Research Article

Design, Synthesis, and Preliminary Evaluation of Substituted Cinnamic Acid Esters as Selective Matrix Metalloproteinase Inhibitors

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Substituted cinnamic acid esters with extended P1′ groups were synthesized using microwave irradiation and tested for their inhibitory activities on matrix metalloproteinase (MMP)-1, MMP-2, and MMP-9. Preliminary structure–activity relationship analysis and docking studies showed that hydroxyl groups in the benzene ring and the presence of extended spatial structures in the carboxylic acid played key roles in the MMP-2 and MMP-9 inhibitory activity and selectivity over MMP-1. Drug Dev Res •• : ••–••, 2012.

Key words: matrix metalloproteinases; tumor; caffeic acid; structure–activity relationship

INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases involved in the breakdown of components of the extracellular matrix that facilitates connective tissue remodeling [Nagase and Woessner, 1999]. This process is important in embryonic development, pregnancy, growth, and wound healing. Normally, MMP activity is tightly controlled by the balance between synthesis of active MMPs and the presence of endogenous inhibitors, e.g., the tissue inhibitor of metalloproteinases [Nagase and Woessner, 1999].

This balance is lost with tumor progression, where an increased expression of certain MMPs accompanies the passage from a benign to a malignant phenotype that is believed to be involved in metastatic tumor dispersion and angiogenesis [MacDougall and Matrisian, 1995; Stetler-Stevenson, 1999]. The MMPs are produced and secreted as inactive zymogens in the extracellular matrix from both tumor cells and surrounding stromal cells that are stimulated by the tumor. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) appear to play a key role in these processes [Crabbe et al., 1994; Aimes and Quigley, 1995].

In the past few years, some potent "broad spectrum" MMP inhibitors (MMPIs) have been tested against tumors [Ray and Stetler-Stevenson, 1996] with some entering clinical trials. None is yet on the market.

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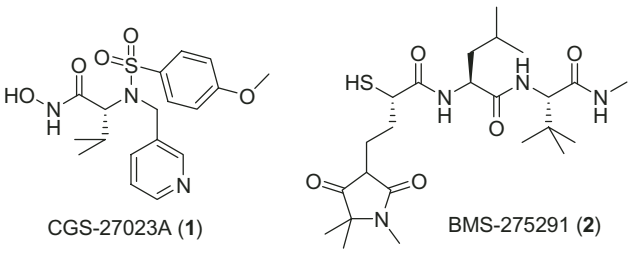

Fig. 1. Structures of CGS-27023A (**1**) and BMS-275291 (**2**).

One problem was the design of the trials themselves. MMPIs had been successful in animal models of early-stage disease but were only tested in late-stage disease in humans [Overall and López-Otín, 2002]. Since they were developed as cytostatic agents, they may be used in a prolonged therapy, and therefore, their bioavailability and slow long-term toxicity are key [Hodgson, 1995]. Another problem was the lack of selectivity with compounds such as CGS-27023A (**1**) (Ciba Geigy) and BMS-275291 (**2**) (Bristol-Myers Squibb) (Fig. 1) showing a severe musculoskeletal syndrome, with fibroproliferative effects in the joint capsule of the knees [Hutchinson et al., 1998; Holmbeck et al., 1999; Steward, 1999]. These effects are thought to occur via impairment of normal tissue remodeling controlled by MMP-1 [Dahlberg et al., 2000]. A lack of activity with respect to MMP-1 is considered to be an important factor in reducing some of the side effects found for "nonselective" MMPIs [Scatena, 2000].

In light of these findings, considerable efforts have been directed to develop a more selective second generation of inhibitors against the specific MMPs believed to be involved in the different pathologies [Li et al., 2009a]. MMPIs may also be derived from natural resources, e.g., herbs, plants, fruits, and other agriculture products.

Caffeic acid (**3**) (Fig. 2), which is found in fruits, vegetables, wine, olive oil, and coffee [Park et al., 2005], inhibits activity of MMP-9 (half maximal (50%) inhibitory concentration IC_{50} = 10–20 nM), but its selectivity for MMP-2 and MMP-9 over MMP-1 was not high [Chung et al., 2004].

