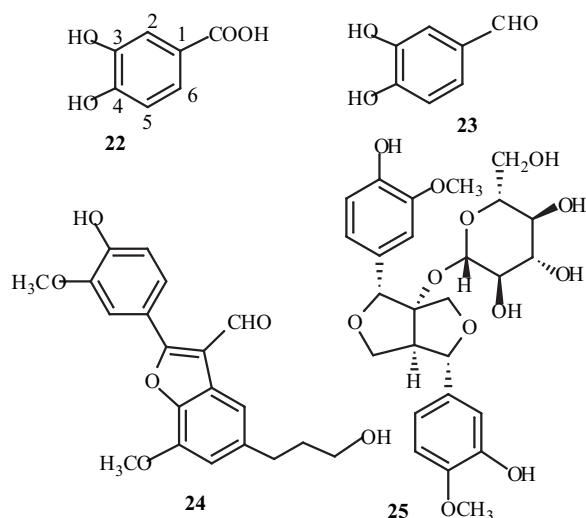


Fig. (4). Structural formulae of caffeic acid tetramers.

species of the same genus. For example, compounds **5**, **6**, **12**, **16**, **18**, **22** and **23** have been detected in twenty-one *Salvia* species (e.g. *S. officinalis*, *S. cavaleriei* and *S. przewalkii*) with the highest content being compound **18** (7.04%) in *S. bowleyana* [30]. Furthermore, caffeic acid is a common phenolic molecule and its derivatives are therefore also found in other genera and families. For examples, rosmarinic acid (**5**) was found to occur in *Rosemarinus* genus of the same family as *Salvia* and several other families, e.g. Boraginaceae and Blechnaceae [14], and lithospermic acid (**13**) was also found in *Lithospermum ruderale* [31] and *Tournefortia sarmentosa* [32] of the Boraginaceae family. Thus the occurrence of caffeic acid derivatives cannot be used as a chemotaxonomical marker to differentiate among species, genera and families. However, compounds **8**, **9**, **17** and **24** have been only reported from *S. miltiorrhiza*. Though all these compounds were initially isolated from the roots, caffeic acid derivatives have also been detected in the leaves and stems [33].



**Fig. (5).** Structural formulae of the miscellaneous group

#### 4 CHEMICAL SYNTHESIS AND BIOSYNTHESIS

The biological activities and structural diversity of this fascinating class of compounds have attracted a number of ingenious strategies toward their chemical synthesis and biosynthesis.

Chemical synthesis of rosmarinic acid (**5**) was achieved by coupling of the protected 3,4-dihydroxyphenyllactic acid and caffeic acid followed by a deprotection. This method can be used to synthesize both (*S*)- and the natural (*R*)-rosmarinic acids [34]. Total synthesis of salvianolic acid F (**9**) was motivated by a program to construct the biologically active salvianolic acid A (**12**). The synthesis of tetramethyl salvianolic acid F was started from isovanillin [35]. However, removal of the protected methyl group may lead to the destruction of the “fragile system”, because compound **9** is very reactive and particularly prone to cyclisation under acidic conditions. Reaction of tetramethyl salvianolic acid F with excess  $\text{BBr}_3$  under  $-40^\circ\text{C}$  led to the isolation of the target molecule in a 10% yield [36]. In order to improve the yield of **9**, a revised strategy including two stages was developed, i.e. i) access to the 1,2,3,4-substitution pattern of

the aromatic ring, and ii) convert to the desired *E,E*-2-styrylcinnamic acid system. Tetramethyl salvianolic acid F was obtained in six steps with 39% overall yield. Considering that the instability of compound **9** was due to a trace amount of acid, the reaction medium was poured into a solution of saturated  $\text{KH}_2\text{PO}_4$  in the final step, and tetramethyl salvianolic acid F was converted into the target molecule using boron tribromide in 26% yield [37].

Compounds **10**, **13-16** and **18-21** all possess a dihydrobenzofuranoid skeleton. However, they cannot be biogenetically regarded as products of oxidative dimerisation of 4-hydroxycinnamic derivatives, in contrast to lignans and neolignans, because the C6-C3 units of these compounds are linked by a C8a-C2 ( $\beta_2$ ) bond. Thus total synthesis of this class of compounds is more challenging [38]. Methylated przewalskinic acid A, the key fragment of **18**, was prepared by condensation of 4-hydroxy-5-methoxybenzofuran-2-one with isovanillin using piperidinium bezoate as the catalyst, followed by cyclization in a mixture of 40% HBr, benzene and chloroform and condensation with malonic acid [39].

