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Design and Synthesis of Novel Aspirin-Caffeic Acid Ester Hybrids for Cardioprotection with Reduced Risk of Hemorrhagic Stroke

ZHI-HAO SHI[1,2], NIAN-GUANG LI[1,*], YU-PING TANG[1,*], QIAN-PING SHI[1], WEI ZHANG[1], PENG-XUAN ZHANG[1], ZE-XI DONG[1], WEI LI[1] and JIN-AO DUAN[1]

[1]National and Local Collaborative Engeering Center of Chinese Medicinal Resources Industrialization and Formulae Innovative Medicine, Nanjing University of Chinese Medicine, Nanjing, P.R. China
[2]Department of Organic Chemistry, China Pharmaceutical University, Nanjing, P.R. China

*Corresponding authors: Tel/Fax: +86 25 85811916; E-mail: linianguang@njutcm.edu.cn; yupingtang@njutcm.edu.cn

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A series of novel aspirin-caffeic acid ester hybrids for cardioprotection with reduced risk of hemorrhagic stroke were designed and synthesized by coupling aspirin and caffeic acid esters inspired by NCX-4016. The synthesized compounds were evaluated for their *in vitro* antiplatelet aggregations induced by ADP and antioxidant activity of the caffeic acid esters which could be released from the hybrids by esterases *in vivo* like NCX 4016 were determined by DPPH assay. The results showed that compound **3d** exhibited potent antiplatelet activity than aspirin and its intermediate caffeic acid isopropyl ester (**2d**) showed good antioxidant activity and thus could be considered to be novel potent cardioprotectic drug candidates with reduced risk of hemorrhagic stroke.

Keywords: Hemorrhagic stroke, Aspirin, Caffeic acid, Synthesis.

INTRODUCTION

Aspirin, which has been known as an antipyretic and analgesic for 100 years, has undergone a resurgence of popularity since it became appreciated in the 1980s as the most cost-effective agent for the secondary prevention of coronary artery disease[1]. Aspirin is also the basic antiplatelet agent for all kinds of acute disease that may cause platelet-dependent thrombotic vessel occlusion[2]. It is recommended as the first-line antiplatelet drug. Aspirin imparts its primary antithrombotic effects through the inhibition of PGH-synthase/COX by the irreversible acetylation of a specific serine moiety[3].

Unfortunately, its long-term administration is accompanied by an increased risk of gastrointestinal ulceration with consequent gastrointestinal bleeding[4]. This side effect is believed to result from the blockade of COX-1. COX-1 is constitutively expressed and has clear physiological functions in many tissues including the gastric mucosa, where it generates cytoprotective meditors such as prostacyclin[5]. So new COX-2 selective inhibitors have been synthesized[6], the consequence of this effort has been the development of compounds such as rofecoxib and celecoxib that are now widely prescribed. However the initial expectation that such new generation of NSAIDs would lack gastrointestinal side effects has been challenged by reports showing that these side effects also occur[7]. A promising alternative solution to minimize the side effects of aspirin on gastric mucosa without compromising its antithrombotic efficacy is a newly designed category of NO-releasing aspirin. NCX-4016 molecule (Fig. 1) is actually consisted of an aspirin group, a spacer joint by an ester linkage and an NO-releasing group[8]. When administered *in vivo*, esterases separate the aspirin moiety from the benzene spacer-NO complex, leading to the release of NO from this complex. Interestingly, NO possesses some properties of prostaglandins within the gastric mucosa, therefore leading to cytoprotection, possibly through the increase of mucosal blood flow and mucous fluid secretion by the gastric epithelial cells.