Selectivity could potentially be achieved by taking advantage of differences in size of the S1′ pocket among the various MMPs. From X-ray crystallographic, nuclear magnetic resonance (NMR) analysis, and homology modeling, MMPs can be classified into two broad structural classes: those with a relatively deep S1′ pocket (MMP-2, 3, 8, 9, and 13) and those with a shallow S1′ pocket (MMP-1 and 7) [Gooley et al., 1994; Lovejoy et al., 1994; Verma and Hansch, 2007]. Consequently, incorporation of an extended P1′ group leads to selective inhibition, whereas the presence of smaller P1′ groups generally leads to broad-spectrum inhibition.

This prompted us to find new caffeic acid derivatives as selective MMP-2 and MMP-9 inhibitors. First, our docking studies of caffeic acid with MMP-2 and MMP-9 showed that carboxylic acid could reacted with the S1′ pocket in MMP-2 and MMP-9 to achieve enhanced inhibitory activities and selectivity over MMP-1 (Fig. 2). Esters of caffeic acid with extended P1′ group (such as branched alkanes and cycloalkanes) improved the inhibitory activity and selectivity. Furthermore, in order to investigate the MMPs inhibitory effects of the substitution on the benzene ring of caffeic acid, its two natural analogs ferulic acid and cinnamic acid were also esterified with an extended P1′ group, based on their inhibitory activities on MMP-1, MMP-2, and MMP-9; structure–activity relationship (SAR) analysis was conducted.

MATERIALS AND METHODS

Synthesis

Reagents and solvents were purchased from commercial sources and used without further purification unless otherwise specified. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation below 45°C at approximately 20 mmHg. All nonaqueous reactions were carried out under anhydrous conditions using flame-dried glassware within an argon atmosphere in dry, freshly distilled solvents, unless otherwise noted. Yields referred to chromatographically, unless otherwise stated. Reactions were monitored by thin-layer chromatography carried out on 0.15~0.20 mm Yantai silica gel plates (RSGF 254) (Yantai Chemical Industry Research Institute, Yantai, Shandong Province, China) using ultraviolet (UV) light as the visualizing agent. Chromatography was performed on Qingdao silica gel (160~200 mesh) (Qingdao Banke separation materials Company Limited, Qingdao, Shandong Province, China) using petroleum ether (60~90) and ethyl acetate as the eluting solvent (Sinopharm Chemical Reagent Company Limited, Shanghai, China).

1H NMR spectra were obtained using a Bruker AV-300 (300 MHz) (Bruker Corporation, Germany). Chemical shifts were recorded in parts per million (ppm) downfield from tetramethylsilane. J-values are given in Hertz (Hz). Abbreviations used were for singlet (s), doublet (d), triplet (t), quartet (q), broad (b), and multiplet (m). Electrospray ionisation tandem mass spectrometry (ESI-MS) spectra were recorded on a Waters Synapt high definition mass spectrometry (HDMS) spectrometer.

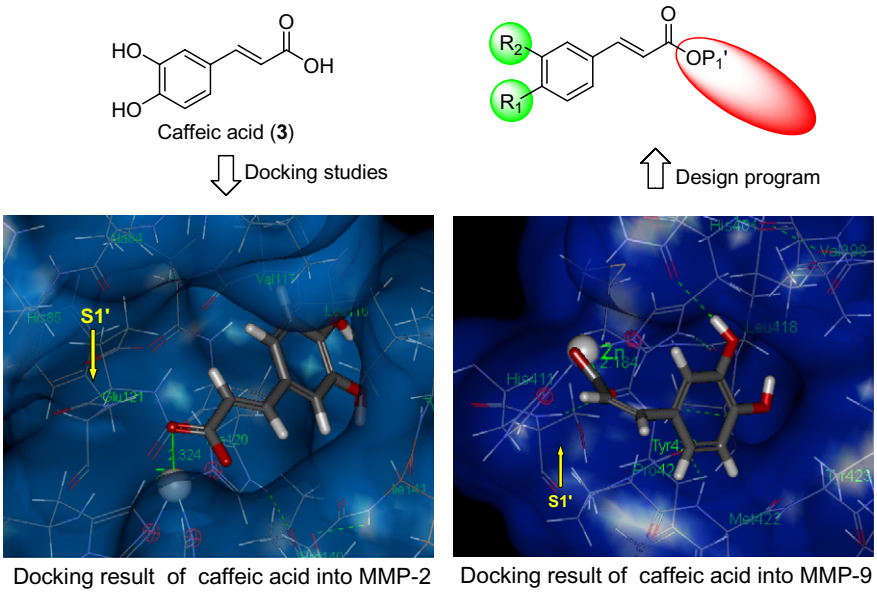

Fig. 2. The structures of caffeic acid, docking studies, and the design program. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

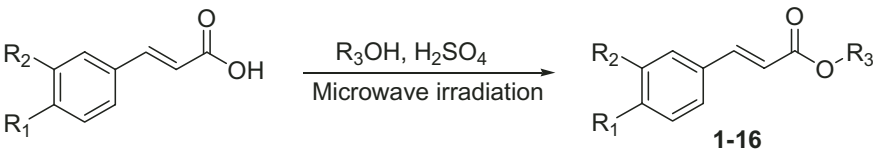

Fig. 3. Synthesis of compounds **4**–**18** under microwave irradiation.