Isolation, structure elucidation and initial synthesis of compound **24** were carried out in 1991 [26]. One year later, the same group reported the total synthesis of **24** as well as its derivatives involving thirteen steps. A key step in this synthetic procedure was the conventional coupling reaction between copper acetylide and aryl bromide, which generated the desired benzofuran skeleton [40]. In 1996, Kuo *et al.* reported a more convenient route featuring the coupling reaction from two monolignols based on free radical chemistry. In this synthetic method, the total number of steps was reduced to eight, and the yield was improved as compared with the first reported total synthesis [41]. Three years later, Scammells's group developed a new synthetic pathway using palladium-catalysed cyclization of *ortho*-hydroxytolans with concomitant carbonylation via insertion of carbon monoxide [42].

Biosyntheses of *Salvia* caffeic acid derivatives were carried out mostly using cells or organs culture system. Production of rosmarinic acid (**5**) and salvianolic acid B (**18**) in callus tissues and regenerated plantlets of *S. miltiorrhiza* were investigated [43]. Callus tissues maintained in 6-benzylaminopurine supplement produced compound **5** in 1.24% yield and compound **18** in 0.10% yield, while the corresponding concentration produced by the regenerated plantlets was 6.96% and 6.05%, respectively. Hairy root cultures of *S. miltiorrhiza* were established by infecting sterile plantlets with *Agrobacterium rhizogenes* ATCC 15834 [44]. As detected by HPLC, these hairy root cultures produced compounds **5** and **18** with the highest yield of 23 mg/L and 64 mg/L, respectively. Furthermore, the contents of these caffeic acid derivatives were found to be enhanced by the addition of a yeast elicitor [45]. A mechanistic study showed that cytochrome P450 protein in yeast elicitor plays an important role in regulation of compound **5** biosynthesis [46].

#### 5 ANALYTICAL METHODS

The rapid qualitative and quantitative analyses of structurally closely related compounds have been an important issue of medicinal chemistry. Several analytical

methods including TLC, HPLC, LC-MS and HSCCC have been developed for the determination of caffeic acid derivatives. TLC remains a common analytical method with advantages of low cost and easy operation. A TLC-densitometric method has been reported for the parallel determination of caffeic acid (**1**) and rosmarinic acid (**5**) in five *Salvia* species [47]. HPTLC procedure for the separation and detection of seven phenolics [caffeic acid (**1**), rosmarinic acid (**5**), methyl rosmarinate (**6**), salvianolic acids A (**12**), B (**18**), C (**16**) and protocatechualdehyde (**23**)] in *S. miltiorrhiza* has been developed. Solvent system I consisting of chloroform-ethyl acetate-benzene-formic acid (2.4:2:1:0.6) was used to separate compound **23**, and then solvent system II comprising chloroform-ethyl acetate-benzene-formic acid-methanol (1.5:2:1:1:0.1) was used to

separate the other six components. All these caffeic acid derivatives were detected at the wavelengths of  $\lambda_S = 300$  nm and  $\lambda_R = 240$  nm [48].

HPLC is also a commonly used technique. It can provide both qualitative and quantitative surveys of the composition in the extract. An isocratic reverse-phase HPLC method was described to measure compound **12** in rat plasma using a mobile phase of acetonitrile-water (1:2.65, adjusted pH to 2.5 with formic acid). The recovery efficiency of this method was 98.9-106.9% [49]. A gradient reverse-phase HPLC method was developed for the simultaneous separation and determination of compound **5**, **18** and another four related phenolic compounds. The mobile phase consists of solvents I (methanol/water/formic acid, 14.0:85.2:0.8) and II (methanol/water, 65:35). The gradient procedure was started

