Caffeic Acid, A Versatile Pharmacophore: An Overview

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Abstract: The caffeic acid scaffold, which is abundant in nature, is extremely versatile and is found in a number of biologically active molecules. The purpose of this review is to provide an overview of the pharmacological activity of synthetic caffeic acid analogs including recent reports of anti-inflammatory, anti-cancer, and antiviral activities of these compounds.

Keywords: Caffeic acid, anticancer, antioxidant, anti-inflammatory, integrase inhibitors, structure-activity relationships.

1. INTRODUCTION

For decades, natural products have been a wellspring of drugs and drug leads. Several small molecules introduced as drugs worldwide can be traced to or were inspired by natural products [1]. In the search for new compounds of therapeutic interest, phenolic acids, which are widely distributed in plants [2], are very important for their interaction with several biological targets. A number of these compounds are linked to several cell wall components such as arabinoxylans and proteins [3,4]. Phenolic acids were also reported to prevent the oxidation of various food ingredients, particularly fatty acids and oils [5,6]. Additionally, fortification of diets with food materials rich in phenolic acids was shown to provide antimutagenic, antiglycemic, and antioxidative benefits, which can be exploited in developing health foods [7].

Caffeic acid, the major representative of hydroxycinnamic acids and phenolic acid in general, is widely distributed in plants and is usually found as various simple derivatives such as glycosides, amides, esters and sugar esters. Among possible modifications of the structure of caffeic acid, transformation into esters or amides gives new analogs with various and interesting biological activities. Caffeic acid phenylethyl ester (CAPE), one of the most active compounds in propolis, is the perfect example of this biological activity diversity. CAPE has shown antioxidant, anti-inflammatory, antitumoral, and antifungal activities [8-10].

As shown in the following sections, derivatives of caffeic acid have many interesting properties from a therapeutic point of view. This review mainly focuses on the diverse pharmacological properties associated with synthetic caffeic acid derivatives. In the subsequent paragraphs, recent findings concerning these molecules are presented and the description is categorized by biological activity.

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2. PHARMACOLOGICAL PROFILE

2.1. Anti-Cancer

Several phenolic compounds have been investigated for their potential use as cancer chemopreventive agents [11,12]. Epidemiological studies indicate that a diet rich in fruits and vegetables reduces cancer risk in humans, suggesting that certain dietary constituents may thus be effective in preventing cancer [13,14].

Caffeic acid **1** and several structurally related phenolic acid derivatives **2**, **3**, and **4** were synthesized (Fig. **1**) and screened for their potential antiproliferative and cytotoxic properties [15]. The phenols **1**, **2**, **3**, and **4** were tested in four different human cancer cell lines: cervix (HeLa), mammary gland adenocarcinomas (MDA-MB-231), lymphoblastic leukemia (MOLT-3), and non-neoplasic fibroblasts from human embryonic lung tissue (L-132 cell line). Caffeic acid **1** was found to exhibit an antiproliferative effect against all the tested cancer cell lines, particularly toward HeLa. Hydrocaffeic acid **2** exhibits an irreversible antiproliferative activity toward all the cell lines, including the non-neoplasic L-132. Surprisingly, in the absence of **2**, cell growth inhibition continued to increase, which is a significant advantage for its potential use as an anti-cancer drug. 3,4-dihydroxyphenylethanoic acid **3** showed specific antiproliferative and cytotoxic effects on MOLT-3 cells. Finally, 3,4-dihydroxybenzoic acid **4** displayed a reversible antiproliferative effect toward all the tested cancer cell lines.

The tested phenolic acids displayed a protective effect toward either cancerous or healthy cell lines. Caffeic acid **1** was found to protect specifically the non-neoplasic L-132 cells. Additionally, these compounds do not display significant toxicity toward healthy cells. The length of the carbon chain between the aromatic ring and the terminal carboxylic group and the presence of a double bond in the carbon chain affect the anticancer activity in a different way. The number of OH ring substituents seems to be a major determinant; triphenols analogues were found by Gomes and co-workers to be more effective than diphenols for all types of cancer lines investigated [15].

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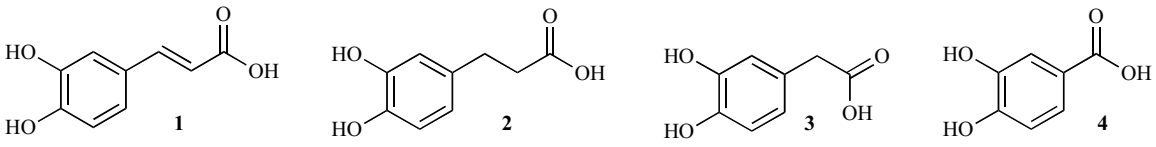

Fig. (1).

Fiuza and co-workers have also investigated the toxicity of some caffeic acid esters **5a-5c** (Fig. **2**) on the human cervix adenocarcinoma cell line HeLa (epithelial-like adherent line) and non-neoplasic cells-fibroblasts from human embryonic lung tissue (L-132) [16]. The length of the alkyl chain was found to induce a clear change in the respective antiproliferative properties: the propyl ester **5b** displayed a considerably pronounced antitumoural effect towards both HeLa and L-132 cell lines than the octyl **5c** and methyl **5a** analogues [16].

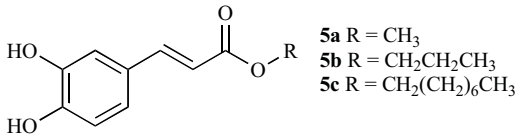

Fig. (2).

Inspired by the isolated methyl caffeate **5a** from the *Nototerygium incisum* plant, Nam and coworkers have described the synthesis and cytotoxicity of a series of **5a** analogues (Fig. **3**) [17]. The methyl caffeate **5a** showed significant cytotoxicity against various cancer cell lines [18]. The synthesized caffeates (Fig. **3**) were obtained by simple esterification or by a Wittig reaction and were assayed against three cancer cell lines including murine melanoma (B16), human colon cancer (HCT116) and human epidermoid carcinoma (A431) (Table **1**). When compared to adriamycin, caffeic acid **1**, and **5a**, the 2,5-dihydroxy-substituted pattern seemed to be most favourable for cytotoxic activity, followed by the 3,4- and 2,3-dihydroxy-substituted ones. Compounds **6a** and **6f** were the most potent among the cinnamates; their IC_{50} values were lower than 0.3 μg/mL in all the three cell lines tested. Compound **6c** bearing a 3,5-dihydroxy substituent was found to be inactive. Comparison of the two 2,5-dihydroxy-substituted products showed that the *E* configuration is essential for the cytotoxicity; compound **6b**, a *Z*-isomer of cytotoxic **6a**, was inactive at the highest concentration assayed (30 μg/mL). Saturation of the double bond and methylation of the hydroxy groups abolished cytotoxicity (Table **1**).

Caffeic acid phenethyl ester (CAPE) **7** (Fig. **4**) is an active component of propolis obtained from honeybee hives

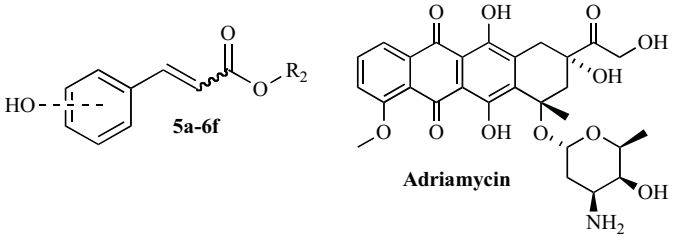

Fig. (3).

Table 1. Cytotoxic Activity of Methyl Caffeate Analogues 5a-6f and Adriamycin

Compounds	*E/Z*	Phenyl substitution	R_2	Cytotoxicity (IC_{50} μg/mL)		
				B16	HCT116	A431
5a	*E*	3,4-(OH)	Me	2.96	3.01	1.79
6a	*E*	2,5-(OH)	Me	0.12	0.17	0.15
6b	*Z*	2,5-(OH)	Me	30.0	>30	>30
6c	*E*	3,5-(OH)	Me	>30	>30	>30
6d	*E*	2,3-(OH)	Me	4.02	4.15	4.06
6e	*E*	3,4-(OH)	Et	1.75	1.99	1.81
6f	*E*	3,4-(OH)	Et	0.21	0.19	0.17
1	*E*	3,4-(OH)	H	>30	>30	>30
Adriamycin				0.11	0.17	0.21

and it exhibits interesting anti-cancer activities [9]. CAPE was reported to selectively inhibit virus-transformed and oncogene-transformed rodent cells and human tumour cells, including colon adenocarcinoma (HT-29 and HCT116) [19], glioblastoma multiforme (GBM-18) [**20**], melanoma (HU-1, SK-MEL-28 and SK-MEL-MO) [21,22], human oral cancer cells (GNM and TSCCa) [23], human breast carcinoma (MCF-7), the invasive phenotype of SK-Hep1 human hepatocellular carcinoma cells [24,25], and Fischer rat embryo fibroblasts (CREF) [21, 26, 27]. This active compound was also reported to stop the growth of human leukaemia HL-60 cells [28] and inhibit the synthesis of DNA, RNA and protein in HeLa cells [29]. Natarajan and co-workers reported that **7** can block NF-κB which is activated by tumour necrosis factor (TNF) [30].

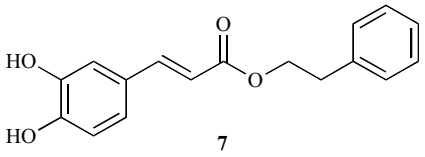

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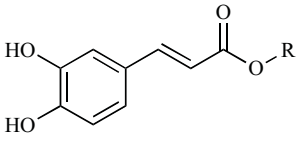
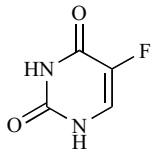
Fig. (4).

The phenethyl **7**, benzyl **7a** and cinnamyl caffeates **7b**, obtained from the MeOH extract of the Netherlands propolis by Banskota and co-workers, showed potent antiproliferative activity toward HT-1080 and B16-BL6 melanoma cell lines and most selectively toward murine colon 26-L5 carcinoma [31]. Starting from these interesting results, several CAPE analogs have been prepared and assayed by Nagaoka and co-workers (Fig. **5**) [32]. Once again, CAPE analogues showed selective antiproliferative activity toward colon 26-L5 carcinoma cell line. Antiproliferative activity of esters **7c**, **7d**, **7e** and **7f** showed stronger activity than the positive controls, 5-fluorouracil (5-FU), adriamycin, and caffeic acid **1** (Table **2**). The antiproliferative activities of *cis* and *trans* isomers **7d** and **7e** were similar against all tested cell lines. At the exception of **7g** against HT-1080, antiproliferative activity was increased from benzyl caffeate **7a** to 8-phenyloctyl caffeate **7g** when compared with CAPE **7** and caffeic acid **1** (Table **2**). Further elongation of the alkyl chain decreased the activity. In esters with a straight alkyl chain, the C8–C12 chain appeared to be the most effective structure for antiproliferative activity [31, 33] (Table **2**).

Starting from vanillin analogues, several caffeates (Fig. **6**) have been prepared by Xia and co-workers. Using the MTT assay, they tested the effect of these analogues on the growth of human hepatocellular carcinoma BEL-7404, human breast MCF-7 adenocarcinoma, human lung A549 adenocarcinoma and human gastric cancer BCG823 cell lines [34]. The best caffeates are summarized in Table **3**. Compounds **7** and **7a** possessed stronger BEL-7404 and MCF-7



7 R = $CH_2CH_2C_6H_5$
7a R = $CH_2C_6H_5$
7b R = $CH_2CH{=}CHC_6H_5$
7c R = $(CH_2)_4C_6H_5$
7d R = $(CH_2)_6CH{=}CHC_6H_5$ (*Z*)
7e R = $(CH_2)_6CH{=}CHC_6H_5$ (*E*)
7f R = $(CH_2)_2$-c-Hex
7g R = $(CH_2)_8C_6H_5$
1 R = H

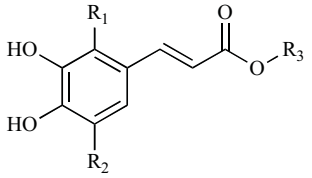


5-FU

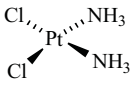
Fig. (5).

Table 2. Antiproliferative Activity of Ester Analogues 7, 7a-7g, 5-FU, and Adriamycin

Compounds	Human (EC_{50} μM)		Murine (EC_{50} μM)	
	HT-1080	A-549	Colon 26-L5	B16-BL6
7	13.7	44.0	1.76	3.16
7a	13.3	18.9	0.28	2.03
7b	9.45	18.9	0.11	1.92
7c	13.3	31.6	0.02	1.99
7d	10.5	22.3	0.02	1.77
7e	10.2	33.5	0.02	1.49
7f	11.8	28.1	0.03	2.00
7g	20.2	22.4	0.09	1.88
1	257	288	43.6	314
5-FU	5.00	3.61	0.06	8.76
Adriamycin	0.06	0.20	0.04	0.22



7 $R_1 = R_2 = H$; $R_3 = CH_2CH_2C_6H_5$
7a $R_1 = R_2 = H$; $R_3 = CH_2C_6H_5$
8a $R_1 = R_2 = H$; $R_3 =$ Phenyl
8b $R_1 = R_2 = H$; $R_3 =$ Cyclohexyl
8c $R_1 = NO_2$; $R_2 = H$; $R_3 = CH_2CH_2C_6H_5$
8d $R_1 = H$; $R_2 = NO_2$; $R_3 = CH_2CH_2C_6H_5$
8e $R_1 = R_2 = H$; $R_3 =$ Bornyl
8f $R_1 = H$; $R_2 = NO_2$; $R_3 =$ Bornyl



Cisplatin

Fig. (6).