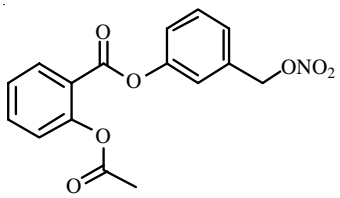


Fig. 1. Structure of NCX 4016

Recently, a meta-analysis included 287 randomized trials that together enrolled 212,000 high-risk patients by the Antithrombotic Trialists' Collaboration[1] indicated that antiplatelet

therapy by aspirin reduced the risk of serious vascular events by 25 % and the risk of non-fatal myocardial infarction (MI) alone by 34 %. However, there was a proportional increase in fatal or nonfatal hemorrhagic stroke of 22 %.

Atherosclerosis played a vital role in stroke, among major risk factors for the development of atherosclerosis are increased levels of low-density lipoprotein (LDL), oxidative modification of low-density lipoprotein and an impairment of endothelial derived relaxing factor (EDRF, nitric oxide, NO)-mediated bioactions[9,10].

The accumulation of low-density lipoprotein and reactive oxygen species (ROS) in the sub-endothelial space induces a high degree of low-density lipoprotein oxidation. According to the oxidative hypothesis of atherosclerosis, this is an early event in a complex process that leads to the formation of foam cells that constitute a fatty streak, a forerunner to the development of mature atherosclerotic plaques[11]. An increasing number of studies seem to indicate that the administration of exogenous antioxidants might decrease the impact of atherosclerosis in animals and humans through the regulation and protection of several aspects of endothelial function[12-14].

On these bases and inspired by the invention of NCX-4016, here we designed and synthesized a novel series of hybrid structures in which some selected antioxidants were linked to aspirin moieties through appropriate spacers. As antioxidants, we chose caffeic acid (3,4-dihydroxycinnamic acid), which is one of the most common phenolic acids and occurs in fruits[15], grains[16] and dietary supplements[17] for human consumption as simple ester with quinic acid or saccharides. The intake of caffeic acid from foods, mainly from tomatoes and potatoes, was estimated to be about 0.2 mg/kg body weight per day. It has been reported that caffeic acid possesses several pharmacological properties like antioxidant[18], free radical scavenging[19] and lipoxygenase inhibitor[20]. However, caffeic acid has only limited solubility in hydrophobic media, which reduces its antioxidant effects in inhibiting autooxidation of fats and oils[21]. The strategy of esterification of hydrophilic caffeic acid with lipophilic molecules, such as aliphatic alcohols, could be employed to alter its solubility in a hydrophobic medium. So in order to improve the solubility of the target compounds in ester, we synthesized a series of caffeic acid esters before they are combined with aspirin. As a result, the aspirin-caffeic acid ester hybrids **3a-h** were synthesized and their activities were evaluated.

EXPERIMENTAL

Reagents and solvents were purchased from commercial sources and used without further purification unless otherwise specified. Air- and moisture-sensitive liquids and solutions were transferred *via* syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation below 45 °C at approximately 20 mm Hg. All non-aqueous reactions were carried out under anhydrous conditions using flame-dried glassware within an argon atmosphere in dry, freshly distilled solvents, unless otherwise noted. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.15-0.20 mm Yantai silica gel plates (RSGF 254) using UV light as the visualizing agent. Chromatography was performed on Qingdao silica gel (160-200 mesh) using petroleum ether (60-90) and ethyl acetate as the eluating solvent. ^{1}H NMR spectra were obtained using a Bruker AV-300 (300 MHz). Chemical shifts were recorded in ppm downfield from tetramethylsilane. ESI-MS spectra were recorded on a Waters Synapt HDMS spectrometer.

General procedures for the esterification of caffeic acid through microwave irradiation: To a stirring mixture of caffeic acid (940 mg, 5 mmol) in alcohol (10 mL) was added the concentrated sulfuric acid (0.067 mL, 1.25 mmol) and the reaction mixture was refluxed for 3-6 min in a sealed reaction vessel of Discover (CEM, USA) under microwave irradiation, where the power was set at 200 W, the temperature was set at some centigrade which was 20 above the boiling point of the alcohol and the PSI was set at 180. After cooling to 25 °C, ethyl acetate was added and washed with water and brine. The ethyl acetate layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluted with petroleum ether-EtOAc (v:v = 8:1) to afford caffeic acid esters.