**Table 1** Caffeic Acid Derivatives Isolated from *S. miltiorrhiza*

caffeic acid derivatives	Formula	M.W.	References
Caffeic acid ( <b>1</b> )	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	180	[6]
3-(3,4-dihydroxyphenyl)lactic acid ( <b>2</b> )	C <sub>9</sub> H <sub>10</sub> O <sub>5</sub>	198	[7]
3-(3,4-dihydroxyphenyl)lactamide ( <b>3</b> )	C <sub>9</sub> H <sub>11</sub> NO <sub>4</sub>	197	[8]
Isoferulic acid ( <b>4</b> )	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	194	[9]
Rosmarinic acid ( <b>5</b> )	C <sub>18</sub> H <sub>16</sub> O <sub>8</sub>	360	[10], [11], [12]
Methyl rosmarinate ( <b>6</b> )	C <sub>19</sub> H <sub>18</sub> O <sub>8</sub>	374	[12]
Salvianic acid C ( <b>7</b> )	C <sub>18</sub> H <sub>18</sub> O <sub>9</sub>	378	[13]
Salvianolic acid D ( <b>8</b> )*	C <sub>20</sub> H <sub>18</sub> O <sub>10</sub>	418	[9]
Salvianolic acid F ( <b>9</b> )*	C <sub>17</sub> H <sub>14</sub> O <sub>6</sub>	314	[14]
Prolithospermic acid ( <b>10</b> )	C <sub>18</sub> H <sub>14</sub> O <sub>8</sub>	358	[15], [16]
Salvianolic acid G ( <b>11</b> )	C <sub>18</sub> H <sub>12</sub> O <sub>7</sub>	340	[17]
Salvianolic acid A ( <b>12</b> )	C <sub>26</sub> H <sub>22</sub> O <sub>10</sub>	494	[18]
lithospermic acid ( <b>13</b> )	C <sub>27</sub> H <sub>22</sub> O <sub>12</sub>	538	[12]
lithospermic acid monomethyl ester ( <b>14</b> )	C <sub>28</sub> H <sub>24</sub> O <sub>12</sub>	552	[12]
lithospermic acid dimethyl ester ( <b>15</b> )	C <sub>29</sub> H <sub>26</sub> O <sub>12</sub>	566	[12]
Salvianolic acid C ( <b>16</b> )	C <sub>26</sub> H <sub>20</sub> O <sub>10</sub>	492	[19]
Salvianolic acid E ( <b>17</b> )*	C <sub>36</sub> H <sub>30</sub> O <sub>16</sub>	718	[9]
Salvianolic acid B ( <b>18</b> )	C <sub>36</sub> H <sub>30</sub> O <sub>16</sub>	718	[19]
Ethyl lithospermic acid B ( <b>19</b> )	C <sub>38</sub> H <sub>34</sub> O <sub>16</sub>	746	[9]
Magnesium lithospermic acid B ( <b>20</b> )	Mg <sup>2+</sup> [C <sub>36</sub> H <sub>28</sub> O <sub>16</sub> ] <sup>2-</sup>	740	[20]
Ammonium-potassium lithospermic acid ( <b>21</b> )	NH <sub>4</sub> <sup>+</sup> K <sup>+</sup> [C <sub>36</sub> H <sub>28</sub> O <sub>16</sub> ] <sup>2-</sup>	773	[20]
Protocatechuic acid ( <b>22</b> )	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>	154	[21]
Protocatechuic aldehyde ( <b>23</b> )	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>	138	[21]
5-(3-hydroxypropyl)-7-methoxy-2-(3'-methoxy-4'-hydroxyphenyl)-3-benzo[b]furancarbaldehyde ( <b>24</b> )*	C <sub>20</sub> H <sub>20</sub> O <sub>6</sub>	356	[22]
1-hydroxypinoresinol-1-O-β-D-glucoside ( <b>25</b> )	C <sub>26</sub> H <sub>32</sub> O <sub>12</sub>	536	[27]

\* Caffeic acid derivatives unique in *S. miltiorrhiza*  
M.W. molecular weight.

from 0% of II for 2 min and then a linear gradient from 30% to 45% of II for 8 min. The flow rate was set at 1.0 ml/min and peaks were measured at a wavelength of 280 nm. The developed method was successfully applied for the determination of caffeic acid derivatives in the dried root of *S. miltiorrhiza* and its transformed cells [50]. LC-MS has proven to be a particular useful technique due to its high sensitivity and great potential to provide structure information. An LC-MS method was established for the rapid analysis of phenolic compounds from *S. miltiorrhiza*. Compounds **2**, **23**, **1**, **13**, **18** and **16** were detected in the water extract successively [51].