Table 3. Antiproliferative Activity of Ester Analogues 7, 7a, 8a-8f, and Cisplatin

Compounds	(IC_{50} μM)			
	BEL-7404	**MCF-7**	**A549**	**BCG832**
7	5.5	26.7	83.6	44.6
7a	17.4	14.1	27.3	48.1
8a	44.3	5.9	75.9	56.5
8b	15.5	30.3	89.3	97.6
8c	59.7	12.3	11.4	22.9
8d	10.2	28.8	8.7	27.8
8e	28.2	>300	19.5	100.6
8f	28.7	40.7	52.9	116.6
Cisplatin	23.8	29.5	32.3	6.4

inhibitory growth activities respectively than the positive control cisplatin. These two compounds differ only by the number of carbon atoms between the oxygen atom and the phenyl group. In comparison with CAPE **7** and bornyl caffeates **8e** and **8f**, the 3,4-dihydroxy-substituted pattern seemed to be most favourable for the A549 cells cytotoxic activity. Addition of NO_2 group in position 2 or 5, such as **7** to **8c** and **8d**, **8e** to **8f** did not increase the antitumour activity (Table **3**) [34].

In a recent study from our laboratory, the synthesis of twelve caffeoyl/cinnamoyl clusters and their anti-cancer effect were investigated [35]. As shown in Fig. (**7**), azide or alkyne functionalized cinnamoyl or caffeoyl moieties were attached to selected core molecules *via* Huisgen 1, 3 dipolar cycloaddition. This allowed for variation of the introduced cinnamoyl or caffeoyl moieties in order to compare their effects on cell proliferation in cancerous (MCF7) and non cancerous (MCF10A) human mammary epithelial cell lines. Trimer compounds **9a**, **9b** and tetramer **10a** decreased proliferation rates of MCF-7 cells by 36, 23 and 47%, respectively, but had no effect on MCF10A proliferation. This result is highly encouraging since selective toxicity or growth inhibition toward cancerous compared to non-cancerous cells is a crucial characteristic of compounds in the development of anti-cancer drugs.

Tumour progression is often correlated with an overexpression of matrix metalloproteinases (MMPs). These enzymes are produced and secreted as zymogens in the extracellular matrix (ECM) by the tumour cells themselves or by surrounding stromal cells [36]. Several proteases, such as MMP and furin-like serine proteases are responsible for the specific activation of the latent proMMP forms. Among these, MMP-2, MMP-9, and the membrane-associated type (MT)-1 MMP are thought to play a significant role in several degenerative processes [37], and are directly involved in metastatic tumour dispersion and angiogenesis [38,39]. More

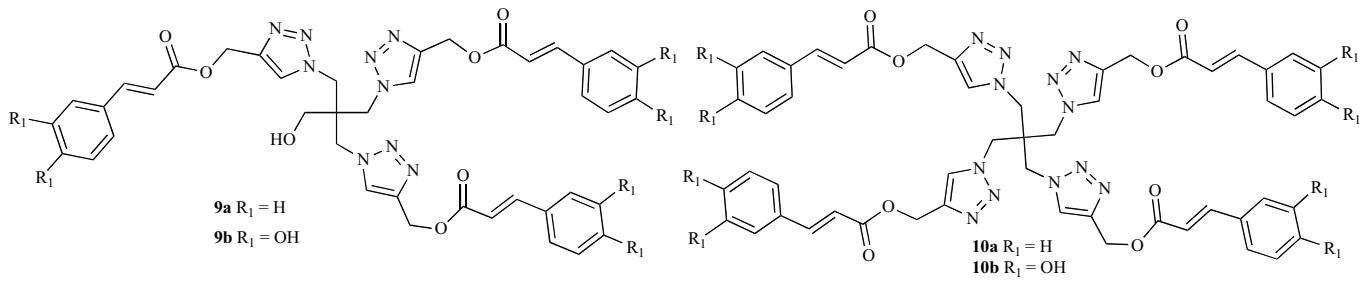
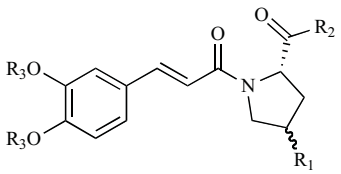


Fig. (7).



11a R_1 = NHCOCH=CHC_6H_3(OMe)-4' (*E*); R_2 = NHOH; R_3 = Me; IC_{50} = 6.7 nmol
11b R_1 = $NHCO(CH_2)_4CH_3$; R_2 = NHOH; R_3 = Me; IC_{50} = 7.8 nmol
11c R_1 = $NHCOC_5H_4$N-3'; R_2 = NHOH; R_3 = Me; IC_{50} = 9 nmol
11d R_1 = $NHCO(CH_2)_2C_6H_5$; R_2 = NHOH; R_3 = Me; IC_{50} = 9.4 nmol
11e R_1 = $NHSO_2C_6H_4CH_3$-*p*; R_2 = NHOH; R_3 = Me; IC_{50} = 9.7 nmol
11f R_1 = $NHCOCH_2C_6H_5$; R_2 = NHOH; R_3 = Me; IC_{50} = 11.5 nmol

Fig. (8).

recently, MMP-2 has also been shown to play important roles in tumour cells resistance to apoptosis, in the activation of EGF receptors and in cellular proliferation [40]. Taken together, these data indicate that MMP-2, MMP-9 and MT1-MMP may represent important targets against which new potential anti-cancer drugs could be developed [41]. It was demonstrated that CAPE inhibit the invasive phenotype through down regulation of MMP-2 and MMP-9 by blocking NF-κB activation [24,30]. Li and co-workers have described the preparation and evaluation of caffeoyl pyrrolidine derivatives as MMP inhibitors (Fig. **8**) [42]. The caffeoyl derivatives were obtained by formation of an amide bond between 4-substituted proline and acylated or methylated caffeic acid chloride. Inhibition activities of synthesized compounds on gelatinase (MMP-2 and -9) were tested by using succinylated gelatin as a substrate. The pyrrolidine derivatives **11a-11f** (Fig. **8**) displayed high anti-MMP-2 and -9 activities *in vitro*. Compound **11f**, with a phenylethanoylamide at C-4 of the pyrrolidine ring showed high *in vivo* anti-metastasis activity with no toxic effects on mice bearing H22 tumor cells [42].

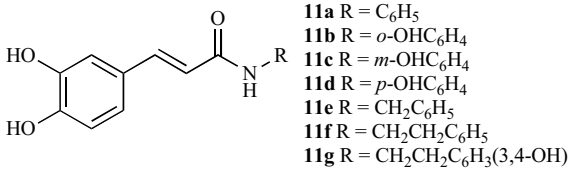
2.2. Antioxidant Activity/Scavenging Activity

CAPE exerts an antioxidant effect at the transcriptional level by inhibiting NF-κB and decreasing the expression of pro-inflammatory genes. CAPE antioxidant activity also stems from multiple other mechanisms such as free radical scavenging, metal ion chelation and inhibition of specific enzymes that induce free radical or lipid peroxidation [43,44]. Based on these facts, several groups explored the synthesis of caffeic acid or CAPE analogues in order to discover an efficient antioxidant molecule.

Rajan and coworkers synthesized a series of caffeic acid amides (Fig. **9**) and tested their antioxidant activity using the lipid peroxidation system. Aliphatic amines derivatives showed IC_{50} in the low micromolar range (2.2-6.1μM) similar to trolox, a standard antioxidant or caffeic acid, the parent compound (Table **4**). Even more potent were the aromatic amine derivatives with submicromolar IC_{50} **11a-g** (Table **4**). Among these aromatic amines, compounds **11b-c** (IC_{50} = 0.29-0.63μM) with an aminophenol moiety were 10 times more potent than either trolox or caffeic acid (Table **4**). The number of catechol moieties, lipophilicity and the ability to stabilize radicals were the main factors explaining the difference in antioxidant activity of those compounds. Future studies with a more thorough array of antioxidant tests would bring valuable information on the antioxidant profile, safety and structure-activity relationship of these compounds [45].

Son and Lewis synthesized four amide analogues of CAPE **7**, compounds **12-15** (Fig. **10**) to evaluate and compare their free radical scavenging and antioxidative activity against two parent compounds, CAPE **7** and caffeic acid **1**, and the reference synthetic compound Trolox.

The free radical scavenging activity of the seven compounds ranked as followed: **15** > **14** > **13** > **12** > Trolox > **7** > **1** (Table **5**). Structural parameters dictating free radical scavenging activity were the number of hydroxyl or catechol groups present and the number of other H-donating groups (amide, sulfhydryl). Compound **15** which combines both structural characteristics, was the most active compound (IC_{50}= 23.26 μM), being twice as potent as CAPE **7**, caffeic acid **1** or trolox. Antioxidative activity was assessed using



11a R = C_6H_5
11b R = *o*-OHC_6H_4
11c R = *m*-OHC_6H_4
11d R = *p*-OHC_6H_4
11e R = $CH_2C_6H_5$
11f R = $CH_2CH_2C_6H_5$
11g R = $CH_2CH_2C_6H_3$(3,4-OH)

Trolox

Fig. (9).

Table 4. Lipid Peroxidation Inhibition Activity of Caffeic Acid Amides 11a-11c, 1, and Trolox

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
11a	0.38	**11d**	0.63
11b	0.29	**11e**	1.02
11c	0.37	**11f**	0.85
1	3.3	**11g**	0.59
Trolox	2.8		

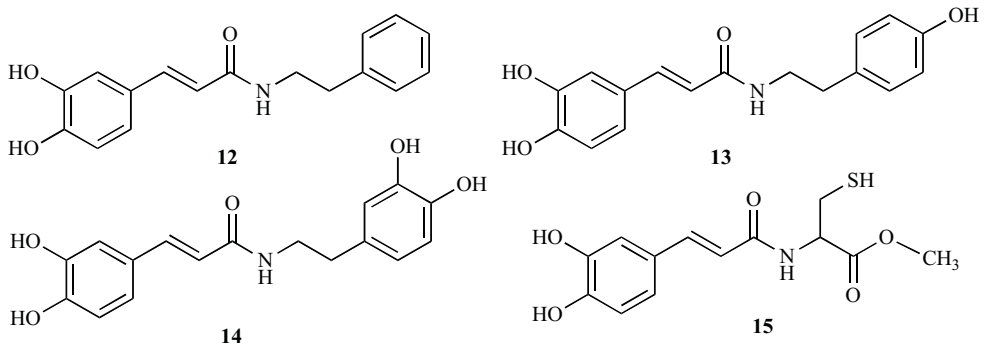


Fig. (10).

Table 5. Radical Scavenging Activity of 12-15, 1, and Trolox (% Inhibition with 50μM of Antioxidants)

Compound	IC_{50} (μM)	Inhibition (%)
7	51.80	48
12	43.07	55
13	41.62	59
14	24.22	91
15	23.26	94
1	54.3	44
Trolox	46.39	53

the AAPH (2, 2,-azobis(2-amidinopropane) dihydrochloride)-induced lipid peroxidation test in order to mimic cell membrane peroxidation *in vivo*. Antioxidative activity ranking for the seven tested compounds was **7** > **12** > **14** > **13** ≥ **15** > **1** > Trolox and depended on the number of hydroxyl and catechol moieties and the hydrophobicity of the antioxidant [46].

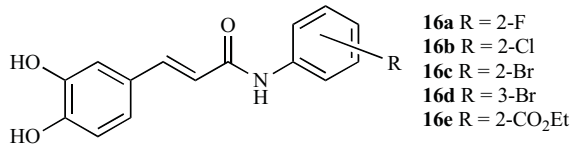


Fig. (11).

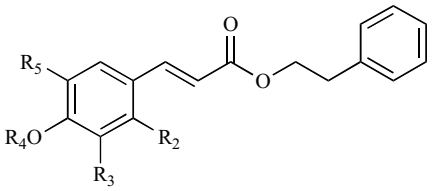
Amide analogues of caffeic acid **1** were also synthesized by Hung and co-workers (Fig. **11**) to evaluate their potential as anti-platelet aggregation and antioxidative agents. Their rationale was based on previous studies showing that antioxidant agents inhibit thromboxane A_2 an inducer of platelet aggregation [47]. Reaction of caffeic acid **1** with various anilines generated compounds **16a-e** (Fig. **11**). Their bioactivities were evaluated by measuring the inhibition against platelet aggregation and malondialdehyde (MDA) formation both induced by arachidonic acid (AA). Radical scavenging activity was tested with the DPPH (2, 2–diphenyl-l-picrylhydrazyl) and ABTS (2, 2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) assays. All the synthesized amides analogues of caffeic acid had better inhibitory activity than caffeic acid **1** against AA-induced platelet aggregation (IC_{50} = 5.8-64.4μM compared to >100μM for caffeic acid) and MDA formation (IC_{50} = 2.1-47.7μM compared to >50μM for caffeic acid and 15.5μM for aspirin) (Table **6**). The more potent inhibitors of AA-induced platelet aggregation were bearers of halogen **16a-d** or ester **16e** groups which were relatively more hydrophobic than the corresponding hydroxyl or methoxy groups. As mentioned in previous studies, relative lipophilicity is an important parameter in predicting *in vivo* activity of antioxidant molecules. Caffeic acid **1** and its amide derivatives **16a-e** showed improved radical scavenging activity (EC_{50} = 11.1-38.8 μM) when compared to vitamin E, a natural antioxidant (EC_{50} = 110.6 μM) or Trolox (EC_{50} = 41.7 μM), a standard synthetic antioxidant. However comparison of radical scavenging activity of the parent compound, caffeic acid **1** and its amide derivatives showed no significant differences (Table **6**) [48].