Cinnamic acid esters **4**–**8**, ferulic acid esters **9**–**13** and caffeic acid esters **14**–**18** were prepared as shown in Figure 3. Although these compounds could be synthesized with the acid directly refluxed with alcohols in the presence of various catalysts including sulfuric acid, hydrogen chloride, boron trifluoride, aluminum chloride, trifluoroacetic anhydride, polyphosphate ester, neodymium oxide, dicyclohexylcarbodiimide, graphite bisulfate, etc [Olah et al., 1978; Li, 1999; Zhang, 1999], the disadvantages of using these catalysts include long-reaction time, low yield, and expensive reagents. In recent years, reactions using microwave irradiation were, in general, not only faster compared with the conventional heating reactions but also potentially more efficient, cleaner, and safer [Kappe, 2004]. Further improvements offer enhanced reaction rates, higher yields, and greater selectivity for the targeted product under milder reaction conditions [Li et al., 2009b]. A highly efficient synthesis of compounds **4**–**18** under microwave irradiation was adopted and implemented with reactions times ranging from 3–6 min (Table 1) methods [Nagaoka et al., 2002] and compounds **4**–**18** were obtained in higher yields (>90%) (Table 1).

EXPERIMENTS

General Procedures for the Esterification of Substituted Cinnamic Acid Esters under Microwave Irradiation

To a stirring mixture of substituted cinnamic acid (5 mmol) in alcohol (10 ml) was added the concentrated sulfuric acid (0.067 ml, 1.25 mmol), and the reaction mixture was refluxed for 3–6 min in a sealed reaction vessel (Discover CEM, Matthews, North Carolina, USA) under microwave irradiation, where the power was set at 200 W, the temperature was set at 10°C above the boiling point of the respective alcohol, and the pounds per square inch (PSI) was set at 180. After cooling to 25°C, ethyl acetate was added and the solution washed with water and brine. The ethyl acetate layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using

TABLE 1. The Structures, Reaction Temperature, Reaction Time, and Yields of Compounds 4–18 under Microwave Irradiation

Product	R_1	R_2	R_3	Temp. (°C)	Time (min)	Yield (%)
4	H	H	$CH(CH_3)_2$	92	3	94
5	H	H	$CH_2CH(CH_3)_2$	118	3	94
6	H	H	$CH_2CH_2CH(CH_3)_2$	142	4	93
7	H	H	$CH(CH_2)_4$	150	5	92
8	H	H	$CH(CH_2)_5$	171	5	91
9	OH	OCH_3	$CH(CH_3)_2$	92	4	93
10	OH	OCH_3	$CH_2CH(CH_3)_2$	118	4	93
11	OH	OCH_3	$CH_2CH_2CH(CH_3)_2$	142	5	91
12	OH	OCH_3	$CH(CH_2)_4$	150	6	91
13	OH	OCH_3	$CH(CH_2)_5$	171	6	90
14	OH	OH	$CH(CH_3)_2$	92	4	93
15	OH	OH	$CH_2CH(CH_3)_2$	118	4	92
16	OH	OH	$CH_2CH_2CH(CH_3)_2$	142	5	91
17	OH	OH	$CH(CH_2)_4$	150	6	90
18	OH	OH	$CH(CH_2)_5$	171	6	90

20% ethyl acetate in petroleum ether to afford the substituted cinnamic esters.