(*E*)-Methyl-3-(3,4-dihydroxyphenyl)acrylate (2a)[22]: Yield 94 %; IR (KBr, ν_{max}, cm^{-1}): 3473, 3098, 1676, 1443, 1279, 583; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.69 (s, 3H, OCH_3), 6.27 (d, *J* = 15.9 Hz, 1H, C=CH), 6.76 (d, *J* = 8.1 Hz, 1H, Ar-H), 6.98-7.05 (m, 2H, 2Ar-H), 7.49 (d, *J* = 15.9 Hz, 1H, CH=C), 9.13 (s, 1H, OH), 9.57 (s, 1H, OH); ESI-MS: *m/z* 195 $[M + H]^+$.

(*E*)-Ethyl-3-(3,4-dihydroxyphenyl)acrylate (2b)[22]: Yield 93%; IR (KBr, ν_{max}, cm^{-1}): 3453, 3088, 1855, 1668, 1607, 1531; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.26 (s, 3H, CH_3), 4.15 (q, *J* = 7.1 Hz, 2H, $COOCH_2$), 6.25 (d, *J* = 15.9 Hz, 1H, C=CH), 6.76 (d, *J* = 8.2 Hz, 1H, Ar-H), 6.98-7.04 (m, 2H, 2Ar-H), 7.47 (d, *J* = 15.9 Hz, 1H, CH=C), 9.31 (s, 2H, 2OH); ESI-MS: *m/z* 209 $[M + H]^+$.

(*E*)-Propyl-3-(3,4-dihydroxyphenyl)acrylate (2c)[22]: Yield 93 %; IR (KBr, ν_{max}, cm^{-1}): 3457, 2968, 1666, 1607, 1530, 1446; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.92 (t, *J* = 7.4 Hz, 3H, CH_3), 1.64 (q, *J* = 7.1 Hz, 2H, CH_2), 4.01 (t, *J* = 6.6 Hz, 2H, $COOCH_2$), 6.26 (d, *J* = 15.9 Hz, 1H, C=CH), 6.76 (d, *J* = 8 Hz, 1H, Ar-H), 6.99-7.04 (m, 2H, Ar-H), 7.47 (d, *J* = 15.9 Hz, 1H, CH=C), 9.11 (s, 1H, OH), 9.56 (s, 1H, OH); ESI-MS: *m/z* 223 $[M + H]^+$.

(*E*)-Isopropyl-3-(3,4-dihydroxyphenyl)acrylate (2d)[23]: Yield 92 %; IR (KBr, ν_{max}, cm^{-1}): 3464, 3312, 2976, 1682, 1599, 1530, 1445; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.23 (m, 6H, $2CH_3$), 4.99 (m, 1H, COOCH), 6.23 (d, *J* = 15.9 Hz, 1H, C=CH), 6.76 (d, *J* = 8 Hz, 1H, Ar-H), 6.98-7.04 (m, 2H, Ar-H), 7.45 (d, *J* = 15.9 Hz, 1H, CH=C), 9.11 (s, 1H, OH), 9.56 (s, 1H, OH); ESI-MS: *m/z* 223 $[M + H]^+$.

(*E*)-Butyl-3-(3,4-dihydroxyphenyl)acrylate (2e)[22]: Yield 93 %; IR (KBr, ν_{max}, cm^{-1}): 3484, 3340, 2953, 1601, 1533; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.91 (t, *J* = 7.3 Hz, 3H, CH_3), 1.36 (m, 2H, CH_2), 1.59 (m, 2H, CH_2), 4.11 (t, *J* = 6.6 Hz, 2H, $COOCH_2$), 6.26 (d, *J* = 15.9 Hz, 1H, C=CH), 6.76 (d, *J* = 8 Hz, 1H, Ar-H), 6.99-7.04 (m, 2H, Ar-H), 7.46 (d, *J* = 15.9 Hz, 1H, CH=C), 9.13 (s, 1H, OH), 9.59 (s, 1H, OH); ESI-MS: *m/z* 237 $[M + H]^+$.