CAPE **7** is known for its cytoprotective properties in cases such as ischemia/reperfusion injury where reactive oxygen species (ROS) are produced. Based on these premises, Wang and co-workers studied the cytoprotective effect of novel fluorinated derivatives of CAPE (Fig. **12**) on human vascular endothelial cells (HUVEC) under menadione-induced oxidative stress. A series of fluorinated analogues of CAPE **7** was synthesized (compounds **17a-f**) and structure activity relationship (SAR) studies were also performed to assess the influence of the F atom position on the catechol ring of the CAPE **7** analogues.

Preliminary cytotoxicity tests showed that most of the compounds **17a-b**, **17e-f**, and CAPE **7** were cytotoxic (less

Table 6. Inhibition of AA-Induced Aggregation, Anti-MDA Formation, and Radical Scavenging of 16a-16e and 1

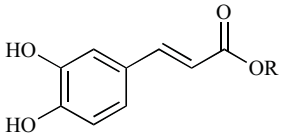
Compound	Anti-AA-induced (μM)	Anti-MDA formation (μM)	Radical scavenging (μM)
16a	21.4	8.7	16.3
16b	18.6	3.1	14.4
16c	5.8	9.3	12.7
16d	6.7	8.2	11.1
16e	17.9	106	13.8
1	>100	>50	15.5

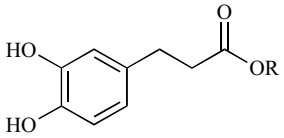


17a $R_2 = R_4 = H$; $R_3 = OMe$; $R_5 = F$
17b $R_2 = R_4 = H$; $R_3 = OH$; $R_5 = F$
17c $R_2 = R_3 = R_4 = H$; $R_5 = F$
17d $R_2 = F$; $R_3 = H$; $R_4 = Me$; $R_5 = OMe$
17e $R_2 = F$; $R_3 = H$; $R_4 = H$; $R_5 = OH$
17f $R_2 = F$; $R_3 = H$; $R_4 = Me$; $R_5 = OH$

Fig. (12).

1 R =H
5a R = CH_3
5b R = CH_2CH_3
6g R = $CH_2CH_2CH_3$

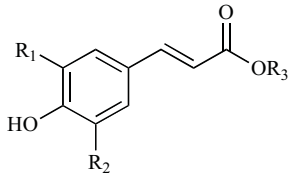




2 R =H
18a R = CH_3
18b R = CH_2CH_3
18c R = $CH_2CH_2CH_3$

Fig. (13).

than 90% viability) at concentrations higher than 15μg/mL while **17c** and **17d** showed no cytotoxicity at all the tested concentrations. At sub-cytotoxic concentrations and in conditions of menadione-induced oxidative stress, analogues **17a**, **17c**, **17d,** and **17f** displayed a continued level of cytoprotection of the HUVEC cells when compared to CAPE **7**. However there was no general pattern in activity since some compounds **17c** and **17d** offered cytoprotection only at concentrations starting around 10μM, which is already a cytotoxic concentration for compound **17e**. Nevertheless, CAPE **7** and its fluorinated analogues displayed cytoprotection that other closely structurally related antioxidants do not show. Overall, fluorinated compounds showed no significant improvement in cytoprotection compared to CAPE **7**. Evalua-



19 $R_1 = OCH_3$; $R_2 = H$; $R_3 = H$
20 $R_1 = OH$; $R_2 = Br$; $R_3 = H$
19a $R_1 = OCH_3$; $R_2 = Br$; $R_3 = H$
6f $R_1 = OH$; $R_2 = H$; $R_3 = CH_2CH_3$
19b $R_1 = OCH_3$; $R_2 = Br$; $R_3 = CH_2CH_3$
20a $R_1 = OH$; $R_2 = Br$; $R_3 = CH_2CH_3$
19c $R_1 = OCH_3$; $R_2 = Br$; $R_3 = CH_2CH_3$

Fig. (14).

Table 7. Antioxidant Activity and Redox Potential of 1, 6f, 19, 19a-19c, 20, 20a, and Trolox

Compound	DPHH*	Ep(V)	Compound	DPHH*	Ep(V)
1	0.978	+0.183	**6f**	0.877	+0.175
19	0.781	0.335	**19b**	0.593	+0.368
20	1.007	+0.182	**20a**	0.938	+0.174
19a	0.808	+0.335	**19c**	0.558	+0.365
Trolox	1.000	-			

tion of the effect of the fluorine position on the catechol ring showed that fluorination at the position 2 was important for cytoprotective properties. When moved to the 3-position **17b** these effects were lost [49]. In a follow-up study, compound **17e** stability was evaluated in rat plasma to assess whether the presence of the fluorine atom on the catechol moiety could increase its stability *in vivo*. CAPE **7** has an ester link which is potentially sensitive to esterase present in biological fluids. Results showed that compound **17e** was more stable than CAPE **7**, in absence of NaF, an esterase inhibitor, at various temperatures and concentrations [50].

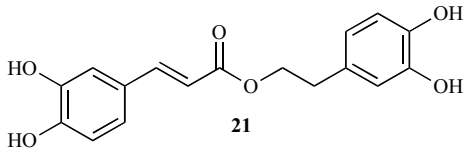
To further assess the structural parameters related to antioxidant activity, Silva and co-workers synthesized three alkyl (methyl **5a**, ethyl **5b**, and propyl **6g**) derivatives of caffeic acid and three alkyl (methyl **18a**, ethyl **18b**, and propyl **18c**) derivatives of dihydrocaffeic acid **2** (Fig. **13**). Since the dihydrocaffeic acid and its analogues lack the ethylene moiety present in caffeic acid **1**, the role and importance of that structural parameter could be assessed.

The radical scavenging activity of both classes of molecules was evaluated with the DPPH assay and expressed as $1/IC_{50}$. The latter is the concentration needed to reduce 50% of the initial amount of DPPH and is expressed as the molar ratio of each compound to radical. With the exception of caffeic acid ($1/IC_{50}$ = 2.50), most of the tested compounds including dihydroxycaffeic acid **2**, showed better radical scavenging activities ($1/IC_{50}$ = 3.77-7.58) than (±)-α-tocopherol ($1/IC_{50}$ = 3.94), a reference compound. The ethylene moiety didn't influence radical scavenging activity since dihydrocaffeic acid **2** was more potent than caffeic acid **1**. For these derivatives, increasing the alkyl chain in a modest fashion; from methyl to propyl has no significant effect on radical scavenging activity [51].

In a recent study, Gaspar and co-workers used alkyl ester analogues and halogenated alkyl ester analogues of caffeic acid **1** and ferulic acid **19** to gain further insights on the parameters regulating antioxidant activity (Fig. **14**). To this effect, the DPHH and ABTS assays were used to evaluate the total antioxidant capacity radical of these compounds. Redox potential was also measured.

Results showed a relationship between antioxidant activity and redox potential (Table **7**). The latter reflects the capacity of antioxidant molecules to accept electrons coming, for example from radicals. A lower redox potential equates to higher antioxidant activity. Caffeic acid **1** and its derivatives with lower redox potentials (Ep = +0.174 to +0.183 V) were more efficient at scavenging radicals than their ferulic acid **19** counterparts (Ep = +0.335 to +0.368 V). No significant differences in antioxidant activity were noticed between caffeic acid and its derivatives. The catechol moiety of caffeic acid, CAPE **7** and other derivatives is important for antioxidant activity due to its ability to generate stabilized resonance structures of the phenoxy radical intermediate with formation of an *o*-quinone [52]. The introduction of a methoxy group at the *meta* position in ferulic acid and its derivatives lead to an increase in redox potential and decreased antioxidant activity. The introduction of an electron withdrawing group such as Br in an *ortho* position did not affect the redox potential or antioxidant activity [53].

Zhang and co-workers synthesized a caffeic acid derivative (Fig. **15**), namely the caffeic acid 3,4-dihydroxyphenethy ester **21**. It is a hydroxylyated derivate of CAPE **7** and is potentially more active because of the introduction of the two hydroxyl groups on the phenylethyl moiety. Using a Knoevenagel condensation, the authors manage to synthesize the caffeic acid 3,4-dihydroxyphenethyl ester **21** with a high yield.

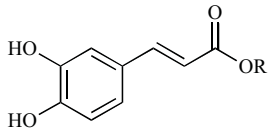


21

Fig. (15).

Compound **21** radical scavenging activity was evaluated with the DPHH assay and appears to show strong radical scavenging activity with an EC_{50} = 4.7 µg/ml. The study did not mention which compounds were used as reference [54].

Jayaprakasam and co-workers synthesized a series of caffeic acid **1** and ferulic acid **19** analogues with variable alkyl chain length (Fig. **16**). They tested their anti-inflammatory activity by measuring COX (cyclooxygenase)-1 and COX-2 inhibition. *In vitro* assays of COX inhibition showed that both COX-1 and COX-2 were inhibited at 25µg/ml by ferulic and caffeic acid alkyl esters. The most potent caffeates were compounds **22**, **23**, and **5c**, which inhibit COX-1 by 79%, 92%, and 83% respectively while caffeic acid **1** was inactive. Compounds **22**, **23**, and **5c** showed inhibitory activities of 59%, 64%, and 61% respectively for COX-2 compared to less than 50% for caffeic acid. Compounds **22**, **23** and **5c** displayed a medium alkyl chain length (C_4-C_8), no branching, and the presence of a catechol moiety. The branched esters (iso-alkyl, *n*-propyl and isopropyl), were less potent than their linear counterparts, showed no inhibition against COX-1 but were selectively active against COX-2 although at a lower level than compounds **22**, **23**, and **5c**. The ferulic acid esters, linear or branched, up to chain length C_8 showed potent although non-selective COX-2 inhibition (60-80%). For caffeic acid analogues, an increase in chain length over C_{18} caused a sharp decrease of COX-2 inhibition and absence of inhibition against COX-1. Ferulates having a chain length > C_{12} showed a marked decrease in both COX-1 and COX-2 inhibition.



22 R = $CH_2(CH_2)_2CH_3$
23 R = $CH_2(CH_2)_3CH_3$
5c R = $CH_2(CH_2)_6CH_3$

Fig. (16).

The antioxidant activity of these compounds was measured by the lipid peroxidation assay. In the lipophilic medium used for this test, the more lipophilic, long-chain alkyl esters (C_{16}-C_{22}) were the most potent for the caffeic acid derivatives. As such, compounds **22**, **23**, and **5c** with medium alkyl chain length (C_4-C_8) were less active (< 20% inhibition) compared to caffeic acid (> 80% inhibition). Surprisingly for the ferulic acid derivatives, the shorter chains

(C_3-C12) were more potent. No real structure-activity conclusions were derived from this study but differences in conformational change and interactions with the COX enzymes might be plausible explanations for the differences between the caffeic and ferulic alkyl esters [55].

Phase 2 enzymes (e.g. quinone reductase) in mammals are part of a defence system against toxic agents such as electrophiles or reactive oxygen species (ROS). Some natural and synthetic agents can induce the activity of these enzymes and increase the level of protection in malignant conditions characterized by high generation of ROS, radicals or electrophile species. One such class of molecules, Michael reaction acceptors include caffeic acid and coumaric acid. Dinkova-Kostova and co-workers synthesized methyl esters of caffeic acid **5a**, *o*-coumaric acid **24a**, *m*-coumaric acid **24b**, and *p*-coumaric acid **24c** (Fig. **17**). The concentration of compounds required to double quinone reductase specific activities in murine hepatoma cells was expressed as CD.

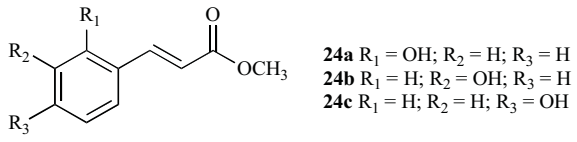


Fig. (17).

Caffeic and *p*-coumaric acid were inactive as inducers of quinone reductase, and their esters showed weak activity against this same enzyme. However, o-coumaric acid and its methyl ester had good inhibition activity (CD = 19 μM and 15 μM respectively). These observations underlined the importance of the position of a hydroxyl group at the *ortho* position of the aromatic ring [56].

Caffeic acid **1** is prone to oxidative dimerization [57]. In natural compounds such as caffeoylquininic acid, where two or three caffeoyl moieties might be present, such dimerization could influence the antioxidant activity of those molecules. To address this issue, Saito and co-workers synthesized 6 regio- and stereoisomers of dicaffeoyloxycyclohexanes **25a-f** and 2, 4-di-*O*-caffeoyl-1, 6-anhydro-β-D-glucose **27** as model compounds to evaluate the effect of intramolecular coupling between two adjacent caffeoyl residues (Fig. **18**). The radical scavenging activity of these compounds was evaluated with the DPPH and ABTS (2, 2'-azinobis assays) and compared against cyclohexyl caffeate **26**. Trolox was used as a reference for these antioxidant tests.

The radical scavenging activity in both assays (DPPH and ABTS) ranked as followed: **27** > **25a** > **25b** > **25d** > **25c**, **25f**, **25e**. These results underlined the importance of the orientation or the distance between two adjacent caffeoyl moieties on antioxidant activity. Thus, in compounds **25c**, **25f** and **25e** radical scavenging activity was at its lowest since the two caffeoyl residues were too far to interact. The highest activity was shown by compound **25d** where the diaxial conformations of the two caffeoyl residues maximize their interaction [58].

Caffeic and hydroxycinnamic acids are natural antioxidants present in plants while carnosine (CAR), a dipeptide (alanyl-L-histidine) and gluthathione (GSH), a tripeptide (l-glutamyl-l-cysteinyl-glycine) are their counterparts in human cells. In a recent study, Kwak and co-workers [59], sought to combine the best of two worlds by conjugating those natural peptides with hydrocinnamic acid derivatives. The goal was to generate molecules with improved antioxidant activity by comparison to the two parent compounds [59]. Prior to conjugation the C-terminus of both antioxidative peptides were converted to amides to increase the peptides stability. Carnosine and a glutathione analogue GS(Bzl)H were conjugated to caffeic acid (Fig. **19**). The antioxidant activity was measured by the DPHH assay and the lipid peroxidation system for the conjugates and the parent compounds.