- Isopropyl cinnamate (**4**) [Maki et al., 2009]: yield 94%; 1H NMR ($CDCl_3$, AV-300), δ: 1.31(d, J = 6.2 Hz, 6H), 4.12(m, 1H), 6.42(d, J = 16.1 Hz, 1H), 7.37(m, 3H), 7.52(m, 2H), 7.66(d, J = 16.1 Hz, 1H); ESI-MS m/z: 191 $[M + H]^+$, 213 $[M + Na]^+$.
- Isobutyl cinnamate (**5**) [Tani et al., 2004]: yield 94%; 1H NMR ($CDCl_3$, AV-300), δ: 0.91(m, 6H), 1.71(m, 2H), 3.91(m, 1H), 6.46(d, J = 16.0 Hz, 1H), 7.37(m, 3H), 7.52(m, 2H), 7.68(d, J = 16.0 Hz, 1H); ESI-MS m/z: 205 $[M + H]^+$, 227 $[M + Na]^+$.
- Isopentyl cinnamate (**6**) [Narasimhana et al., 2004]: yield 93%; 1H NMR ($CDCl_3$, AV-300), δ: 0.92(m, 6H), 1.57(m, 2H), 1.70(m, 1H), 4.19 (m, 2H), 6.47(d, J = 16.0 Hz, 1H), 7.36(m, 3H), 7.53(m, 2H), 7.67(d, J = 16.0 Hz, 1H); ESI-MS m/z: 205 $[M + H]^+$, 227 $[M + Na]^+$.
- Cyclopentyl cinnamate (**7**): yield 92%; 1H NMR ($CDCl_3$, AV-300), δ: 1.61(m, 4H), 1.92(m, 4H), 3.93(m, 1H), 6.41(d, J = 15.9 Hz, 1H), 7.37(m, 3H), 7.51(m, 2H), 7.65(d, J = 15.9 Hz, 1H); ESI-MS m/z: 217 $[M + H]^+$, 239 $[M + Na]^+$.
- Cyclohexyl cinnamate (**8**) [Sova et al., 2006]: yield 91%; 1H NMR ($CDCl_3$, AV-300), δ: 1.52(m, 2H), 1.73(m, 4H), 1.90(m, 4H), 4.13(m, 1H), 6.53(d, J = 15.9 Hz, 1H), 7.37(m, 3H), 7.52(m, 2H), 7.66(d, J = 15.9 Hz, 1H); ESI-MS m/z: 231 $[M + H]^+$, 253 $[M + Na]^+$.
- (E)-isopropyl-3-(3,4-dihydroxyphenyl)acrylate (**9**) [Murakami et al., 2000]: YIELD 93%; 1H NMR ($CDCl_3$, AV-300), δ: 1.32 (d, J = 6.2 Hz, 6H), 3.92 (s, 3H), 5.14 (m, 1H), 6.27 (d, J = 15.9 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 7.03 (m, 2H), 7.60 (d, J = 15.9 Hz, 1H); ESI-MS m/z: 223 $[M + H]^+$, 245 $[M + Na]^+$.
- (E)-isobutyl-3-(3,4-dihydroxyphenyl)acrylate (**10**) [Murakami et al., 2000]: yield 93%; 1H NMR ($CDCl_3$, AV-300), δ: 0.98 (d, J = 6.8 Hz, 6H), 2.03 (m, 1H), 3.92 (s, 3H), 3.98 (d, J = 6.6 Hz, 2H), 6.30 (d, J = 15.9 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 7.05 (m, 2H), 7.62 (d, J = 15.9 Hz, 1H); ESI-MS m/z: 237 $[M + H]^+$, 259 $[M + Na]^+$.
- (E)-isopentyl-3-(3,4-dihydroxyphenyl)acrylate (**11**) [Murakami et al., 2000]: yield 91%; 1H NMR ($CDCl_3$, AV-300), δ: 0.95 (d, J = 6.6 Hz, 6H), 1.56 (m, 2H), 1.81 (m, 1H), 3.91 (s, 3H), 4.22 (t, 2H), 6.29 (d, J = 15.9 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 7.07 (m, 2H), 7.61 (d, J = 15.9 Hz, 1H); ESI-MS m/z: 251 $[M + H]^+$, 273 $[M + Na]^+$.
- (E)-cyclopentyl-3-(4-hydroxy-3-methoxyphenyl) acrylate (**12**): yield 91%; 1H NMR ($CDCl_3$, AV-300), δ: 1.62(m, 4H), 1.78(m, 4H), 3.92(s, 3H), 4.13(m, 1H), 6.26(d, J = 15.9 Hz, 1H), 6.91(d, J = 8.2 Hz, 1H), 7.02(d, J = 2.0 Hz, 1H), 7.06(dd, J_1 = 2.0 Hz, J_2 = 8.2 Hz, 1H), 7.57(d, J = 15.9 Hz, 1H); ESI-MS: m/z 263 $[M + H]^+$, 285 $[M + Na]^+$.
- (E)-cyclohexyl-3-(4-hydroxy-3-methoxyphenyl)acrylate (**13**): yield 90%; 1H NMR ($CDCl_3$, AV-300), δ: 1.52(m, 2H), 1.73(m, 4H), 1.88(m, 4H), 3.87(m, 1H), 3.92(s, 3H), 6.27(d, J = 15.7 Hz, 1H), 6.90(d, J = 8.2 Hz, 1H), 7.03(d, J = 2.0 Hz, 1H), 7.06(dd, 1H, J_1 = 2.0 Hz, J_2 = 8.2 Hz, 1H), 7.59(d, J = 15.7 Hz, 1H); ESI-MS: m/z 277 $[M + H]^+$, 299 $[M + Na]^+$.
- (E)-isopropyl 3-(3,4-dihydroxyphenyl)acrylate (**14**) [de Campos Buzzi et al., 2009]: yield 93%; 1H NMR