(*E*)-Isobutyl-3-(3,4-dihydroxyphenyl)acrylate (2f)[23]: Yield 91 %; IR (KBr, ν_{max}, cm^{-1}): 3724, 3087, 1666, 1606, 1530, 1445; ^1H NMR (DMSO-d_6, 300 MHz) δ: 0.92 (d, *J* = 6.8 Hz, 6H, $2CH_3$), 1.93 (m, 1H, CH), 3.90 (d, *J* = 6.6 Hz, 2H, $COOCH_2$), 6.27 (d, *J* = 15.9 Hz, 1H, C=CH), 6.76 (d, J = 8 Hz, 1H, Ar-H), 7-7.05 (m, 2H, Ar-H), 7.48 (d, *J* = 15.9 Hz, 1H, CH=C), 9.12 (s, 1H, OH), 9.58 (s, 1H, OH); ESI-MS: *m/z* 237 $[M + H]^+$.

(*E*)-Pentyl-3-(3,4-dihydroxyphenyl)acrylate (2g)[24]: Yield 91 %; IR (KBr, ν_{max}, cm^{-1}): 3342, 2861, 1684, 1599, 1532, 1445; ^1H NMR (DMSO-d_6, 300 MHz) δ: 0.88 (m, t, *J* = 7.1 Hz, 3H, CH_3), 1.33 (m, 4H, $2CH_2$), 1.61 (m, 2H, CH_2), 4.10 (t, *J* = 6.6 Hz, 2H, $COOCH_2$), 6.25 (d, *J* = 15.9 Hz, 1H, C=CH), 6.75 (d, *J* = 8 Hz, 1H, Ar-H), 6.99-7.04 (m, 2H, Ar-H), 7.46 (d, *J* = 15.9 Hz, 1H, CH=C), 9.11 (s, 1H, OH), 9.58 (s, 1H, OH); ESI-MS: *m/z* 251 $[M + H]^+$.

(*E*)-Isopentyl-3-(3,4-dihydroxyphenyl)acrylate (2h)[23]: Yield 90 %; IR (KBr, ν_{max}, cm^{-1}): 3483, 2955, 1684, 1601, 1532, 1447; ^1H NMR (DMSO-d_6, 300 MHz) δ: 0.90 (m, 6H, $2CH_3$), 1.52 (m, 2H, CH_2), 1.68 (m, 1H, CH), 4.14 (t, *J* = 6.8 Hz, 2H, $COOCH_2$), 6.25 (d, *J* = 15.9 Hz, 1H, C=CH), 6.75 (d, *J* = 8 Hz, 1H, Ar-H), 6.99-7.04 (m, 2H, Ar-H), 7.46 (d, *J* = 15.9 Hz, 1H, CH=C), 9.12 (s, 1H, OH), 9.58 (s, 1H, OH); ESI-MS: *m/z* 251 $[M + H]^+$.

General procedures for the condensation of compound 2 with aspirin: To a stirring solution of aspirin (540 mg, 3 mmol), N,N-dicyclohexylcarbodiimide (DCC) (618 mg, 3 mmol) and N,N-(dimethylamino)pyridine (DMAP) (73.2 mg, 0.6 mmol) in dry CH_2Cl_2 (50 mL) at room temperature was added compound **2** (1 mmol), then the reaction mixture was stirred at room temperature for 4-10 h, at last the reaction mixture was filtered. Removal of the solvent under *vacuo* followed by silica gel column chromatographic purification of the residue using petroleum ether-EtOAc (v:v = 8:1) afforded the compounds (yields 57-73 %).