Radical scavenging activities of compounds **28** and **29** respectively 89% and 81% were similar to caffeic acid **1**

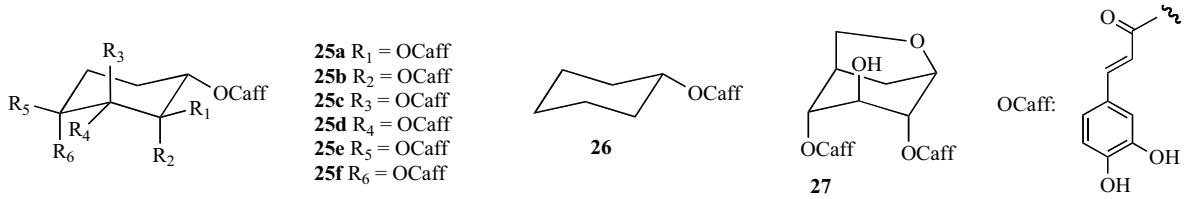


Fig. (18).

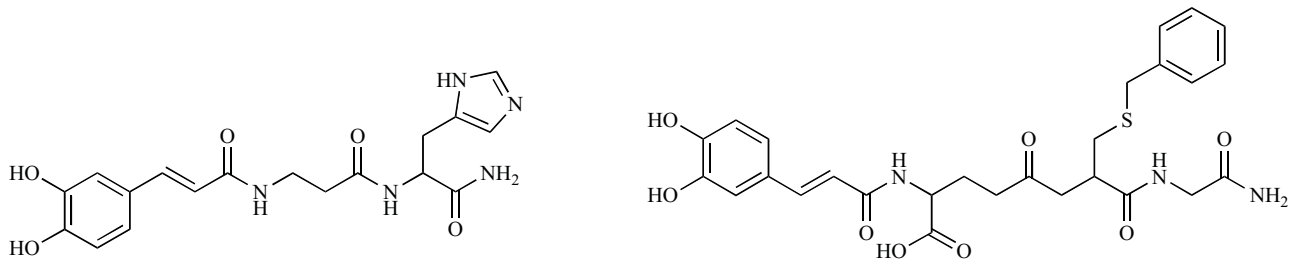
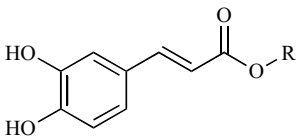


Fig. (19).

7 R = $(CH_2)_2C_6H_5$
32a R = $(CH_2)_6C_6H_5$
32b R = $(CH_2)_{12}C_6H_5$
7d R = $(CH_2)_6CH{=}CHC_6H_5$ (*Z*)
7e R = $(CH_2)_6CH{=}CHC_6H_5$ (*E*)



32c R = $(CH_2)_{10}CH{=}CHC_6H_5$ (*Z*)
32d R = $(CH_2)_{10}CH{=}CHC_6H_5$ (*E*)
32e R = $(CH_2)_3CH_3$

Fig. (20).

Table 8. NO Production Inhibition and Cytotoxicity of Caffeic Acid Esters 7, 7d, 7e, and 32a-32e

Compound	NO inhibition IC_{50} (μM)	Cell viability (%)	Compound	NO inhibition IC_{50} (μM)	Cell viability (%)
7	13.8	76.7	**7e**	3.29	4.3
32a	4.80	5.9	**32c**	5.13	4.3
32b	5.39	7.2	**32d**	6.76	4.4
7d	5.36	4.8	**32e**	8.86	76.6

(91%) but significantly higher than their parent compounds carnosine or GS(Bzl)H (<10%). Conjugation of the peptides increased their radical scavenging activity without affecting significantly the initial activity derived from the hydroxycinnamic moiety. Results from the lipid peroxidation test showed that caffeic acid, a relatively hydrophilic molecule, had the lowest inhibitory activity (29%). Conjugation resulted in higher activities as demonstrated by compounds **28** (66%) and **29** (54%). Lipophilic factors cannot account for these observations since the caffeic acid-peptides conjugates are more hydrophilic than free caffeic acid. In fact, both conjugates have hydrophilic and hydrophobic moieties allowing them to be fairly efficient in aqueous and hydrophobic environments. In particular, the conjugate carnosine-caffeic acid **28** synergistically increased the antioxidant activity of caffeic acid. Its radical scavenging activity is similar to free caffeic acid but its lipid peroxidation inhibitory activity was better than caffeic acid.

In a follow-up study, the same group synthesized a library of caffeic acid-histidine dipeptide conjugates in order to identify conjugates with a better antioxidant profile than carnosine-caffeic acid **28**. Radical scavenging activity assayed by DPHH at a molar ratio DPHH/compound = 0.25 showed in general a synergistic effect by conjugating the dipeptides to caffeic acid. In most cases the conjugates activity was similar or better than the activity of free caffeic acid due to the trapping capacity of the imidazole ring of histidine. The most potent conjugate was CA-Pro-His-NH_2 **28** (70% of radical scavenging activity vs 50% for caffeic acid). The best inhibitor of lipid peroxidation was again CA-Pro-His-NH_2 **28** (87%) and its activity, in this case, was similar to the reference compound, butylhydroxyanisole (BHA), (86%) a strong synthetic antioxidant. Switching the position of the His residue in the dipeptide moiety did not affect significantly radical scavenging activity or lipid peroxidation, the only exception being CA-Pro-His-NH_2 **28**. Radical scavenging and inhibition of lipid peroxidation were higher in **28** than in CA-His-Pro-NH_2. In this particular case, the Proline moiety in **28** presents a tilted structure where one hydroxyl group is in the immediate vicinity of the imidazole ring. This conformation provided additional stability to the hydroxyl radical of caffeic acid by the imidazole ring after quenching of free radicals [60].

2.3. Anti-Inflammatory Effects

During inflammation, external agents such as mitogens or pro-inflammatory interleukins activate NF-κB, an eukaryotic transcription factor regulating the expression of genes such as COX-2, iNOS and 5-LO. The products of these genes are key components in the inflammatory response. Cyclooxygenase type 2 (COX-2) is implicated in the synthesis of prostaglandins (PGs), inducible nitric oxide synthase (iNOS) is involved in the production of nitric oxide (NO) and 5-lipoxygenase (5-LO) transforms arachidonic acid to leukotrienes. PGs play key roles in cell proliferation, mitosis, apoptosis and immune response [61-63] and numerous studies have linked elevated levels of PGs to many types of cancers (breast, colon lung) [64-66]. NO is a pro-inflammatory agent which plays many roles in carcinogenesis and mutagenesis [67,68]. Leukotrienes (LTs) are a class of chemotactic metabolites involved in a wide array of pathological conditions such as inflammation and allergic disorders [69]. Thus, PGs, NO and LTs are involved in the inflammatory process and their production is under some kind of regulation by NF-κB [70,71].

Caffeic acid **1** and CAPE **7** are known to inhibit NF-κB [72,73] thus slowing down the transcription of certain pro-inflammatory genes. In the particular case of caffeic acid methyl vanillate ester (CAMVE), a synthetic caffeic acid analogue, the mechanism of NF-κB inhibition was demonstrated [74] and is not cell specific [75]. Over the years many analogues of caffeic acid **1** and CAPE **7** were synthesized in order to improve the anti-inflammatory potential of these natural molecules.

2.3.1. Nitric Oxide Inhibition (NO)/Anti-Nitrosating Agents

Nitric oxide (NO) synthesis is one of the hallmarks of inflammatory processes in conditions such as sepsis, ulcerative colitis and arthritis [76,77]. NO also acts as a defense molecule by nitrosating DNA and protein tyrosine residues and inducing lipid peroxidation [78, 79]. NO's deleterious effects are usually kept in check by tight regulation and an antioxidant system including GSH (reduced gluthathione).

With the advent of natural anti-oxidant products, alternative solutions were explored for new anti-inflammatory treatments. CAPE, a natural catechol, is one such molecule. Most available studies based on the development of derivatives with anti-nitric oxide properties were centered around this molecule.

Nagaoka and co-workers synthesized a series of phenyl alkyl caffeic acid ester derivatives (Fig. **20**) to assess their potential as nitric oxide inhibitors. Their cell model was the LPS-activated macrophage-like J774.1 cells which reflect inflammatory conditions and produce NO detected as nitrites in the cell medium. Among the caffeic acid analogues synthesized, compounds **7d**, **7e**, and **32a-d** were more potent than CAPE (**7**, IC_{50} = 13.8 μM) itself and polymixin B (IC_{50} = 27.8 μM) the positive control (Table **8**). Increasing the length of the alkyl chain decreased the IC_{50} and enhanced the anti-NO inhibitory activity. Nevertheless when alkyl length was more than C_5, those interesting properties were offset by the high cytotoxicity at concentration of 100 μM. Therefore, high anti-NO activity measured for these compounds with long alkyl chains might only reflect cellular death. Only compound **32e** manage to combine potent anti-NO activity and an acceptable level of cytotoxicity. However, compared to CAPE **7**, there was no significant improvement (Table **8**) [80].

In a similar study with LPS-activated RAW264.7 macrophage cells, Uwai and co-workers. tested the effect of different alkyl side chains on NO production. They synthesized a series of caffeic acid esters (Fig. **21**).

33a X = O; R = $(CH_2)_{10}CH_3$
33b X = O; R = $(CH_2)_{11}CH_3$
33c X = O; R = -c-Hexyl
7a X = O; R = $CH_2C_6H_5$
33d X = O; R = $CH_2CH_2CH{=}C(CH_3)_2$
33e X = NH; R = $(CH_2)_{10}CH_3$
33f X = CH_2; R = $(CH_2)_{10}CH_3$

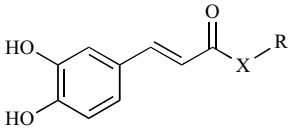


Fig. (21).

Cytotoxicity studies showed an increase in toxic effects (lower EC_{50}) with increasing alkyl chain length. Undecyl caffeate **33a** (EC_{50} = 1.188 μM) and dodecyl caffeate **33b** (EC_{50}: 1.000 μM) being the most cytotoxic of all the synthesized compounds. Bulkier lipophilic compounds such as cyclohexyl **33c**, benzyl **7a**, and prenyl **33d** esters showed low toxicity (high EC_{50}= 28-38 μM). The authors explained these apparently contradictory results by the relation between ClogP and cytoxicity. Their results showed a biphasic correlation between ClogP and cytotoxicity for caffeic acid esters. Cytotoxicity increased depending on lipophilicity to an optimal ClogP value of 4.36. NO inhibition studies for alkyl chains of 0 to 11 carbon atoms showed a direct relationship between chain length and inhibitory effects on NO production. Undecyl caffeate **33a** was the more potent NO inhibitor (EC_{50}= 0.018 μM), 9000 times more potent than caffeic acid (EC_{50}= 165 μM). This ester derivative of caffeic acid, despite its high cytotoxicity, was investigated further to evaluate the stability of the ester link in biological fluids and its importance in NO inhibition. To this effect, an amide derivative **33e** and a ketone derivative **33f** of undecyl caffeate were synthesized. The results showed that the ester linkage was not hydrolyzed by cells and more importantly that the ester linkage of undecyl caffeate has a higher NO inhibitory activity compared to the amide and ketone derivatives [81].

Octyl caffeate, a caffeic acid ester derivative was investigated to assess its anti-oxydant properties and the molecular mechanisms involved. Using rat aortic smooth muscle cells (RASMC) treated with LPS/IFN-γ, Hsiao and co-workers showed that octyl caffeate is a potent inhibitor of NO production *in vitro*. At the non-cytotoxic concentration of 50μM of octyl caffeate, inhibition was characterized by a decrease of 93% in nitrite production, a decrease of 83 % and 84 % in iNOS and iNOS mRNA expression respectively. It was also shown that in the presence of octyl caffeate the expression of MAPKs signaling molecules ERK1, ERK2 and JNK, well-known activators of iNOS gene transcription, was greatly reduced. These results suggest that the MAPK pathway is greatly affected by the presence of octyl caffeate [82].

Caffeic acid and its esters (e.g. chlorogenic acid) are known inhibitors of nitrosation reactions [83]. Based on this information, De Lucia *et al.* synthesized glutathionyl deriva-

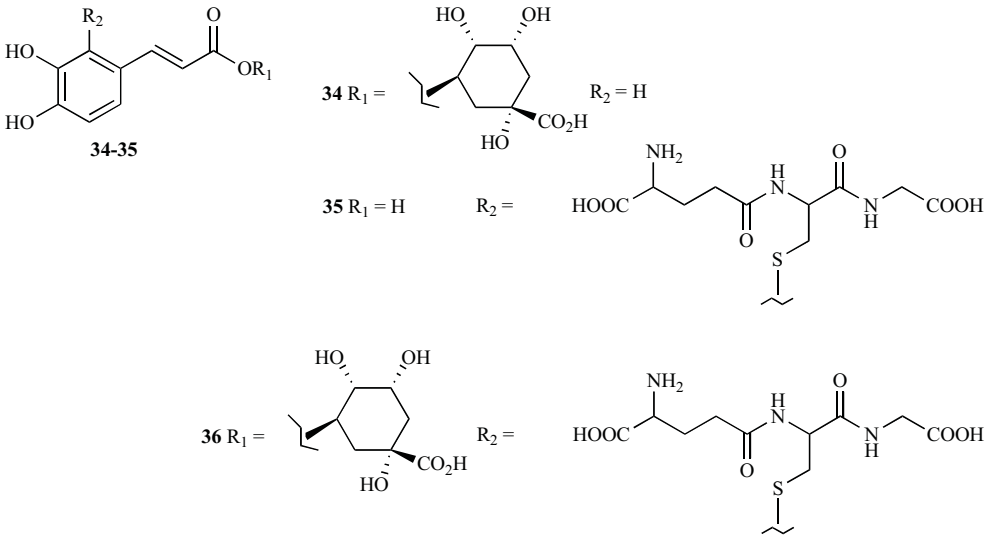


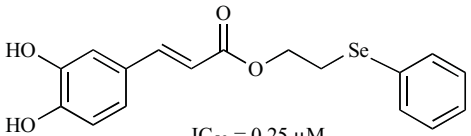
Fig. (22).

tives of caffeic acid (**1**) and chlorogenic acid (**34**) to assess their anti-nitrosation activities (Fig. **22**). It was surmised that the addition of the glutathionyl moiety, a physiological antioxidant tripeptide, to either caffeic acid or chlorogenic acid would create a synergistic effect increasing the overall activity. *In vitro* nitrosation studies, based on inhibition of tyrosine nitration by these compounds, showed some startling results. Both glutathionyl conjugates **35** (60% inhibition) and **36** (40% inhibition) showed at 100 μM a lower inhibition of nitration when compared respectively to their parent compounds **1** (80%) and **34** (95%).