($CDCl_3$, AV-300), δ: 1.23 (m, 6H, $2CH_3$), 4.99 (m, 1H, COOCH), 6.23 (d, J = 15.9 Hz, 1H, C = CH), 6.76 (d, J = 8.0 Hz, 1H, Ar-H), 6.98-7.04 (m, 2H, Ar-H), 7.45 (d, J = 15.9 Hz, 1H, CH = C), 9.11 (s, 1H, OH), 9.56 (s, 1H, OH); ESI-MS: m/z 223 $[M + H]^+$, 245 $[M + Na]^+$.

- (E)-isobutyl 3-(3,4-dihydroxyphenyl)acrylate (**15**) [de Campos Buzzi et al., 2009]: yield 92%; 1H NMR ($CDCl_3$, AV-300), δ: 0.92 (d, J = 6.8 Hz, 6H, $2CH_3$), 1.93 (m, 1H, CH), 3.90 (d, J = 6.6 Hz, 2H, $COOCH_2$), 6.27 (d, J = 15.9 Hz, 1H, C = CH), 6.76 (d, J = 8.0 Hz, 1H, Ar-H), 7.00–7.05 (m, 2H, Ar-H), 7.48 (d, J = 15.9 Hz, 1H, CH = C), 9.12 (s, 1H, OH), 9.58 (s, 1H, OH); ESI-MS: m/z 237 $[M + H]^+$, 259 $[M + Na]^+$.
- (E)-isopentyl 3-(3,4-dihydroxyphenyl)acrylate (**16**) [de Campos Buzzi et al., 2009]; yield 91%; 1H NMR ($CDCl_3$, AV-300), δ: 0.90 (m, 6H, $2CH_3$), 1.52 (m, 2H, CH_2), 1.68 (m, 1H, CH), 4.14 (t, J = 6.8 Hz, 2H, $COOCH_2$), 6.25 (d, J = 15.9 Hz, 1H, C = CH), 6.75 (d, J = 8.0 Hz, 1H, Ar-H), 6.99-7.04 (m, 2H, Ar-H), 7.46 (d, J = 15.9 Hz, 1H, CH = C), 9.12 (s, 1H, OH), 9.58 (s, 1H, OH); ESI-MS: m/z 251 $[M + H]^+$, 273 $[M + Na]^+$.
- (E)-cyclopentyl 3-(3,4-dihydroxyphenyl)acrylate (**17**): yield 90%; 1H NMR ($CDCl_3$, AV-300), δ: 1.52(m, 4H), 1.64(m, 4H), 4.12(m, 1H), 6.26(d, J = 15.9 Hz, 1H), 6.91(d, J = 8.1 Hz, 1H),7.02(d, J = 2.0 Hz, 1H), 7.06(dd, 1H, J_1 = 2.0 Hz, J_2 = 8.1 Hz, 1H), 7.57(d, J = 15.9 Hz, 1H); ESI-MS m/z: 249 $[M + H]^+$, 271 $[M + Na]^+$.
- (E)-cyclohexyl 3-(3,4-dihydroxyphenyl)acrylate (**18**) [Uwai et al., 2008]: yield 90%; 1H NMR ($CDCl_3$, AV-300), δ: 1.33(m, 4H), 1.56(m, 2H), 1.75(m, 2H), 1.92(m, 2H), 4.14(m, 1H), 6.26(d, J = 15.9 Hz, 1H), 6.88(d, J = 8.3 Hz, 1H), 7.00(dd, 1H, J_1 = 2.0 Hz, J_2 = 8.3 Hz, 1H), 7.08(d, J = 2.0 Hz, 1H), 7.57(d, J = 15.9 Hz, 1H); ESI-MS m/z: 263 $[M + H]^+$, 285 $[M + Na]^+$.