High speed counter current chromatography (HSCCC) was applied for both analysis and purification of compounds from *S. miltiorrhiza* [52]. A two-phase solvent system *n*-hexane-ethyl acetate-ethanol-water (3:7:1:9, vol./vol.) was successfully performed to yield 342 mg salviannic acid B (**18**) at 98% purity from 500 mg of crude extract [52].

## 6 BIOLOGICAL ACTIVITIES

Phenolic acids are thought to play a positive role in the prevention of human diseases [53]. As a popular traditional

**Table 2** Some Reported Bioactivities of Caffeic acid Derivatives from *S. miltiorrhiza*

Caffeic acid derivatives	Biological activity	References
<b>1</b>	Anti-lipid-peroxidation	[55]
<b>2</b>	Anti-lipid-peroxidation	[55]
<b>5</b>	Anti-lipid-peroxidation Radical scavenging activity Antioxidation of low density lipoprotein Anti-thrombosis Inhibition of HIV-1 integrase Inhibition of HIV-1 reverse transcriptase Inhibitory activities against adenylate cyclase	[55], [56] [57], [58] [60] [71] [85] [86] [16]
<b>6</b>	Inhibitory activities against adenylate cyclase	[16]
<b>10</b>	Anti-lipid-peroxidation Anti-hypertension	[55] [74]
<b>12</b>	Anti-lipid-peroxidation Radical scavenging activity Antioxidation of low density lipoprotein Protection against cerebral and heart ischemia-reperfusion Inhibitory activity against liver fibrosis and hepatoprotection Inhibition of the side effects of antitumor drugs Inhibition of gastric H <sup>+</sup> , K <sup>+</sup> -ATPase Inhibition of 5-lipoxygenase Inhibition of aldose reductase	[55], [56] [57] [59], [60] [65], [67] [76], [77], [78], [79] [88], [89] [91] [95] [96]
<b>13</b>	Antioxidation of low density lipoprotein Inhibition of HIV-1 integrase Inhibition of adenylate cyclase	[60] [83], [84] [16]
<b>14</b>	Inhibition adenylate cyclase	[16]
<b>16</b>	Radical scavenging activity	[57], [58]
<b>18</b>	Anti-lipid-peroxidation Radical scavenging activity Inhibition of atherosclerotic lesions Protection against cerebral and heart ischemia-reperfusion Anti-hypertension Inhibitory activity against liver fibrosis and hepatoprotection Inhibition of HIV-1 integrase Inhibition of the side effects of antitumor drugs Inhibit of amyloid beta-protein fibril formation Inhibition of 5-lipoxygenase Inhibition of aldose reductase	[55], [56] [57], [58] [61] [66], [68], [69] [72] [80], [81], [82] [83], [84] [90] [93] [95] [96]
<b>20</b>	Anti-hypertension Improving effects on uremic symptoms	[73] [94]
<b>21</b>	Improving effects on uremic symptoms	[94]
<b>23</b>	Anti-lipid-peroxidation	[55]
<b>24</b>	Ligand of adenosine A <sub>1</sub> receptor	[26]
<b>25</b>	Radical scavenging activity	[27]

herbal medicine and a rich source of caffeic acid derivatives, the active components of *S. miltiorrhiza* responsible for its clinical usages were extensively studied. The natural abundance of these compounds allowed systematic studies on their biological properties. Accordingly, a wide variety of activities including antioxidant, anti-ischemia-reperfusion, anti-thrombosis, anti-hypertension, anti-fibrosis, antiviral and antitumor of some representatives have been reported (Table 2).

### 6.1 Antioxidant

The oxidative damages happen when the reactive oxygen species, e.g. superoxide ( $O_2^{\bullet-}$ ) and hydroxyl ( $HO^{\bullet}$ ), attack the lipid in cell membranes, proteins and DNA to cause membrane injury and protein and DNA modification [54]. The antioxidant effects of caffeic acid derivatives include three aspects: i) anti-lipid-peroxidation; ii) radical scavenging; and iii) antioxidation of low density lipoprotein.