Steric factors might explain the decrease of reactivity of the propenoate chain following conjugation with glutathione. The presence of the bulky GSH group could break the coplanarity of the important double bond side chain with the catechol ring and hinder efficient electron delocalization over the phenylpropenoate moiety [84].

2.3.2. 5-Lipoxygenase Inhibition

5-Lipoxygenase (5-LO) is the key enzyme in the metabolism of arachidonic acid (AA) to leukotriene A4 (LTA4). Further metabolism of LTA4 produces LTB4, a potent chemotactic agent for leukocytes that is thought to be a key component in a variety of diseases [85, 86] including inflammatory bowel disease and atherosclerosis. LTA4 can also be converted to the peptidoleukotrienes LTC4, LTD4, and LTE4 [87] which are implicated in allergic hyper reactivity disorders such as asthma [86]. Elevated levels of these LTs, associated with several inflammatory and allergic disorders, have been found in various pathologic tissues [86]. In search of pharmacological strategies that potently suppress LTs synthesis, a large number of different types of low molecular weight inhibitors have been developed in the past years [88]. Among the known inhibitors of the 5-LO are a variety of polyhydroxylated natural products such as caffeic acid (**1**), and CAPE (**7**) [88-90].



37 IC_{50} = 0.25 μM
K_i = 0.22 μM

Fig. (23).

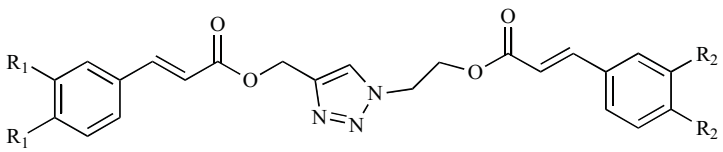
Based on the biological properties of selenium [91,92], Lin and co-workers described the synthesis and biological evaluation of the CAPE selenium-containing analog **37** (Fig. **23**). 2-phenylselenoethanol, a key intermediate, was prepared from 2-chloroethanol with phenylselenol produced *in situ* by a reduction of diphenyl diselenide. Compound **37** was found to be a very effective antioxidant and 5-lipoxygenase inhibitor with activity comparable to or better than CAPE **7** (IC_{50} = 0.22 μM; K_i 0.30 μM) [93].

In an application of clusters or dendrimers, our group applied the versatile "click chemistry" conditions to an azido or alkyne-functionalized caffeoyl derivatives for novel dimeric analogues synthesis (Fig. **24**) [94]. Multivalent interactions have several advantages over monomeric ones and are often used by nature to control a wide variety of cellular processes [95]. Such inhibitors may provide therapeutic benefit for the pharmacological treatment of inflammatory and allergic disorders, cardiovascular diseases, and cancer. In dose-response studies, some compounds showed concentration-dependent inhibition of 5-LO product synthesis with IC_{50} values below 1 μM (**38a** IC_{50} = 0.68 μM; **38c** IC_{50} = 0.74 μM; **10** IC_{50} = 0.66 μM) [94] that were comparable to the inhibitory activity of zileuton (IC_{50} = 0.5–1 μM) [**96**]. On the basis of corrected values on a per caffeoyl residue, dimer **38c** (Fig. **24**) and tetramer **10b** (Fig. **7**), readily surpassed the activity of caffeic acid **1** (IC_{50} = 25 μM) by more than 10-fold.

The inhibitory capacities of these compounds were then evaluated in intact HEK293 cells [35]. These cells have the cellular machinery required for leukotriene biosynthesis. Compound **38b** caused almost complete 5-LO inhibition at 10 mM, and was more effective than the known 5-LO inhibitor zileuton [96]. Inhibition tests with diacetylated precursors of molecules **38a** and **38b** resulted in the complete inhibition of 5-LO metabolites in intact cells. These results could suggest that these molecules infiltrate into intact cells more efficiently than **38a** and **38b**, resulting in a more efficient inhibition of 5-LO following esterase action, or a possible interference with 5-LO associated proteins (e.g. coactosin-like protein or FLAP). From this laboratory, small libraries of caffeic acid esters and amides were constructed for the establishment of a quantitative structure activity relationship (QSAR) model toward *5*-LO [97].

2.4. Anti-Human Immunodeficiency Virus (HIV)

The HIV reverse transcriptase and protease enzymes are two targets which currently provide the basis for most AIDS therapies. To augment these approaches, inhibitors directed at new enzyme targets such as the HIV integrase are needed [98]. The attractiveness of the HIV integrase is attributed to the absolute requirement for its participation in HIV replication [99-102] and to the fact that it is not indigenous in humans. HIV-1 integration encompasses a series of molecular



38a R_1 = H, R_2 = OH
38b R_1 = OH, R_2 = H
38c R_1 = OH, R_2 = OH

Fig. (24).

events which follow the completion of reverse transcription in the cytoplasm of the infected cell and end with the initiation of the transcription from the proviral DNA [103].

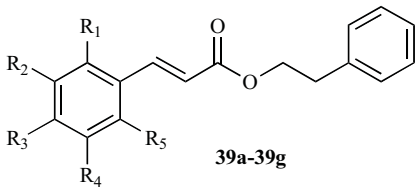


Fig. (25).

Catechol containing bis-aryl moieties, separated by a linker, are a significant structural component in many potent HIV integrase inhibitors. A recurrent structural pattern in many active compounds is the presence of multiple aromatic rings, with polyaryl hydroxylation in the 1,2-position. Bruke and co-workers described several compounds based on CAPE **7** structure (which exhibit an IC_{50} value of **7** μM, Table **9**). These analogues were essentially designed to examine the phenyl ring substitution and conformational orientation (Fig. **25**) [104]. As shown in Table **9**, the number and nature of ring substituents affected potency. Replacement of the hydroxyl groups of CAPE (**7**) with either one or two methyl ethers (**39a-c**) resulted in loss of potency. Additionally, altering the 3, 4-dihydroxy pattern substitution to the 2,5-dihydroxy pattern resulted in a complete loss of activity (**7** and **39d**). Adding a third hydroxyl to give either the 2,3,4-trihydroxy or the 3,4,5-trihydroxy derivative increased potency (**5** and **6**); however, the 2,4,5- trihydroxy derivative **39g** was less potent than the 2,3,4-trihydroxy, 3,4,5-trihydroxy derivative, and the parent CAPE **7**. CAPE **7**, methyl **5a** and ethyl **6e** ester analogs were also reported by Ho and co-workers to significantly inhibit the HIV replication [105].

5-Nitrocaffeic acid phenethyl ester **8d** (IC_{50} = 25 μM), bornyl caffeate **8e** (IC_{50} = 19.9 μM), and bornyl 2-nitrocaffeate **8f** (IC_{50} = 13.5 μM) (Fig. **5**) described by Xia and co-workers showed a good HIV integrase inhibitory activity. While adding a third strong election-withdrawing NO_2

Table 9. 7 and 39a-39g Structures (Fig. 25) and Inhibition of HIV Replication

Compounds	R_1	R_2	R_3	R_4	R_5	IC_{50} (μM)
7	H	OH	OH	H	H	7
39a	H	OCH_3	OH	H	H	>100
39b	H	OH	OCH_3	H	H	60
39c	H	OCH_3	OCH_3	H	H	>100
39d	OH	H	H	OH	H	>100
39e	OH	OH	OH	H	H	2
39f	H	OH	OH	OH	H	2
39g	H	OH	OH	H	OH	55

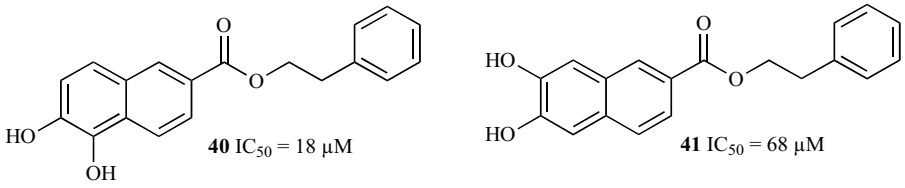


Fig. (26).

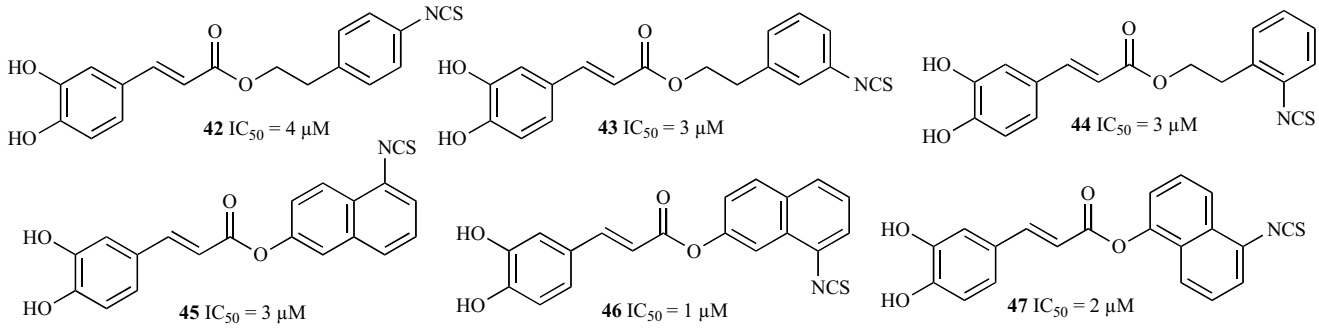


Fig. (27).

group on 3,4-dihydroxyl pattern resulted in potent activity, such as compounds **8e** and **8f**. Aryl ring or multi-ring compound seemed to be required [34]. For the conformational orientation evaluation, 5,6-dihydroxy and 6,7-dihydroxy bicyclic analogues (**40** and **41**, (Fig. **26**))**,** which differ in the placement of hydroxyl substituents, were prepared and evaluated by Bruke and co-workers. These two bicyclic derivatives represent the conformationally constrained analogues of the CAPE **7** rotamers. The 6,7-dihydroxy isomer **41** was less potent than the 5,6-dihydroxy derivative **40** and CAPE **7** (Fig. **25**).

Bruke and co-workers have also described the preparation and evaluation of new CAPE **7** isothiocyanates derivatives as irreversible HIV-1 integrase affinity ligands (Fig. **27**) [106].

The choice of isothiocyanate substitution was based on its demonstrated utility in the preparation of affinity ligands directed against a number of other protein targets. CAPE **7** (IC_{50} = 5 μM) as well as isothiocyanate analogues **42**-**47**, provided approximately equivalent IC_{50}, indicating that changes to the phenethyl portion of CAPE had little effect on potency. Introduction of the isothiocyanate group onto various positions of the phenethyl ring or replacement of the phenyl ring with naphthyl rings, failed to significantly affect inhibitory potency. This observation might indicate a degree of insensitivity of this region of the molecule toward structural modification [106].

The investigations of new integrase inhibitors with structural modifications overcoming the *in vitro* toxicities of CAPE **7** and its analogues lead to the discovery of compounds with multiple caffeoyl groups appended to a carboxylic acid-containing framework. 4,5-di-*O*-caffeoylquinic acid **48** and L-chicoric acid **49** (Fig. **28**) (dicaffeoyltartaric acid) inhibit integration in biochemical assays and block HIV replication in cell culture [107-112].

Through a series of analogues, Bruke and co-workers have shown that both L- and D-chicoric acids (**49** and **50**) are nearly identical in potency against integrase (IC_{50} values of approximately 1 μM) [113]. Additionally, removal of both carboxylic functionalities results in no loss of inhibitory potency (**51**, Fig. **29**). Modification of **51** (IC_{50} = 0.9 μM) by the addition of a methylene (Fig. **29**) or replacing the caffeoyl ester linkage with an amide linkage resulted in significant losses of activity. Furthermore, replacement of one caffeoyl ester linkage by an amide linkage combined with removal of one carboxyl group provided compound **52** (IC_{50} = 3.3 μM) with good activity compared to the parent chicoric acid. The inactivity of **53** (IC_{50} = 333 μM) indicates that two caffeoyl moieties are important for potency within the series.