BIOLOGICAL SCREENING

MMP-1, MMP-2, and MMP-9 Inhibition Assay

The substituted cinnamic acid esters were assayed for the inhibitory activities against MMP-1 MMP-2, and MMP-9 in 96-well microtiter plates using succinylated gelatin as the substrate [Baragi et al., 2000]. The compounds and enzyme were dissolved in sodium borate buffer (pH 8.5, 50 mM) and incubated at 37°C for 30 min. The substrate was added and incubated at 37°C for another 60 min. The 100% and blank groups were also carried out in which the 100% group contained no compound and the blank group contained only the enzyme. Then 0.03% picrylsulfonic acid solution was added and incubated at room temperature for additional 20 min. The resulting solutions were measured at optical density 450 (OD_{450}) and the values used to calculate the inhibitory rates using the equation $[OD_{450}(100\%) - OD_{450}\ (\text{compound})] / [OD_{450}(100\%) - OD_{450}(\text{blank})] \times 100\%$. IC_{50} values were obtained from the aforementioned inhibitory rates using OriginPro 7.5 software (OriginLab corporation, Hampton, Massachusetts, USA).

TABLE 2. The Inhibitory Activity of Compounds 4–18 toward Some of the Principal matrix Metalloproteinases (MMPs), the MMP-2 Selectivity,[a] and the MMP-9 Selectivity[a] (in Parentheses)

	IC_{50}[b] (nM)		
Product	MMP-1	MMP-2	MMP-9
4	1503.2 ± 18.4	84.2 ± 3.0 (18)	98.4 ± 2.1 (15)
5	1488.8 ± 12.5	71.9 ± 2.9 (21)	78.7 ± 2.4 (19)
6	1631.7 ± 16.4	89.3 ± 2.4 (18)	86.4 ± 2.8 (19)
7	1510.9 ± 11.7	68.8 ± 2.0 (22)	71.5 ± 2.4 (21)
8	1797.6 ± 12.8	62.7 ± 2.4 (29)	58.6 ± 2.1 (30)
9	5761.6 ± 21.6	61.7 ± 2.9 (93)	88.9 ± 2.7 (65)
10	5812.8 ± 22.4	44.8 ± 2.1 (129)	41.2 ± 2.1 (142)
11	5410.3 ± 24.3	49.2 ± 2.1 (110)	65.2 ± 2.3 (83)
12	5971.4 ± 25.6	38.3 ± 1.9 (157)	48.7 ± 2.4 (122)
13	6111.7 ± 31.7	30.8 ± 1.8 (197)	28.2 ± 1.6 (218)
14	5892.6 ± 26.9	20.3 ± 1.3 (295)	53.5 ± 2.4 (111)
15	6132.3 ± 32.7	16.2 ± 1.0 (383)	25.2 ± 1.7 (245)
16	6270.8 ± 41.6	17.1 ± 0.5 (369)	66.2 ± 2.6 (95)
17	6766.9 ± 37.6	12.4 ± 0.5 (564)	13.4 ± 1.4 (521)
18	6972.9 ± 48.7	10.8 ± 0.4 (633)	6.8 ± 0.7 (996)
Caffeic acid	238.9 ± 2.2	24.3 ± 0.4 (10)	21.2 ± 1.3 (11)

[a]Selectivity for MMP-1 over each of the other MMPs, is expressed as the ratio of the IC_{50} value for MMPs over the value for MMP-1.
[b]IC_{50} values are the mean of four experiments, standard deviation is given.

RESULTS AND DISCUSSION

MMP-1, MMP-2, and MMP-9 Inhibitory Activity

Table 2 summarizes the results obtained in the assay for the inhibitory activities on MMP-1, MMP-2, and MMP-9; caffeic acid was used as the reference control, selectivity indices for MMP-2 and MMP-9 over MMP-1 are also reported, expressed as ratios of their inhibitory indices (Table 2). The results showed that these substituted cinnamic acid esters exhibited highly selective inhibition against MMP-2 and MMP-9 as compared with MMP-1, thus confirming our strategy for designing MMP-2, MMP-9 inhibitors (Table 2).