The effects of caffeic acid (**1**), danshensu (**2**), rosmarinic acid (**5**), salvianolic acid A (**12**), B (**18**), prolithospermic acid (**10**) and protocatechuic aldehyde (**23**) on the peroxidation damage to live microsomes, hepatocytes and erythrocytes of rats were examined. The results showed that all seven compounds exhibited inhibitory activity against the lipid peroxidation induced by iron/cysteine and the erythrocytes hemolysis induced by hydrogen peroxide. Among seven compounds, compound **12** was the most potent [55]. Compounds **5**, **12** and **18** also inhibited the peroxidation of rat microsomes of the brain and kidney in the order of **12** > **18** > **5** [56].

Caffeic acid derivatives possess radical scavenging activity. Compounds **5**, **12**, **16** and **18** have been found to lower the production of superoxide anion radical ( $O_2^{\bullet-}$ ) in the xanthine oxidase system [57]. The effects of these compounds on the hydroxylation of salicylic acid in both EDTA- $H_2O_2$ - $Fe^{2+}$  and Vitamin C- $H_2O_2$ - $Fe^{2+}$  systems were studied. All compounds were shown to dose-dependently inhibit the hydroxylation of salicylic acid except compound **12**, which promoted hydroxylation at lower concentration, but inhibited it at higher concentration. Caffeic acid derivatives were thus considered to be hydroxyl radical scavengers [58].

Oxidized low density lipoprotein (LDL) is involved in the development of atherosclerosis. Oxidative modulation of serum LDL is related to the oxygen free radicals. The effect of compound **12** on human LDL oxidative modulation mediated by  $Cu^{2+}$  was studied. The results showed that compound **12** markedly reduced the production of malondialdehyde (MDA) and lipofuscin, as well as consumption of vitamin E during LDL oxidation. Compound **12** also dose-dependently decreased the generation of free radicals [59]. The same model was used to evaluate the anti-lipid-peroxidative activity of compounds **5**, **12**, **13** and **9**. The results showed that all compounds exhibited more potent activity than probucol (cholesterol-lowering agent) except for **9** [60]. Treatment of a compound **18** enriched fraction of *S. miltiorrhiza* significantly reduced atherosclerotic lesions in the NZW rabbits and apoE (-) mice animal models [61].

### 6.2 Protective Effects Against Cerebral And Heart Ischemia-Reperfusion

The protective effect of total salvianolic acids against cerebral ischemia-reperfusion injury was studied. The cerebral ischemia-reperfusion model was made by ligating bilateral common carotid arteries in mice. Spectrophotometric assay was used to measure the activity of superoxide dismutase (SOD), content of MDA and glutathione peroxidase (GSH-Px) in the experimental mice brain. The results showed that total salvianolic acids could remarkably improve the function of learning and memory, and also reduce the error number. The changes of SOD, MDA and GSH-Px in the cerebrum were significantly inhibited [62], and the infarction size in focal injured rat induced by two hours ischemic and twenty-four hours reperfusion was decreased (5 mg/kg and 10 mg/kg, i.v.). It was proposed that the total salvianolic acids had a protective effect against the cerebral ischemia-reperfusion injury via its antioxidant activity [63].

Inhibition of cerebral ischemia-reperfusion was also tested on a middle cerebral artery occlusion animal model. Injection of total salvianolic acids (12.5-25 mg/kg, i.p. 2 h after middle cerebral artery occlusion) was found to decrease the infarction area and inhibit the glutamate release from brain synaptosomes [64].

Salvianolic acid A (**12**) was shown to improve the impairment of memory function induced by cerebral ischemia-reperfusion in the step-down and step-through tests. The mean error number of 3 and 10 mg/kg (i.v.) treated groups were 1.29 and 1.15, in contrast to 3.8 of the control group [65], and the latency of the treated group was longer than that of control. Salvianolic acid B (**18**) was assessed by a learning and memory dysfunction model induced by transient cerebral ischemia in mice. At the same dose as **12**, the error number was also decreased and latency was increased in the treated group as compared with those of the control [66].