In another round of SAR, King and co-workers found that the L-chicoric acid **49** (IC_{50} = 0.18 μM) was 2-3 times less potent than the D- **50** (IC_{50} = 0.07 μM) and *meso*- **54** (IC_{50} = 0.08 μM) isomers (Fig. **30**) [114]. To determine the importance of carboxyl groups for bioactivity, (L)- dicaffeoylglyceric acid **55** with a single carboxyl group, was evaluated. As demonstrated by compound **55** (IC_{50} = 0.52 μM), only one carboxylic acid was required for integrase inhibition activity (Fig. **30**). The activity of the 3,4-dihydroxyphenylacetyl, 3,4- dihydroxybenzoyl, and 3,4-dihydroxydihydrocinnamoyl L-chicoric acid analogues, with modified linker group separating the catechol from the central core, indicates that length (0, 1, or 2 carbons) or hybridization (sp^3 or sp^2) is not critical. Since only the 3,4-dihydroxyphenyl isomer was active, the 3,4-biscatechol moiety was required for integrase inhibition which indicates that activity is not only due to antioxidant or metal-chelating properties. Additionally, the blocking or replacement of phenolic hydroxyls abolished the activity [114]. As shown in Fig. (**30**), the dicaffeoyl derivatives of L-serine **56a**, D,L-isoserine **56b**, and L-lysine **56c** prepared by Reinke and co-workers are potent inhibitors of integrase [115].

Replacement of L-tartaric acid central core with a more rigid 5-membered heterocycles has been tried by Hwang and co-workers to improve the activity profile [116]. The nature of the five-membered heterocyclic ring linker did not

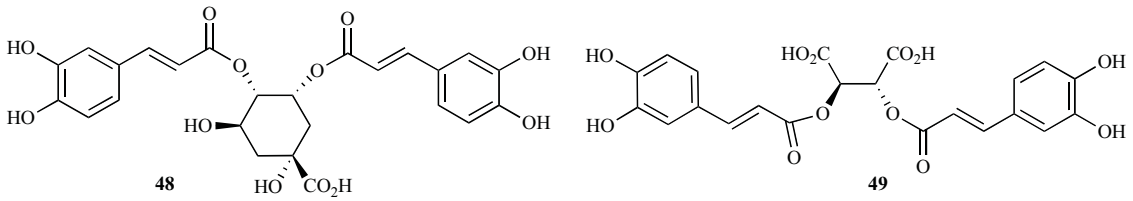


Fig. (28).

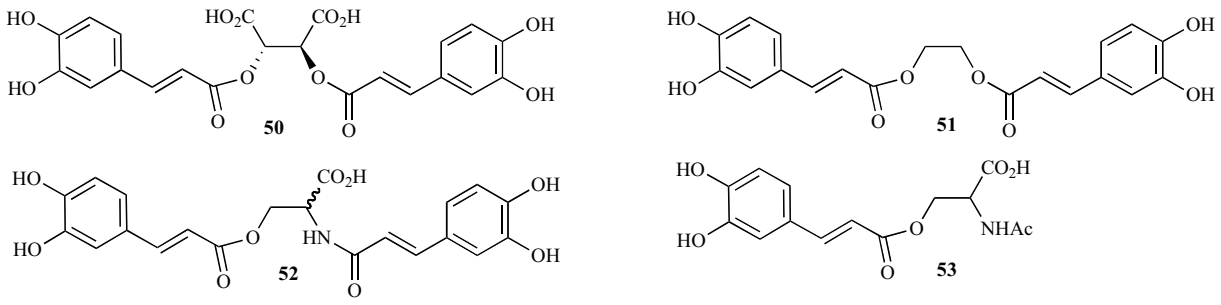


Fig. (29).

49 (*L*) IC_{50} = 0.18 μM
50 (*D*) IC_{50} = 0.07 μM
54 (*meso*) IC_{50} = 0.08 μM

55 IC_{50} = 0.52 μM

56a X = NH, Y = O, n = 0, IC_{50} = 0.18 μM
56b X = O, Y = NH, n = 0, IC_{50} = 0.12 μM
56c X = NH, Y = NH, n = 2, IC_{50} = 0.19 μM

Fig. (30).

Table 10. Inhibitory Activity Against HIV Replication of 49, 57, and 58

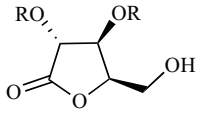
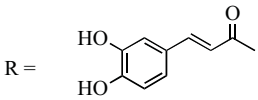
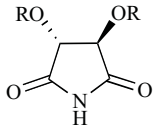
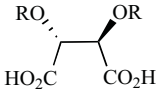
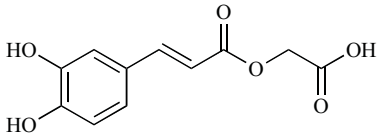
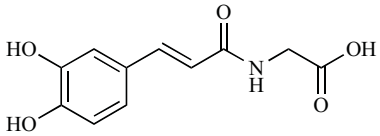
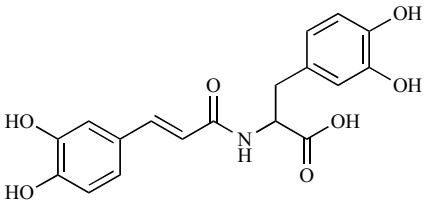
Compounds	IC_{50} (μM)		
57		22.4	
58		25.2	
49		24.9	

Table 11. Inhibitory Activity Against HIV Replication of 59a-59c and 49

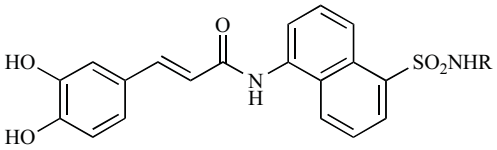
Compounds		IC_{50} (μM)
59a		10.5
59b		171.6
59c		12.0
49		15.7

influence the inhibitory activity. Among synthesized compounds, **57** and **58**, which contains a dihydrofuran-2(3H)-one and a pyrrolidine-2,5-dione ring respectively as a linker, showed the best inhibitory activity (Table **10**).

In order to simplify the L-chicoric acid **49** structure, Lee and co-workers designed caffeoylglycolic and caffeoylamino acid derivatives as new HIV-1 integrase inhibitors [117]. The inhibitory activity of caffeoylglycolic acid **59a**, which contain only one caffeoyl group was comparable to that of L-chicoric acid. The inhibitory activity was decreased with the caffeoylamino acid analog **59b**. On the other hand, the 3,4-dihydroxybenzyl-substituted caffeoylamino acid **59c** was

equipotent to L-chicoric acid, underlining the importance of the catechol group for activity (Table **11**).

Caffeic amides connected with naphthalenesulfonamides as HIV integrase inhibitors have been reported by Xu and co-workers and compared to L-chicoric acid **49** [118]. Some compounds showed moderate HIV integrase inhibitory activities and were comparable to that of L-chicoric acid. Caffeic amides **60a** and **60b** containing N-phenethyl and N-benzyl sulfonamide groups showed the best inhibitory activity (Fig. **31**). Compared to the sulfonamide derivative **60b**, introduction of a *p*-fluoro group in **60d** increased the inhibitory activity (Fig. **30**).



60a R = CH_2CH_2Ph; IC_{50} = 4.5 μg/mL
60b R = CH_2Ph; IC_{50} = 8.6 μg/mL
60c R = Ph; IC_{50} = 12.2 μg/mL
60d R = CH_2PhF; IC_{50} = 7.9 μg/mL

Fig. (31).

3. CONCLUSIONS

Caffeic acid is a unique scaffold that is associated with several biological activities. The radical quenching properties of the 3,4-dihydroxy pattern substitution in many caffeic acid derivatives have raised interest in using these compounds as drugs or food supplements. The anticancer, anti-inflammatory, and anti-HIV activities of a variety of synthetic caffeic acid derivatives have been presented in this review. Few ketone analogues of caffeic acid were synthesized and tested. Based on the fact that ketone analogues are less sensitive to hydrolysis in biological medium than esters and amides, this might represent a new avenue for the synthesis of more stable caffeic acid analogues.The literature was analysed to provide a meaningful overview of the structural requirements for activity, wherever possible.

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REFERENCES

[1] Newman, D.J. Natural Products as Sources of New Drugs over the Period 1981-2002. *J. Nat. Prod.*, **2003**, *66* (7), 1022-1037.

[2] Deshpande, S.S.; Sathe, S.K.; Salunkhe, D.K. Chemistry and safety of plant polyphenols. *Adv. Exp. Med. Biol.*, **1984**, *177*, 457-495.

[3] Harris, P.J.; Hartley, R.D. Detection of bound ferulic acid in cell walls of the gramineae by ultraviolet fluorescent microscopy. *Nature,* **1976**, *259*, 508-510.

[4] Hartley, R.D.; Morrison, W.H.; Himmelsbach, D. S.; Borneman, N. S. Cross-linking of cell wall phenolics to arabinoxylans in graminaceous plants. *Phytochemistry,* **1990**, *29*, 3701-3709.

[5] Cuvelier, M.E.; Richard, H.; Berset, C. Comparison of the antioxidant activity of some acid phenols: structure-activity relationship. *Biosci., Biotechnol., Biochem*., **1992**, *56* (2), 324-325.

[6] Maillard, M.N.; Soum, M.H.; Boivia, P.; Berset, C. Antioxidant activity of barley and malt: relationship with phenolic content. *Lebensm.-Wiss.-Technol.,* **1996**, *3*, 238-244.

[7] Friedman, M. Chemistry, biochemistry and dietary role of potato polyphenols A review. *J. Agric. Food Chem*., **1997**, *45*, 1523-1540.

[8] Michaluart, P.; Masferrer, J.L.; Carothers, A.M.; Subbaramaiah, K. ; Zweifel, B.S.; Koboldt, C.; Mestre, J.R.; Grunberger, D.; Sacks, P.G.; Tanabe, T.; Dannenberg, A.J. Inhibitory Effects of Caffeic Acid Phenethyl Ester on the Activity and Expression of Cyclooxygenase-2 in Human Oral Epithelial Cells and in a Rat Model of Inflammation. *Cancer Res*., **1999**, *59*, 2347-2352.

[9] Liao, H.F.; Chen, Y.Y.; Liu, J.J.; Hsu, M.L.; Shieh, H.J.; Liao, H.J.; Shieh, C.J.; Shiao, M.S.; Chen, Y.J. Inhibitory effect of caffeic acid phenethyl ester on angiogenesis, tumor invasion,and metastasis. *J. Agric. Food Chem.*, **2003**, *51*, 7907-12.

[10] Baranowski, J.D.; Davidson, P.M.; Nagel, C.W.; Brannen, R.L. Inhibition of *Saccharomyces cerevisiae* by naturally occurring hydroxy cinnamates. *J. Food Sci*., **1980**, *45*, 592-594.

[11] Huang, W.Y.; Cai, Y.Z.; Zhang, Y. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutr. Cancer*, **2010**, *62* (1), 1-20.

[12] Slavin, J.L. Mechanisms for the impact of whole grain foods on cancer risk. *J. Am. Coll. Nutr.*, **2000**, *19*, 300S-307S.

[13] Fresco, P.; Borges, F.; Marques, M.P.; Diniz, C. The anticancer properties of dietary polyphenols and its relation with apoptosis. *Curr. Pharm. Des.*, **2010**, *16* (1), 114-1134.

[14] Del Rio, D.; Costa, L.G.; Lean, M.E.; Crozier, A. Polyphenols and health: what compounds are involved? *Nutr. Metab. Cardiovasc. Dis.*, **2010**, *20* (1), 1-6.

[15] Gomes, C.A.; da Cruz, T.G.; Andrade, J.L.; Milhazes, N.; Borges, F.; Marques, M.P.M. Anticancer activity of phenolic acids of natural or synthetic origin: A structure-activity study. *J. Med. Chem.*, **2003**, *46*, 5395-5401.

[16] Fiuza, S.M.; Gomes, C.; Teixeira, L.J., da Cruz, M.T.G.; Cordeiro, M.N.D.S.; Milhazes, N.; Borgesa, F.; Marquesa, M.P.M. Phenolic acid derivatives with potential anticancer properties - a structure–activity relationship study. Part 1: Methyl, propyl and octyl esters of caffeic and gallic acids. *Bioorg. Med. Chem.*, **2004**, *12*, 3581-3589.

[17] Nam, N.H.; You, Y.J.; Kim, Y.; Hong, D.H.; Kimb, H.M.; Ahna, B.Z. Syntheses of Certain 3-Aryl-2-propenoates and Evaluation of Their Cytotoxicity. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 1173-1176.

[18] Nam, N.H.; Huong, H.T.T.; Kim, H.M.; Ahn, B.Z. Cytotoxic constituents from Notopterygium incisum. *Kor. J. Pharmacogn*., **2000**, *31*, 77-81.

[19] Rao, C.V.; Desai, D.; Kaul, B.; Amin, S.; Reddy, B.S. Effects of caffeic acid esters on carcinogen-induced mutagenicity and human colon adenocarcinoma cell growth. *Chem. Biol. Interact*., **1992**, *84*, 277-290.

[20] Guarini, L.; Su, Z.Z.; Zucker, S.; Lin, J.; Grunberger, D.; Fisher, P.B. Growth inhibition and modulation of antigenic phenotype in human melanoma and glioblastoma multiforme cells by caffeic acid phenethyl ester (CAPE). *Cell Mol. Biol.,* **1992**, *38*, 513-527.

[21] Grunberger, D.; Banerjee, R.; Eisinger, K.; Oltz, E.M.; Efros, L.; Caldwell, M.; Estevez, V.; Nakanishi, K. Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. *Experientia,* **1988**, *44*, 230-232.

[22] Su, Z.Z., Grunberger, D., Fisher, P.B. Suppression of adenovirus type 5 EIA-mediated transformation and expression of the transformed phenotype by caffeic acid phenethyl ester (CAPE). *Mol. Carcinog.*, **1991**, *4*, 231-242.

[23] Lee, Y.J.; Liao, P.H.; Chen, W.K.; Yang, C.C. Preferential cytotoxicity of caffeic acid phenethyl ester analogues on oral cancer cells. *Cancer Lett.*, **2000**, *153*, 51-56.