For MMP-2, caffeic acid esters **14–18** showed good potency compared with caffeic acid, with IC_{50} values less than 20 nM, while the IC_{50} value of caffeic

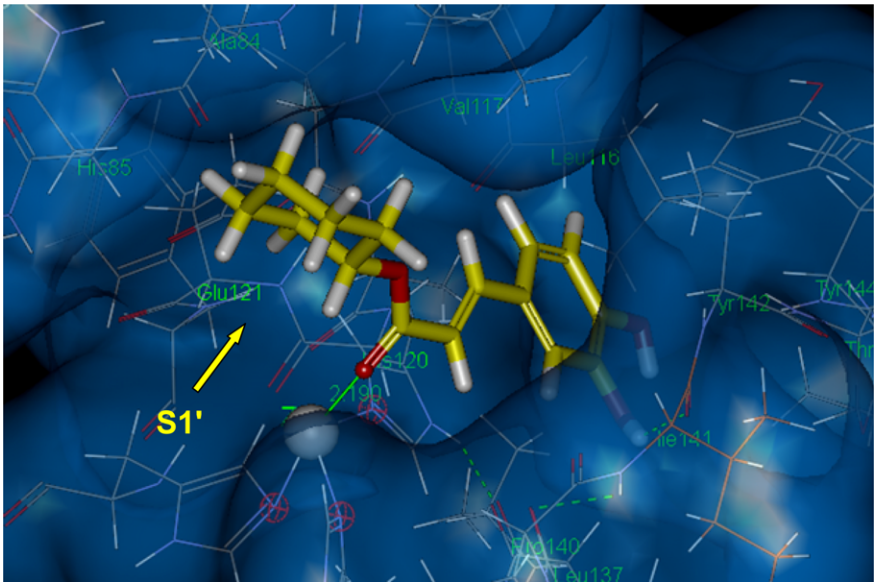

Fig. 4. Docking result of compound **18** with matrix metalloproteinase 2. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

acid was 24.3 nM. Among the caffeic acid esters, the most active compound on MMP-2 was **18** (IC_{50} = 10.8 nM), approximately twice as potent as caffeic acid (IC_{50} = 24.3 nM). At MMP-2, the ferulic acid esters **9**–**11** were less active by greater than two orders of magnitude than caffeic acid, **12** and **13** showed inhibitory activity practically equal to that of caffeic acid. Among the cinnamic acid esters, no compound was equal or more active than caffeic acid. At MMP-9, the caffeic acid cyclohexane ester **18** was the most active of the synthesized esters (IC_{50} = 6.8 nM), some threefold more active than caffeic acid (IC_{50} = 21.2 nM). The caffeic acid cyclopentane ester **17** had good inhibitory activity (IC_{50} = 13.4 nM), while the isobutane analog **15** had inhibitory activity equal to that of caffeic acid (IC_{50} = 25.2 nM). Among the ferulic acid esters, only the cyclohexane ester **13** showed inhibitory activity equally to that of caffeic acid (IC_{50} = 28.2 nM). Among the cinnamic acid esters **4**–**8**, no compound was equal or more active than caffeic acid and were twofold to fourfold two less active than caffeic acid. All compounds showed minimal inhibition of MMP-1 (IC_{50} = 1488.8 nM [**5**] to 6972.9 nM [**18**]). Caffeic acid had an IC_{50} value of 238.9 nM. An analysis of the selectivity indices reported in parentheses in Table 2 for the synthesized esters and caffeic acid indicated that all the synthesized compounds had higher selectivity profiles than caffeic acid, with **18** having the highest selectivity profile (MMP-1/MMP-2 = 633, MMP-1/MMP-9 = 996), whereas **17** (similar in its inhibitory potency toward MMP-2 to **18**) had lower MMP-1/MMP-2 and MMP-1/MMP-9 selectivity ratios that were 564 and 521, respectively.

Docking Studies

In the MMP inhibition tests, **18** showed the strongest inhibitory activity on MMP-2 and MMP-9 and the highest selectivity against MMP-1 so **18** was selected for the subsequent molecular docking experiment with MMP-2 (Fig. 4) and MMP-9 (Fig. 5). The MMP-2 docking showed that the cyclohexane group in **18** occupied the deep S1′ pocket (composed of Alanine (Ala) 84, Histidine (His)85, Leucine (Leu)116, Valine (Val) 117, His120, Glutamate (Glu)121) of MMP-2, and the carbonyl group chelated the active site zinc ion with a distance of 2.19 Å. The MMP-9 docking showed that the cyclohexane group in **18** occupied the deep S1′ pocket (composed of His411, Leu418, Tyrosine (Tyr)420, Proline (Pro)421, Methionine (Met)422) of MMP-9, and the carbonyl group chelated the active site zinc ion with a distance of 2.218 Å. These results indicated that compound **18** interacted well with MMP-2 and the MMP-9 active site, especially the deep S1′ pocket and zinc ion, consistent with MMP-2 and MMP-9 assay results.