The protective effect of compound **12** on the cardiac ischemia-reperfusion induced injury was studied on an isolated rat heart. The ventricular fibrillation was reduced at the concentration of 0.1  $\mu$ mol/L. The production of MDA was significantly inhibited and the surface damage of cardiac leaking in Langendorff rat heart was decreased. These results indicated that compound **12** possessed anti-arrhythmic effect in the ischemia-reperfusion rat heart and protective effect on the cardiac myocytes [67]. Compound **18** also showed antioxidant-based protective effect against myocardial ischemia-reperfusion [68]. It was found to reduce  $62 \pm 10\%$  damage of the rabbit heart when infused at 5.5  $\mu$ mol/kg as compared with the saline control [69].

### 6.3 Anti-Thrombosis

The effects of total salvianolic acids on thrombosis and the plasma endothelin content after cerebral ischemia were studied using a middle cerebral artery occlusion and thrombosis model. The results showed that total salvianolic acids inhibited the formation of thrombosis, and decreased the plasma endothelin content and the high level of thromboxane B2 (TXB2) after cerebral ischemia. Inhibition of thrombosis was proposed to be another anti-cerebral-

ischemia mechanism of salvianolic acids [70]. Rosmarinic acid (**5**) was also found to show mild antithrombotic effect. The venous thrombosis was inhibited by 41.9 and 54.8% at the dosages of 50 and 100 mg/kg, respectively, while the blood platelet aggregation was suppressed by 30.4 and 46.4%, respectively [71].

#### 6.4 Anti-Hypertension

Intravenous injection of compound **18** (10-30 mg/kg) decreased the blood pressure in a dose-dependent manner. The antihypertensive effect may be due to the endothelium-dependent vasodilation of resistant artery [72]. Oral administration of magnesium lithospermate B (**20**) lowered the systolic, mean and diastolic blood pressures in the hypertensive rats [73]. Prolithospermic acid (**10**) could cause a slow but sustained relaxation of rat aortic strips precontracted with norepinephrine [74].

#### 6.5 Inhibitory Activity Against Hepatic Fibrosis and Hepatoprotection

Hepatic fibrosis occurs as a result of injury to the liver parenchyma and biliary system [75]. Salvianolic acid A (**12**) was found to inhibit the activities of serum alanine transaminase (ALT) and aspartate transaminase (AST), alleviate liver fibrogenesis, maintain the deposition of type I and III collagen in liver matrix, and decrease the intracellular collagen synthetic rate and pro-collagen mRNA expression markedly [76]. It could inhibit the NIH/3T3 fibroblast proliferation [77] and the activation of hepatic stellate cells [78]. Compound **12** was also shown to possess better efficacy against liver injury and fibrosis induced by CCl<sub>4</sub> than the widely recognized vitamin E [79].

Salvianolic acid B (**18**) could effectively reverse the hepatic fibrosis in chronic hepatitis B. Compound **18** performed better activity than interferon (positive control) in the overall decrease of serum fibrotic markers and ultrasound imaging score, and showed no obvious side effect [80]. A mechanistic study showed that compound **18** could inhibit the hepatic stellate cell proliferation and collagen production as well as the transformation growth factor  $\beta$ 1 (TGF- $\beta$ 1), autocrine and mitogen-activated protein kinase (MAPK) of the cells [81]. Similar to **12**, compound **18** was also found to show potent hepatoprotective activity against liver injuries induced by CCl<sub>4</sub> or D-galactosamine [82].

#### 6.6 Antivirus

The water extract of *S. miltiorrhiza* was found to exhibit potency against HIV-1 integrase. Bioassay guided isolation led to the identification of lithospermic acid (**13**) and lithospermic acid B (**18**), whose IC<sub>50</sub> were 0.83 M and 0.48 M, respectively. These two compounds hold promise as novel therapeutic agents for AIDS based on their high potency and absence of cytotoxicity [83]. A recent patent also claimed the anti-HIV-1 activity of compounds **13** and **18** [84]. Rosmarinic acid (**5**) inhibited the activities of both mutant and wild-type integrase of HIV-1 virus with both IC<sub>50</sub> values below 10  $\mu$ M. Kinetic studies suggested that it binds to the enzyme at a slow rate [85]. Compound **5** was

also found to directly inhibit the reverse transcriptase of HIV-1 virus [86].