(*E*)-Methyl-3-[3,4-*bis*(2-acetoxybenzoyloxy)phenyl]-acrylate (3a): Yield 73 %; IR (KBr, ν_{max}, cm^{-1}): 3433, 3322, 2959, 2850, 2354, 1756; ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.39 (d, 6H, $2COCH_3$), 3.93 (s, 3H, $COOCH_3$), 6.55 (d, *J* = 15.9 Hz, 1H, C=CH), 7.20-7.68 (m, 11H, Ar-H), 7.80 (d, *J* = 15.9 Hz, 1H, CH=C), 8.18 (m, 2H, 2OH); ESI-MS: *m/z* 519 $[M + H]^+$.

(*E*)-Ethyl-3-[3,4-*bis*(2-acetoxybenzoyloxy)phenyl]-acrylate (3b): Yield 70 %; IR (KBr, ν_{max}, cm^{-1}): 3547, 3328, 1756, 1634; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.33 (t, *J* = 7.1 Hz, 3H, CH_3), 2.28 (s, 6H, $2COCH_3$), 4.27 (q, *J* = 7.1 Hz, 2H, $COOCH_2$), 6.43 (d, *J* = 15.9 Hz, 1H, C=CH), 7.08-7.58 (m, 11H, Ar-H), 7.68 (d, *J* = 15.9 Hz, 1H, CH=C), 8.08 (m, 2H, OH); ESI-MS: *m/z* 533 $[M + H]^+$.

(*E*)-Propyl-3-[3,4-*bis*(2-acetoxybenzoyloxy)phenyl]-acrylate (3c): Yield 70 %; IR (KBr, ν_{max}, cm^{-1}): 2965, 2357, 1757, 1605, 1495; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.99 (t, *J* = 7.5 Hz, 3H, CH_3), 1.74 (m, 2H, CH_2), 2.28 (s, 6H, $2COCH_3$), 4.16 (t, *J* = 6.8 Hz, 2H, $COOCH_2$), 6.44 (d, *J* = 15.9 Hz, 1H, C=CH), 7.08-7.58 (m, 11H, Ar-H), 7.68 (d, *J* = 15.9 Hz, 1H, CH=C), 8.04 (m, 2H, OH); ESI-MS: *m/z* 547 $[M + H]^+$.

(*E*)-Isopropyl-3-[3,4-*bis*(2-acetoxybenzoyloxy)phenyl]-acrylate (3d): Yield 57 %; IR (KBr, ν_{max}, cm^{-1}): 3510, 3326, 3074, 2982, 2933; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.31 (d, *J* = 6.2 Hz, 6H, $2CH_3$), 2.28 (s, 6H, $2COCH_3$), 5.14 (m, 1H, COOCH), 6.42 (d, *J* = 15.9 Hz, 1H, C=CH), 7.08-7.58 (m, 11H, Ar-H), 7.66 (d, *J* = 15.9 Hz, 1H, CH=C), 8.08 (m, 2H, OH); ESI-MS: *m/z* 547 $[M + H]^+$.

(*E*)-Butyl-3-[3,4-*bis*(2-acetoxybenzoyloxy)phenyl]-acrylate (3e): Yield 61 %; IR (KBr, ν_{max}, cm^{-1}): 3492, 3064, 2958, 2357, 1755, 1700; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.97 (t, *J* = 7.3 Hz, 3H, CH_3), 1.44 (m, 2H, CH_2), 1.70 (m, 2H, CH_2), 2.28 (s, 6H, $COCH_3$), 4.22 (t, *J* = 6.6 Hz, 2H, $COOCH_2$), 6.43 (d, *J* = 15.9 Hz, 1H, C=CH), 7.09-7.60 (m, 11H, Ar-H), 7.67 (d, *J* = 15.9 Hz, 1H, CH=C), 8.07 (m, 2H, OH); ESI-MS: *m/z* 561 $[M + H]^+$.