Discussion and SAR

In this study, based on docking studies of caffeic acid with MMP-2 and MMP-9, 15 compounds were synthesized and investigated for inhibitory activity on MMP-1, MMP-2, and MMP-9. Selected for docking experiments for the molecular modeling investigation was **18**.

Compounds **4**–**8** were cinnamic acid esters with no OH group or OCH_3 group on the benzene ring,

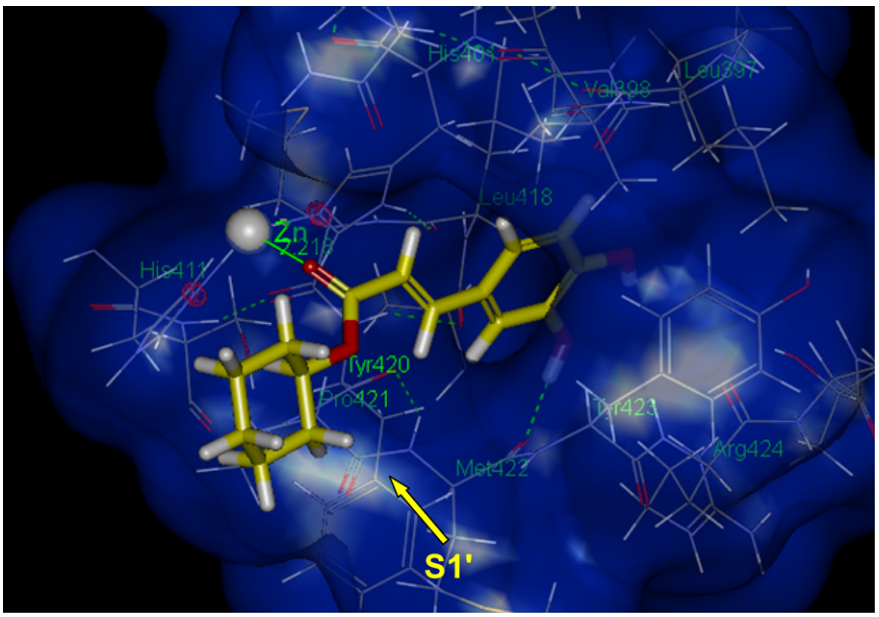

Fig. 5. Docking result of compound **18** with matrix metalloproteinase 9. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

compounds **9–13** were ferulic acid esters with one OH group and one OCH_3 group on the benzene ring, and compounds **14–18** were caffeic acid esters with two OH groups on the benzene ring, when OH group in the benzene ring was replaced by the OCH_3 group or by the H group, MMP inhibition activities and selectivity reduced, such as **13**, with one OCH_3 group and one OH group in the benzene ring and **8** with H groups in the benzene ring, showed weaker MMP inhibition activities and selectivity as compared with **18**, which had two OH groups in the benzene ring. These results showed that the two OH groups were important for MMP inhibition and selectivity.

In the three series, substituted cinnamic acid esters, when the carboxylic acid group was converted to cyclic ester, the inhibitory activity for MMP-2 and MMP-9 increased, e.g., **8**, a cyclohexyl ester, had stronger inhibitory activity on MMP-2 and MMP-9 than **5**, a isobutanol ester. This result indicated that the extended spatial structures, e.g., cyclohexyl and cyclopentyl, were important for the improvement of the activity and selectivity in inhibiting MMP-2 and MMP-9.

CONCLUSIONS

In this study, we examined the effects of 15 substituted cinnamic acid esters that were synthesized using microwave irradiation on MMP-1, MMP-2, and MMP-9 inhibition activity. Preliminary SAR analysis showed that hydroxyl groups in the benzene ring and the presence of extended spatial structures in the carboxylic acid played key roles in the MMP-2 and MMP-9 inhibitory activity and selectivity. These findings would facilitate the design of compounds with higher potency to serve as selective MMP-2 and MMP-9 inhibitors that could provide information for the exploitation and utilization of caffeic acid as MMPI for metastatic tumor treatment.

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