[24] Lee, K.W.; Kang, N.J.; Kim, J.H.; Lee, K.M.; Lee, D.E.; Hur, H.J.; Lee, H.J. Caffeic acid phenethyl ester inhibits invasion and expression of matrix metalloproteinase in SK-Hep1 human hepatocellular carcinoma cells by targeting nuclear factor kappa B. *Genes Nutr.*, **2008**, *2*, 319-322.

[25] Chen, M.J.; Chang, W.H.; Lin, C.C.; Liu, C.Y.; Wang, T.E.; Chu, C.H.; Shih, S.C.; Chen, Y.J. Caffeic acid phenethyl ester induces apoptosis of human pancreatic cancer cells involving caspase and mitochondrial dysfunction. *Pancreatology*, **2008**, *8* (6), 566-576.

[26] Chiao, C.; Carothers, A.M.; Grunberger, D.; Solomon, G.; Preston, G.A.; Barrett, J.C. Apoptosis and altered redox state induced by caffeic acid phenethyl ester (CAPE) in transformed rat fibroblast cells. *Cancer Res.*, **1995**, *55* (16), 3576-3583.

[27] Su, Z.Z.; Lin, J.; Prewett, M.; Goldstein, N.I.; Fisher, P.B. Apoptosis mediates the selective toxicity of caffeic acid phenethyl ester (CAPE) toward oncogenetransformed rat embryo fibroblast cells. *Anticancer Res*., **1995**, *15*, 1841-1848.

[28] Chen, J.H.; Shao, Y.; Huang, M.T.; Chin, C.K.; Ho, C.T. Inhibitory effect of caffeic acid phenethyl ester on human leukemia HL-60 cells. *Cancer Lett*., **1996**, *108*, 211-214.

[29] Huang, M.T.; Ma, W.; Yen, P.; Xie, J.G.; Han, J.; Frenkel, K.; Grunberger, D.; Conney, A.H. Inhibitory effects of caffeic acid phenethyl ester (CAPE) on 12-O-tetradecanoylphorborl-13-acetate-induced tumor promotion in mouse skin and the synthesis of DNA, RNA, and protein in HeLa cells. *Carcinogenesis*, **1996**, *17*, 761-765.

[30] Natarajan, K.; Singh, S.; Burke, T.R.Jr.; Grunberger, D.; Aggarwal, B.B. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kB. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 9090-9095.

[31] Banskota, A.H.; Nagaoka, T.; Sumioka, L.Y.; Tezuka, Y.; Awale, S.; Midorikawa, K.; Matsushige, K.; Kadota, S. Antiproliferative activity of the Netherlands propolis and its active principles in cancer cell lines. *J. Ethnopharmacol.*, **2002**, *80*, 67-73.

[32] Nagaoka, T.; Banskota, A.H.; Tezuka, Y.; Saiki, I.; Kadota, S. Selective Antiproliferative Activity of Caffeic Acid Phenethyl Ester Analogues on Highly Liver-Metastatic Murine Colon 26-L5 Carcinoma Cell Line. *Bioorg. Med. Chem.*, **2002**, *10*, 3351-3359.

[33] Jayaprakasam, B.; Vanisree, M.; Zhang, Y.; Dewitt, D.L.; Nair, M.G. Impact of Alkyl Esters of Caffeic and Ferulic Acids on Tumor Cell Proliferation, Cyclooxygenase Enzyme, and Lipid Peroxidation. *J Agri. Food Chem*., **2006**, *54*, 5375-5381.

[34] Xia, C.H.; Li, H.B.; Liu, F.; Hua, W.X. Synthesis of trans-caffeate analogues and their bioactivities against HIV-1 integrase and cancer cell lines. *Bioorg. Med. Chem. Lett.,* **2008**, *18*, 6553-6557.

[35] Boudreau, L.H.; Picot, N.; Doiron, J.; Villebonnet, B.; Surette, M.E.; Robichaud, G.A.; Touaibia, M. Caffeoyl and cinnamoyl clusters with anti-inflammatory and anti-cancer effects. Synthesis and structure–activity relationship. *New J. Chem*., **2009**, *33*, 1932-1940.

[36] Folguera, A.R.; Pendas, A.M.; Sanchez, L.M.; Lopez-Otin, C. Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. *J. Dev. Biol.*, **2004**, *48*, 411-424.

[37] Birkedal-Hansen, H. Proteolytic remodeling of extracellular matrix. *Curr. Opin. Cell. Biol.,* **1995**, *7*, 728-735.

[38] John, A.; Tuszynski, G. The role of matrix metalloproteinases in tumor angiogenesis and tumor metastasis. *Pathol. Oncol. Res.*, **2001**, *7*, 14-23.

[39] Itoh, T.; Tanioka, M.; Matsuda, H.; Nishimoto, H.; Yoshioka, T.; Suzuki, R.; Uehira, M. Experimental metastasis is suppressed in MMP-9-deficient mice. *Clin. Exp. Metastas*., **1999**, *17*, 177-181.

[40] Ahonen, M.; Poukkula, M.; Baker, A.H.; Kashiwagi, M.; Nagase, H.; Eriksson, J.E.; Kahari, V.M. Tissue inhibitor of metalloproteinases-3 induces apoptosis in melanoma cells by stabilization of death receptors. *Oncogene*, **2003**, *22*, 2121-2134.

[41] Giannelli, G.; Antonaci, S. Gelatinases and their inhibitors in tumor metastasis: from biological research to medical applications. *Histol. Histopathol*., **2002**, 17, 339-345.

[42] Li, Y.L.; Xu, W.F. Design, synthesis, and activity of caffeoyl pyrrolidine derivatives as potential gelatinase inhibitors. *Bioorg. Med. Chem.*, **2004**, *12*, 5171-5180.

[43] Sugiura, M.; Naito, Y.; Yamaura, Y.; Fukaya, C.; Yokohama, K. Inhibitory activities and inhibition specificities of caffeic acid derivatives and related compounds toward 5-lipoxygenase. *Chem. Pharm. Bull.* **1989**, *37*, 1039-1043.

[44] Michaluart, P.; Masferrer, J.L.; Carothers, A.M.; Subbharamaiah, K.; Zweifel, B.S.; Koboldt, C.; Mestre, J.R.; Grunberger, D.; Sacks, P.G.; Tanabe, T.; Dannenberg, A.J. Inhibitiry effects of caffeic acid phenylethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation. *Cancer Res.* **1999**, *59*, 2347-2352.

[45] Rajan, P.; Vedernikova, I.; Cos, P.; Berghe, D.V.; Augustyns, K.; Haemers, A. Synthesis and evaluation of caffeic acid amides as antioxydants. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 215-217.

[46] Son, S; Lewis, B.A.; Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: Structure-activity relationship. *J. Food Agric. Chem.* **2002**, *50*, 468-472.

[47] Tsai, W.J.; Hsieh, H.T.; Chen, C.C.; Kuo, Y.C.; Chen, C.F. Characterization of the anti-platelet effects of (2-S)-5-methoxy-6-methylflavan-7-ol from *Draconis Resina*. *Eur. J. Pharmacol.* **1998**, *346* (1), 103-110.

[48] Hung, C.C.; Tsai, W.J.; Kuo, L.M.Y.; Kuo, Y.H. Evaluation of caffeic acid amide analogues as anti-platelet aggregation and anti-oxidative agents. *Bioorg. Med. Chem.* **2005**, *13*, 1791-1797.

[49] Wang, X.; Stavchansky, S.; Bowman, P.D.; Kerwin, S.M. Cytoprotective effect of caffeic acid phenylethyl ester (CAPE) and catechol ring fluorinated CAPE derivatives against menadione-induced oxidative stress in human endothelial cells. *Bioorg. Med. Chem.* **2006**, *14*, 4879-4887.

[50] Wang, X.; Bowman, P.D.; Kerwin, S.M.; Stavchansky, S. Stability of caffeic acid phenethyl ester and its fluorinated derivative in rat plasma. *Biomed. Chromatogr.* **2007**, *21*, 343-350.

[51] Silva, F.A.M.; Borges, F.; Guimaraes, C.; Lima, J.L.F.C.; Matos, C.; Reis, S. Phenolic acids and derivatives : Studies on the relationship among structure, radical scavenging activity and physico-chemical properties. *J. Agric. Food Chem.* **2000**, *48*, 2122-2126.

[52] Antolovich, M.; Bedgood Jr, D.R.; Bishop, A.G.; Jardine, D.; Prenzler, P.D.; Robards, K. LC-MS investigation of oxidation products of phenolic antioxidants. *J. Agric. Food Chem.* **2004**, *52*, 962-971.

[53] Gaspar, A.; Garrido, E.M.; Esteves, M.; Quezada, E.; Milhazes, N.; Garrido, J.; Borges, F. New insights into the antioxidant activity of hydrocinnamic acids : Synthesis and physicochemical characterization of novel halogenated derivatives. *Europ. J. Med. Chem.* **2009**, *44*, 2092-2099.

[54] Zhang, Z.; Xiao, B.; Chen, Q.; Lian, X.Y. Synthesis and biological evaluation of caffeic acid 3, 4-dihydroxyphenethyl ester. *J. Nat. Prod.* **2010**, *73*, 252-254.

[55] Jayaprakasam, B.; Vanisree, M.; Zhang, Y.; Dewitt, D.L.; Nair, M.G. Impact of alkyl esters of caffeic acid and ferulic acids on tumor cell proliferation, cyclooxygenase enzyme and lipid peroxidation. *J. Agric. Food Chem.* **2006**, *54*, 5375-5381.

[56] Dinkova-Kostova, A.T.; Abeygunawardana, C.; Talalay, P. Chemoprotective properties of phenylpropenoids, bis(benzylidene) cycloalkanones and related Michael reaction acceptors : correlation of potencies as phase 2 enzyme inducers and radical scavengers. *J. Med. Chem.* **1998**, *41*, 5287-5296.

[57] Tazaki, H.; Taguchi, D.; Hayashida, T.; Nabeta, K. Stable isotope-labeling studies on the oxidative coupling of caffeic acid *via* o-quinone. *Biosci. Biotechnol. Biochem.* **2001**, *65* (12), 2613-2621.

[58] Saito, S.; Kurakane, S.; Seki, M.; Takai, E.; Kasai, T.; Kawabata, J. Radical scavenging activity of dicaffeoyloxycyclohexanes : Contribution of an intramolecular interaction of two caffeoyl residues. *Bioorg. Med. Chem.* **2005**, *13*, 4191-4199.

[59] Kwak, S.Y.; Seo, H.S.; Lee, Y.S. Synergistic antioxidative activities of hydroxycinnamoyl-peptides. *J. Pept. Sci.* **2009**, *15*, 634-641.

[60] Seo, H.S.; Kwak, S.Y.; Le, Y. S. Antioxidative activities of histidine containing caffeic acid-dipeptides. *Bioorg. Med. Chem.Lett.* **2010**, *20*, 4266-4272.

[61] Qiao, L.; Kozoni, V.; Tsioulias, G.J.; Koutsos, M.I.; Hanif, R.; Shiff, S.J.; Rigas, B. Selected eicosanoids increase the proliferation rate of human colon carcinoma cell lines and mouse colonocytes *in vivo*. *Biochim. Biophys. Acta* **1995**, *1258*, 215-223.

[62] Tsujii, M.; DuBois, R. N. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandinendoperoxide synthase 2. *Cell* **1995**, *83*, 493-501.

[63] Goodwin, J. S.; Ceuppens J. Regulation of immune response by prostaglandins. *J. Clin. Immunol.* **1983**, *3*, 295-314.

[64] Bennett, A.; Charlier, E.M.; McDonald, A.M.; Simpson, J. S.; Stamford, I.F.; Zebro, T. Prostaglandins and breast cancer. *Lancet* **1977**, *2*, 624-626.

[65] Rigas, B.; Goldman, I.S.; Levine, L. Altered eicosanoid levels in human colon cancer, *J. Lab. Clin. Med.* **1993**, *122*, 518-523.

[66] Bennett, A.; Carroll, M.A.; Stamford, I.F.; Whimster, W.F.; Williams, F. Prostaglandins and human lung carcinomas. *Br. J. Cancer* **1982**, *46*, 888-893.

[67] Maeda, H.; Akaike, T. Nitric oxide and oxygen radicals in infection, inflammation, and cancer, *Biochemistry* **1998**, *63*, 854-865.

[68] Wink, D.; Vodovotz, Y.; Laval, J.; Laval, F.; Dewhirst, M.W.; Mitchell, J.B. The multifaceted roles of nitric oxide in cancer. *Carcinogenesis* **1998**, *19*, 711–721.

[69] Peters-Golden, M.; Henderson, W.R. Jr Leukotrienes *N. Engl. J. Med.* **2007**, *357* (18), 1841-1854.

[70] Santangelo, C.; Vari, R.; Scazzochio, B.; Di Benedetto, R.; Filesi, C.; Masella, R. Polyphenols, intracellular signaling and inflammation. *Ann. Ist. Super. Sanita* **2007**, *43* (4), 394-405.

[71] Lee, S.J.; Kim, C.E.; Seo, K.W.; Kim, C.D. HNE-induced 5-LO expression is regulated by NF-κB/ERK and Sp1/p38 MAPK pathways *via* EGF receptor in murine macrophages. *Cardiovasc. Res.* **2010**, *88*, 352-359.

[72] Chung, T.W.; Moon, S.K.; Chang, Y.C.; Ko, J.H.; Lee, Y.C.; Cho, G.; Kim, S.H.; Kim, J.G.; Kim, C.H. Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells : complete regression of hepataoma growth and metastasis by dual mechanism. *FASEB J.* **2004**, *18*, 1670-1681.

[73] Lee, K.W.; Kang, N.J.; Kim, J,H.; Lee, K.M.; Lee, D.E.; Hur, H.J.; Lee, H.J. Caffeic acid phenethyl ester inhibits invasion and expression of matrix metalloproteinase in SK-Hep1 human hepatocellular carcinoma cells by targeting nuclaer factor kappa B. *Genes Nutr.* **2008**, *2*, 319-322.