(*E*)-Isobutyl-3-[3,4-*bis*(2-acetoxybenzoyloxy)phenyl]-acrylate (3f): Yield 65 %; IR (KBr, ν_{max}, cm^{-1}): 3493, 3424, 3075, 2962, 2610, 2357; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.98 (d, *J* = 6.6 Hz, 6H, $2CH_3$), 2.04 (m, 1H, CH), 2.28 (s, 6H, $COCH_3$), 4.01 (d, *J* = 6.8 Hz, 2H, $COOCH_2$), 6.46 (d, *J* = 15.9 Hz, 1H, C=CH), 7.08-7.60 (m, 11H, Ar-H), 7.68 (d, *J* = 15.9 Hz, 1H, CH=C), 8.05 (m, 2H, OH); ESI-MS: *m/z* 561 $[M + H]^+$.

(*E*)-Pentyl-3-[3,4-*bis*(2-acetoxybenzoyloxy)phenyl]-acrylate (3g): Yield 70 %; IR (KBr, ν_{max}, cm^{-1}): 2952, 2357, 1757, 1607, 1492, 1371; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.93 (t, *J* = 7 Hz, 3H, CH_3), 1.37-1.41 (m, 4H, 2CH2), 1.72 (m, 2H, CH2), 2.29 (s, 6H, $2COCH_3$), 4.21 (t, *J* = 6.7 Hz, 2H, $COOCH_2$), 6.44 (d, *J* = 15.9 Hz, 1H, C=CH), 7.09-7.60 (m, 11H, Ar-H), 7.68 (d, *J* = 15.9 Hz, 1H, CH=C), 8.09 (m, 2H, OH); ESI-MS: *m/z* 575 $[M + H]^+$.

(*E*)-Isopentyl-3-[3,4-*bis*(2-acetoxybenzoyloxy)phenyl]-acrylate (3h): Yield 66 %; IR (KBr, ν_{max}, cm^{-1}): 3525, 2959, 2357, 1757, 1639, 1606, 1496, 1371; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.97 (m, 6H, $2CH_3$), 1.60 (m, 2H, CH_2), 1.76 (m, 1H, CH), 2.28 (s, 6H, $2COCH_3$), 4.25 (d, *J* = 6.8 Hz, 2H, $COOCH_2$), 6.44 (d, *J* = 15.9 Hz, 1H, C=CH), 7.09-7.58 (m, 11H, Ar-H), 7.67 (d, *J* = 15.9 Hz, 1H, CH=C), 8.08 (m, 2H, OH); ESI-MS: *m/z* 575 $[M + H]^+$.

Determination of antiplatelet aggregations activity: New Zealand male Rabbits were housed in a conventional animal facility and were allowed to acclimate for 7 days under normal conditions (21-24 °C temperature and 60-70 % relative humidity) before experimentation. All the protocols for the animal study were approved by the Internal Animal Ethics Committee of SK Chemical and Ethics Committee of Animal Service Center at Nanjing University of Chinese Medicine. Blood was withdrawn from carotid artery of New Zealand rabbits, which was local anaesthetized by pentobarbital sodium (30 mg/kg) and it was drawn into a plastic syringe containing 3.8 % trisodium citrate solution (1:9 citrate/blood, v/v). Platelet rich plasma (PRP) was prepared by centrifugation at room temperature for 10 min at 800 rpm. Platelet poor plasma (PPP) was obtained from the precipitated fraction of platelet rich plasma by centrifugation at room temperature for 10 min at 3000 rpm. Platelet rich plasma (280 μL) plus different samples 10 μL were incubated at 37 °C for 5 min in the aggregometer. After incubation, the tested tube was turned into the test channel and platelet aggregation was induced by the addition of ADP (Final concentration is 5 μM). The aggregation inhibition rate (AIR) was calculated with the following equation. X(%) = (A-B)/A × 100, where X was platelet aggregation inhibition, A was maximal aggregation of the control, B was maximal aggregation of administration group. The IC_{50} value was calculated by the least-squares method[25].