#### 6.7 Antitumor

The aqueous extract of *S. miltiorrhiza* was found to strongly inhibit the proliferation of human hepatoma HepG<sub>2</sub> cells. It was also observed that crude extract treatment caused apoptotic cell death [87]. Salvianolic acid A (**12**) was claimed as an antitumor drug in a Chinese patent. It showed synergistic effects in combination with other antitumor agents e.g. 5-fluorouracil (5-FU), mitomycin C and methotrexate [88]. Furthermore, compound **12** could increase the antitumor effects of 5-FU without increasing its toxicity in an animal study [88]. It was also found to protect rat heart mitochondria against adrimycin induced toxicities, e.g. MDA formation and membrane rigidification [89]. A Japanese patient claimed that salvianolic acid B (**18**) was beneficial to alleviation of the cytotoxicity of cisplatin, an important antitumor drug [90]. Thus compounds **12** and **18** might curtail the side effects of some commonly used antitumor drugs.

#### 6.8 Miscellaneous Activities

Compounds **5**, **13** and their methyl esters exhibited significant inhibitory activities against adenylate cyclase in both rat brain and erythrocytes [16]. Compound **12** showed anti-secretory and anti-ulcer activities by inhibiting the gastric H<sup>+</sup>, K<sup>+</sup>-ATPase [91]. The crude extract of *S. miltiorrhiza* and compound **18** could enhance the angiogenic processes in SVR endothelial cell line through upregulation of vascular endothelial growth factor (VEGF) and VEGF genes [92]. Compound **18** could inhibit the amyloid beta-protein fibril formation and its toxicity towards PC12 cells, which was the major pathological feature of Alzheimer's disease [93]. The magnesium and ammonium potassium salts of compound **18** exhibited beneficial effects on uremic symptoms by significantly decreasing the blood urea nitrogen, creatine, methylguanidine and guanidine succinic acid in rats with chronic renal failure [94]. Compounds **12** and **18** were also found to be inhibitors of 5-lipoxygenase [95] and aldose reductase [96], which are involved in the allergic reaction and the galactose metabolism, respectively.

### 7 SUMMARY

*S. miltiorrhiza* is a rich source of caffeic acid derivatives. They have become the focus of increasing attention from many research groups in recent years, owing to their exciting chemistry and their wide spectrum of biological activities. Tremendous progress has been achieved in the methodology for the isolation, structural identification, analyses and biological activities of these phenolic acids. Most of the biological activities, e.g. antioxidant, anti-ischemia-reperfusion, anti-thrombosis and anti-hypertension, are closely related to the clinical applications of *S. miltiorrhiza* for the treatment of heart and cerebral circulation diseases. Thus a systematic investigation on natural and synthetic products derived from caffeic acid derivatives of *S. miltiorrhiza* not only leads to evaluation of totally new

classes of cardiotoxic agents, but also illustrates the application of modern techniques to the modernization and development of traditional Chinese herbal medicines.

Still, much work remains to be done, particularly in the areas of biochemical pharmacology, chemical synthesis and metabolism of this intriguing class of natural products. Most bioassay studies were performed on salvianolic acid A (**12**) and lithospermic acid B (**18**) and not compared with other related compounds. Thus the active pharmacore and the structure-activity relationship have not been established. Though compounds **12** and **18** are considered to be the major active components of *S. miltiorrhiza*, their total syntheses have yet to be achieved. Furthermore, there is a very large amount of in-vitro data, but few reports of animal studies are available. The bioavailability and metabolism of this class of compounds were not studied except for rosmarinic acid (**5**) [97].

In conclusion, caffeic acid derivatives from *S. miltiorrhiza* are of interest in the context of structural diversity, and also in regard to their broad spectrum of biological activities. Thus this class of compounds and their biological activities are worthy of further studies and development.

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## ABBREVIATIONS

ALT	= Alanine transaminase
AST	= Aspartate transaminase
GSH-Px	= Glutathione peroxidase
HIV	= Human immunodeficiency virus
HPLC	= High performance liquid chromatography
HPTLC	= High performance thin layer chromatography
HSCCC	= High speed counter current chromatography
i.p	= Intraperitoneal
i.v	= Intravenous
LDL	= Low density lipoprotein
MAPK	= Mitogen-activated protein kinase
MDA	= Malondialdehyde
SOD	= Superoxide dismutase
TGF- $\beta$ 1	= Transforming growth factor $\beta$ 1
TLC	= Thin layer chromatography
TXB2	= Thromboxane B2
VEGF	= Vascular endothelial growth factor

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