[74] Bose, J.S.; Gangan, V.; Jain, S.K.; Manna, S.K. Downregulation of inflammatory responses by novel caffeic acid ester derivative by inhibiting NF-κB. *J. Clin. Immunol.* **2009**, *29*, 90-98.

[75] Bose, J.S.; Gangan, V.; Jain, A.K.; Manna, S.K. Novel caffeic acid ester derivative induces apoptosis by expressing FasL and down-regulating NF-κB :Potentiation of cell death mediated by chemotherapeutic agents. *J. Cell Physiol.* **2009**, *218*, 653-662.

[76] Lechner, M.; Lirk, P.; Rieder, J. Inducible nitric oxide synthase (iNOS) in tumor biology : the two sides of the same coin. *Sem. Cancer Biol.* **2005**, *15* (4), 277-289.

[77] Clancy, R.M.; Amin, A.R.; Abramson, S.B. the role of nitric oxide in inflammation and immunity. *Arthritis Rheum.* **1998**, *41* (7), 1141-1151.

[78] Wink, D.A.; Hanbauer, I.; Grisham, M.B.; Laval, F; Nims, R.W.; Laval, J. Chemical biology of nitric oxide: regulation and protective and toxic mechanisms. *Curr. Top. Cell. Regul.* **1996**, *34*, 159-187.

[79] Stamler. J.S. Redox signalling: nitrosylation and related target interactions of nitric oxide. *Cell* **1994**, *78*, 931-936.

[80] Nagaoka, T.; Banskota, A.H.; Tezuka, Y.; Midorikawa, K.; Matushige, K.; Kadota, S. Caffeic acid phenyl ethyl (CAPE) ester : potent nitric oxide inhibitors from the Netherlands propolis. *Biol. Pharm. Bull.* **2003**, *26*, 487-491.

[81] Uwai, K.; Osanai, Y.; Imaizumi, T.; Kanno, S; Takeshita, M.; Ishikawa, M. Inhibitory effect of the alkyl side chain of caffeic acids analogues on lipopolysaccharide-induced nitric oxide production un RAW264.7 macrophages. *Bioorg. Med. Chem.* **2008**, *16*, 7795-7803.

[82] Hsiao, G.; Shen, M.Y.; Chang, W.C.; Cheng, Y.W.; Pan, A.L.; Kuo, Y.S.; Chen, T.F.; Sheu, J.R. A novel antioxidant, octyl caffeate, suppression of LPS/IFN-γ-induced inducible nitric oxide synthase gene expression in rat aortic smooth muscle cells. *Biochem. Pharmacol.* **2003**, *65*, 1383-1392.

[83] De Lucia, M.; Panzella, L.; Crescenzi, O.; Napolitano, A.; Barone, V.; d'Ischia, M. The catecholic antioxidant piceatannol is an effective nitrosation inhibitor *via* an unusual double bond nitration. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2238-2242.

[84] De Lucia, M.; Panzella, M.; Pezzella, A.; Napolitano, A.; d'Ischia, M. Plant catechols and theirs glutathionyl conjugates as anti-nitrosating agents : Expedient synthesis and remarkable potency of 5-S-glutathionylpiceatannol. *Chem. Res. Toxicol.* **2008**, *21*, 2407-2413.

[85] Spanbroek, R.; Habenicht, A.J. The Potential role of antileukotriene drugs in atherosclerosis, *Drug News Perspect*., **2003**, *16*, 485-489.

[86] Helgadottir, A.; Manolescu, A.; Thorleifsson, G.; Gretarsdottir, S.; Jonsdottir, H.; Thorsteinsdottir, U.; Samani, N.J.; Gudmundsson, G.; Grant, S.F.; Thorgeirsson, G.; Sveinbjornsdottir, S.; Valdimarsson, E.M.; Matthiasson, S.E.; Johannsson, H.; Gudmundsdottir, O.; Gurney, M.E.; Sainz, J.; Thorhallsdottir, M.; Andresdottir, M.; Frigge, M.L.; Topol, E.J.; Kong, A.; Gudnason, V.; Hakonarson, H.; Gulcher, J.R.; Stefansson, K. The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. *Nat. Genet*. **2004**, *36*, 233-239.

[87] Maycock, A.L.; Pong, S.S.; Evans, J.F.; Miller, D.K. Biochemistry of the lipoxygenase pathways. In Leukotrienes and Lipoxygenases; Rokach, J., Ed.; Elsevier Science: New York, **1989**; pp 143-208.

[88] Pergola, C.; Werz, O. 5-Lipoxygenase inhibitors: a review of recent developments and patents. *Expert Opin. Therap. Pat*., **2010**, *20* (3), 355-375.

[89] Mirzoeva, O.K.; Calder, P.C.; The effect of propolis and its components on eicosanoid production during the inflammatory response. *Prostaglandins Leukot. Essent. Fatty Acids,* **1996,** *55* (6), 441-449.

[90] Sud'ina, G.F.; Mirzoeva, O.K.; Pushkareva, M.A.; Korshunova, G.A.; Sumbatyan, N.V.; Varfolomeev, S.D. Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. *FEBS Lett.,* **1993***, 329*, 21-24.

[91] Nordberg, J.; Arner, E.S. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic. Biol. Med.*, **2001**, *31*, 1287-1312.

[92] Schewe T. Molecular actions of ebselen--an antiinflammatory antioxidant. *Gen. Pharmacol.*, **1995,** *26*, 1153-1161.

[93] Lin, C.F.; Chang, T.C.; Chiang, C.C.; Tsai, H.J.; Hsu, L.Y. Synthesis of Selenium-Containing Polyphenolic Acid Esters and Evaluation of Their Effects on Antioxidation and 5-Lipoxygenase Inhibition. *Chem. Pharm. Bull.,* **2005**, *53* (11) 1402-1407.

[94] Doiron, J.; Boudreau, L.H.; Picot, N.; Villebonet, B.; Surette, M.E.; Touaibia, M. Synthesis and 5-lipoxygenase inhibitory activity of new cinnamoyl and caffeoylclusters. *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 1118–1121.

[95] Mammen, M.; Choi, S.-K.; Whitesides, G.M. Polyvalent interactions in biological systems: implications for design and use of multivalent ligands and inhibitors. *Angew. Chem. Int. Ed*., **1998**, *37* (20), 2755-2794.

[96] Carter, G.W.; Young, P.R.; Albert, D.H.; Bouska, J.; Dyer, R.; Bell, R.L.; Summers, J.B.; Brooks, D.W. 5-Lipoxygenase inhibitory activity of zileuton. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 929-937.

[97] *Note*: a library of ~50 caffeic acid derivaitives revealed several good inhibitors Touaibia *et al*., manuscript in preparation.

[98] De Clercq, E. Toward improved anti-HIV chemotherapy: Therapeutic strategies for intervention with HIV infections. *J. Med. Chem.,* **1995**, *38*, 2491-2517.

[99] LaFemina, R.L.; Schneider, C.L.; Robbins, H.L.; Callahan, P.L.; LeGrow, K.; Roth, E.; Schleif, W.A.; Emini, E.A. Requirement of active human immunodeficiency virus type 1 integrase for productive infection of human T-lymphoid cells. *J. Virol.*, **1992**, *66(12)*, 7414-7419.

[100] Sakai, H.; Kawamura, M.; Sakuragi, J.; Sakuragi, S.; Shibata, R.; Ishimoto, A.; Ono, H.; Ueda, S.; Adachi, A. Integration is essential for efficient gene expression of human immunodeficiency virus type 1. *J. Virol.*, **1993**, *7*, 1169-1174.

[101] Taddeo, B.; Haseltine, W.A.; Farnet, C.M. Integrase mutants of human immunodeficiency virus type 1 with a specific defect in integration. *J. Virol.,* **1994**, *68*, 8401-8405.

[102] Engelman, A.; Englund, G.; Orenstein, J.M.; Martin, M.A.; Craigie, R. Multiple effects of mutations in human immunodeficiency virus type 1 integrase on viral replication. *J. Virol.,* **1995**, *69*, 2729-2736.

[103] Raffanti, S.; Hass, D.W. In *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*; Hardman, Limbird, Gilman, Eds.; 10th ed. McGraw-Hill, NewYork, **2001**, pp. 1351-1380.

[104] Burke, T.R.Jr.; Fesen, M.R.; Mazumder, A.; Wang, J.; Carothers, A.M.; Grunberger, D.; Driscoll, J.; Kahn, K.; Pommier, Y. Hydroxylated aromatic inhibitors of HIV-1 integrase. *J. Med. Chem., **1995**, 38,* 4171-4178.

[105] Ho, C.C.; Lun, S.S.; Chou, M.Y.; Chen, F.L.; Hu, C.C.; Chen, C.S.; Lu, G.Y, Yang, C.C. Effects of CAPE-like compounds on HIV replication *in vitro* and modulation of cytokines *in vivo*. *J. Antimicrob. Chemother.*, **2005**, *56*, 372-379.

[106] Zhang, X.; Neamati, N.; Lee, Y.K.; Orr, A.; Brown, R.D.; Whitaker, N.; Pommier, Y.; Burke, T.R.Jr. Arylisothiocyanate-Containing Esters of Caffeic Acid Designed as Affinity Ligands for HIV-1 Integrase. *Bioorg. Med. Chem.*, **2001**, *9*, 1649-1657

[107] Mahmood, N.; Moore, P.S.; De Tommasi, N.; De Simone, F.; Hay, A.J.; Pizza, C. Inhibition of HIV infection by caffeolyquinic acid derivatives. *Antiviral Chem. Chemother.,* **1993**, *4*, 235-240.

[108] Robinson, W.E.; Reinecke, M.G.; AbdelMalek, S.; Jia, Q.; Chow, S. A. Inhibitors of HIV-1 replication that inhibit HIV integrase. *Proc. Natl. Acad. Sci. U.S.A.,* **1996**, *93*, 6326-6331.

[109] Neamati, N.; Hong, H.X.; Sunder, S.; Milne, G.W.A.; Pommier, Y. Potent inhibitors of human immunodeficiency virus type 1 integrase: Identification of a novel four-point pharmacophore and tetracyclines as novel inhibitors. *Mol. Pharmacol.,* **1997**, *52*, 1041-1055.

[110] Neamati, N.; Hong, H.X.; Mazumder, A.; Wang, S.M.; Sunder, S.; Nicklaus, M.C.; Milne, G.W.A.; Proksa, B.; Pommier, Y. Depsides and depsidones as inhibitors of HIV-1 integrase: Discovery of novel inhibitors through 3D database searching. *J. Med. Chem.,* **1997**, *40*, 942-951.

[111] McDougall, B.; King, P.J.; Wu, B.W.; Hostomsky, Z.; Reinecke, M.G.; Robinson, W.E. Dicaffeoylquinic and dicaffeoyltartaric acids are selective inhibitors of human immunodeficiency virus type 1 integrase. *Antimicrob. Agents Chemother.,* **1998**, *42*, 140-146.

[112] Robinson, W.E.; Cordeiro, M.; AbdelMalek, S.; Jia, Q.; Chow, S.A.; Reinecke, M.G.; Mitchell, W.M. Dicaffeoylquinic acid inhibitors of human immunodeficiency virus integrase: Inhibition of the core catalytic domain of human immunodeficiency virus integrase. *Mol. Pharmacol.,* **1996**, *50*, 846-855.

[113] Lin, Z.; Neamati, N.; Zhao, H.; Kiryu, Y.; Turpin, J.A.; Aberham, C.; Strebel, K.; Kohn, K.; Witvrouw, M.; Pannecouque, C.; Debyser, Z.; De Clercq, E; Rice, W.G.; Pommier, Y.; Burke, T.R.Jr. Chicoric Acid Analogues as HIV-1 Integrase Inhibitors. *J. Med. Chem.,* **1999,** *42,* 1401-1414.

[114] King, P.J.; Ma, G.; Miao, W.; Jia, Q.; McDougall, B.R.; Reinecke, M.G.; Cornell, C.; Kuan, J.; Kim, T.R.; Robinson, W.E.Jr. Structure-Activity Relationships: Analogues of the Dicaffeoylquinic and Dicaffeoyltartaric Acids as Potent Inhibitors of Human Immunodeficiency Virus Type 1 Integrase and Replication. *J. Med. Chem.,* **1999,** *42,* 497-509.

[115] Reinke, R.A.; King, P.J.; Victoria, J.G.; McDougall, B.R.; Ma, G.; Mao, Y.; Reinecke, M.G.; Robinson, W.E.Jr. Dicaffeoyltartaric Acid Analogues Inhibit Human Immunodeficiency Virus Type 1 (HIV-1) Integrase and HIV-1 Replication at Nontoxic Concentrations. *J. Med. Chem.,* **2002**, *45,* 3669-3683.

[116] Hwang, D.J.; Kim, S.N.; Choib, J.H.; Leea, Y.S. Dicaffeoyl- or Digalloyl Pyrrolidine and Furan Derivatives as HIV Integrase Inhibitors. *Bioorg. Med. Che.*, **2001**, *9*, 1429-1437.

[117] Lee, S.U.; Shin, C.G.; Lee, C.K.; Lee, Y.S. Caffeoylglycolic and caffeoylamino acid derivatives, halfmers of L-chicoric acid, as new HIV-1 integrase inhibitors. *Eur. J. Med. Chem.,* **2007***, 42*, 1309-1315.

[118] Xu, Y.W.; Zhao, G.S.; Shin, C.G., Zang, H.C.; Leec, C.K.; Leed, Y.S. Caffeoyl Naphthalenesulfonamide Derivatives as HIV Integrase Inhibitors. *Bioorg. Med. Chem.*, **2003**, *11*, 3589-3593.

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