Determination of antioxidant activity: DPPH radical scavenging activity was determined using the method of Wang *et al.*[26] with minor modifications. The solution of the sample (10 μL) in ethanol was added to 90 μL of a 0.1 mM DPPH radical in ethanol in a 96-well plate. The sample solution refers to the tested compounds and the reference antioxidants at various concentrations, as well as ethanol as a control. The solutions of the tested compounds had concentrations ranging from 3 to 1000 μg/mL, whereas the concentrations of the solutions of the reference compounds varied from 0.1 to 1000 μg/mL. The reaction leading to the scavenging of DPPH radical was complete within 10 min at 25 °C. The absorbance of the mixture was then measured at 517 nm using a microplate reader. The reduction of DPPH radical was expressed as percentage: Scavenged DPPH (%) = $(1-A_{test}/A_{control}) \times 100$, where A_{test} is the absorbance of a sample at a given concentration after 10 min reaction time and $A_{control}$ is the absorbance recorded for 10 μL ethanol. The IC_{50} value is defined as the concentration of sample that causes 50 % loss of the DPPH radical.

RESULTS AND DISCUSSION

Compounds **3a-h** were prepared as shown in **Scheme-I**. The intermediate compound **2a-h** could be synthesized through the method where the acid directly refluxed with alcohols in the presence of various catalysts such as conc. sulfuric acid, hydrogen chloride, boron trifluoride, aluminum chloride, trifluoroacetic anhydride, polyphosphate ester, neodymium oxide, dicyclohexylcarbodiimide, graphite bisulfate, *etc*[27-29]. The disadvantages of using these catalysts, such as long reaction time, low yield, expensive reagents and tedious operation were difficult to avoid. For instance, in 2002, Kadota's group[22] described a classical esterification of caffeic acid in the presence of methanol and *p*-TsOH to afford methyl caffeate and the same procedure was used in the presence of ethanol to afford ethyl caffeate, however, the yields of methyl caffeate and ethyl caffeate were only 40 and 17 %, respectively.

In recent years, microwave-assisted reactions have received a great deal of attention, because reactions under microwave irradiation were in general not only faster compared with the conventional heating reactions, but also potentially more efficient, cleaner and safer[30]. Further improvements have also been reported which can offer enhanced reaction rates, higher yields and greater selectivity to the targeted product under milder reaction conditions[31]. So a highly efficient synthesis of alkyl caffeates under microwave irradiation was adopted and implemented in this paper, the time of these reactions ranged from 3 to 6 min, which was much shorter than the traditional synthetic methods[22] and the alkyl caffeates (**2a-h**) were obtained in higher yields.

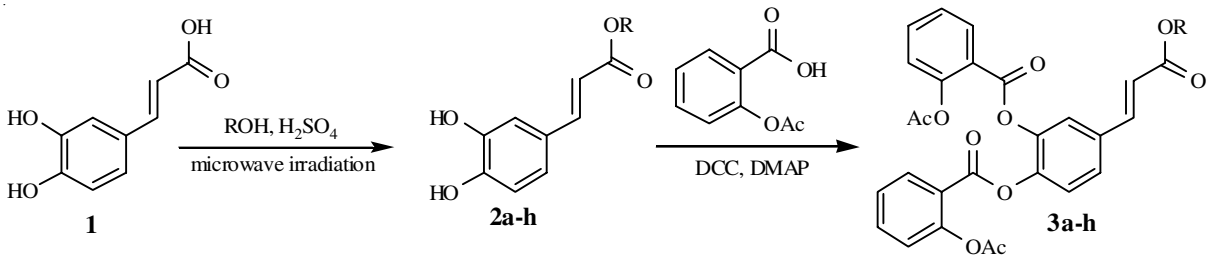
Then compound **2a-h** was condensed with aspirin, respectively in CH_2Cl_2 in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) and a catalytic amount of *N*,*N*-(dimethylamino)pyridine (DMAP) to afford the corresponding target compounds **3a-h**.

Biological activity: The synthesized compounds were evaluated for their antiplatelet aggregations induced by ADP *in vitro*, aspirin was taken as a positive control drug in the assays[25]. Firstly, the results reported in Table-1 showed that for derivatives with same atom numbers, the antiplatelet aggregation activity for caffeic acid esters with branched carbon chains was much stronger than those with linear carbon chains, for instance, the IC_{50} of compound **3e** was 29.2 μM, while the IC_{50} of compound **3f** was 18.3 μM. Secondly, all the target compounds exhibited potent antiplatelet aggregations activity than aspirin, the most potent compound was compound **3d**, which showed about 22 times more potent than aspirin (The IC_{50} for these two compounds were 4.3 mmol/L and 90 mmol/L, respectively).

Furthermore, such as in the case of NCX-4016, when administered *in vivo*, esterases separated the aspirin moiety from the benzene spacer-NO complex, leading to the release of NO from this complex[8]. So in order to pre-evaluate the antioxidant activity of the target hybrids *in vivo*, all the

TABLE-1
in vitro ANTIPLATELET AGGREGATIONS (IC_{50} In μM) INDUCED BY ADP OF TARGET COMPOUNDS AND ANTIOXIDANT ACTIVITY OF INTERMEDIATE CAFFEIC ACID ESTERS IN DPPH ASSAY (IC_{50} In μM)

Compdounds	Antiplatelet	Compounds	Antiplatelet	Compounds	DPPH	Compounds	DPPH
3a	29.1	**3e**	29.2	**2a**	7.16	**2e**	33.78
3b	31.7	**3f**	18.3	**2b**	7.67	**2f**	25.48
3c	33.7	**3g**	46.6	**2c**	25.28	**2g**	14.12
3d	4.3	**3h**	40.4	**2d**	6.98	**2h**	7.4
Aspirin	90.0			**Caffeic acid**	9.3		



R= CH_3, CH_2CH_3, $CH_2CH_2CH_3$, $CH(CH_3)_2$, $(CH_2)_3CH_3$, $CH_2CH(CH_3)_2$, $(CH_2)_4CH_3$, $CH_2CH_2CH(CH_3)_2$

Scheme-I: Synthetic route of aspirin-caffeic acid ester hybrids (**3a-h**)

intermediate caffeic acid esters were evaluated by DPPH assay through the measurement of the hydrogen-donating ability to covert stable DPPH free radical to 1,1-diphenyl-2-picryl-hydrazine[26]. The reaction was accompanied by a change in color from deep-violet to light-yellow and was monitored spectrophotometrically, caffeic acid served as reference antioxidant in this assay. Table-1 showed that the intermediate caffeic acid esters (**2a**) (IC_{50} = 7.16 μM) compound **2b** (IC_{50} = 7.67 μM) compound **2d** (IC_{50} = 6.98 μM) and compound **2h** (IC_{50} = 7.4 μM) exhibited good antioxidant activity in DPPH assay, which were more potent than the reference antioxidant caffeic acid (IC_{50} = 9.3 μM).

Because oxidative damage played a vital role in stroke, so a kind of compound with both antiplatelet aggregation and antioxidant activity would be very important for cardioprotection. In this paper, we found that compound **3d** not only exhibited potent antiplatelet activity than aspirin, but also its intermediate caffeic acid isopropyl ester (**2d**) showed good antioxidant activity and thus compound **3d** could be considered to be novel potent cardioprotectic drug candidates with reduced risk of hemorrhagic stroke.

Conclusion

In conclusion, we have obtained a new series of aspirin-caffeic acid ester hybrids, these new compounds were confirmed that they were actually pharmacodynamic hybrids possessing antiplatelet aggregations induced by ADP. In addition, as NCX 4016, which were separated by esterases to release aspirin and NO leading to antiplatelet and cytoprotection, the intermediate caffeic acid esters showed interestingly antioxidant activity which were very important to prevent stroke through inhibition of atherosclerosis. Further biological evaluations on these compounds, such as the beneficial roles played by caffeic acid esters in the prevention of stroke are currently in progress.

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