Synthesis and Bioactivity Evaluation of Cinnamic Acid Esters from *Oxalis pes-caprace*

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**ABSTRACT**

Synthesis of cinnamic acid esters 4a-c to 7 was achieved starting from appropriately substituted benzaldehydes. While compound 4a-c to 7 was exhibited potent antioxidative activity in both the NBT and DPPH-radical scavenging models among the synthesized cinnamic acid ester derivatives. No ester derivative showed significant 5-Lox, Tyrosine inhibitory and Cytotoxic activities in the present study.

**Keywords**: Oxalis pes-caprace; synthesis; cinnamic acid ester; antioxidative; cytotoxicity.

**Abbreviations**: NBT, nitroblue tetrazolium; DPPH, 1,1-diphenyl-2-picrylhydrazyl; MDC, methylenedichloride; N,N-DMA, N,N-dimethylaniline; DMAP, 4-dimethylaminopyridine.

**INTRODUCTION**

Hydroxycinnamic acid esters are widely distributed in plant kingdom [1,2] and are known to exhibit a wide range of pharmacological activities such as antioxidative [3,4] cytotoxic [5,6], antimicrobial [7,8] and antiviral activities [9]. Recently, Lucio revitera et al. isolated few cinnamic acid acid ester derivatives 4a-c to 7 from *Oxalis pes-caprace* and reported their phytotoxic activities [10]. Since we are interested on the bioactivity of natural phenolic secondary plant metabolites especially hydroxycinnamic acid esters [11,12], here we would like to report the synthesis and bioactivity studies on the synthesized cinnamic acid esters, 4a-c to 7, for the first time.
EXPERIMENTAL SECTION

Melting points were recorded on a V Scientific melting point apparatus, in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer BX1 FTIR Spectrophotometer, $^1$H NMR (400 MHz) & $^{13}$C NMR (100 MHz) spectra on a Bruker 400 MHz NMR spectrometer and the values for chemical shifts ($\delta$) being given in ppm and coupling constants ($J$) in Hertz (Hz). Mass spectra were recorded on Agilent 1100 Series LC/MSD and elemental analysis was carried out on a Vario El Elementar instrument. Column chromatography was carried out using ACME silica gel (100-200 mesh/finer than 200 mesh). 6 was prepared using standard literature procedure [13] in quantitative yield.

RESULTS AND DISCUSSION

3.1. General Procedure

The desired esters were synthesized as shown in Scheme-1 and Scheme-2 starting from 3,4,5-trimethoxybenzaldehyde. 3,4,5-Trimethoxycinnamic acid (4a-c) was prepared using Knoevenagel-Doebner reaction conditions starting from 3,4,5-trimethoxy-benzaldehyde using malonic acid and pyridine in presence of piperidine as a catalyst in 95% yield [13].

Esterification of 4a-c with monomethoxy resorcinol and resorcinol using DCC [14] as dehydrating agent in presence of DMAP as a catalyst (Scheme 1) yielded 3-methoxyphenyl-3-(3',4',5'-trimethoxyphenyl)propionate (1) and 3-hydroxyphenyl-3-(3',4',5'-trimethoxyphenyl) propionate (2) (65% and 56%), respectively.

2-Hydroxyethyl-3-(3',4',5'-trimethoxyphenyl)propionate (3) was prepared by the esterification 6 with ethylene glycol in presence of DCC (Scheme 2, 45%). O-Acetylation of 3 using acetic anhydride in pyridine gave 2-(acetyloxy)ethyl-3-(3',4',5'-trimethoxyphenyl)propionate (4) in 73% yield [15]. 3 was treated with aluminum chloride to obtain 2-hydroxyethyl-3-(4'-hydroxy-3',5'-trimethoxyphenyl)propionate (5) in 50% yield (Scheme 2). The spectral data of all the synthesized esters are well coincides with those obtained from natural source [10]. The details of the experimental procedure were given in experimental section.

The above synthesized cinnamic acid esters were evaluated for their bioactivity regarding antioxidative and 5-Lipooxigenase, tyrosine inhibitory and cytotoxic activities. Antioxidative potency was carried out in both the DPPH free radical inhibition and superoxide radical inhibition (NBT) methods [16]. The only derivative 5 exhibited significant anti-oxidative activity both in NBT and DPPH free radical inhibition models with $IC_{50} = 11$ and 29 $\mu g/ml$, respectively. This clearly indicates the importance of hydroxyl group (s) on both the acid and alcohol part of the cinnamic acid ester derivative to exhibited better anti-oxidative activity. No synthesized cinnamic acid ester analog was showed any significant 5-Lipooxigenases, Tyrosine inhibitory activities even at higher concentrations.

The cytotoxic activity of the above active compound 5 was evaluated using five different human cancer cell lines namely, colorectal carcinoma, HT-29 (A), breast carcinoma, MDA-MB-231 (B), pancreas carcinoma, MIA-PaCa2 (C), prostate adenocarcinoma, DU-145 (D) and oral carcinoma, KB (E). However, this compound did not exhibit any significant inhibition of above mentioned
cancer cell lines (A: 4; B: 17; C: 30; D: 2 and E: 25% of inhibition) even at higher concentration (300µg/ml), respectively.

Reagents and conditions: (i) Malonic acid, Pyridine, Piperidine, 120 °C, 3 h, 95%, (ii) 3-Substitutedphenol, DCC, DMAP, MDC, rt, 1-2 h.

3.2. Synthesis of Cinnamic Acid Ester Derivatives:
To a stirred solution of 2 (1 g, 4.2 mmol) in MDC (25 ml) was added DCC (6.3 mmol) in MDC (5 ml) and stirred for about 15 min at rt. To the above reaction mixture was 3-Substitutedphenol (520 mg, 4.2 mmol) followed by the addition of catalytic amount of DMAP and continued to stir at rt for further 1 h. DHU formed was filtered and filtrate was poured into water, extracted with ethyl acetate (3 x 25 ml). The combined ethyl acetate was washed with water, brine solution and dried over anhydrous Na₂SO₄. The crude obtained by the evaporation of the solvent was chromatographed using hexane: ethyl acetate (70:30) to yield corresponding ester as a colourless solid (939 mg, 65%);

3.2.1. 3-Methoxyphenyl-3-(3,4,5-trimethoxyphenyl)acrylate (4a):
Yield: 65%, mp: 82-84 °C; IR (CHCl₃) νmax cm⁻¹: 1722, 1610, 1582, 1421, 1245, 1128; NMR δH (400 MHz, CDCl₃): 3.81 (3H, s, Ar-OCH₃), 3.90 (6H, s, Ar-OCH₃), 6.53 (1H, d, J = 15.6 Hz, H-3), 6.73 (1H, t, J = 2.4 Hz, H-2’’), 6.78 (2H, m, H-4’’6’’), 6.81 (2H, s, H-2’,6’), 7.30 (1H, t, J = 8.0 Hz, H-5), 7.77 (1H, d, J = 15.6 Hz, H-2); ¹³C NMR (100 MHz, CDCl₃): δ 165.2 (C-9), 160.6 (C-3’’), 153.6 (3C-3,4,5), 151.9 (C-1’’), 146.5 (C-7), 129.8 (C-1), 129.6 (C-5’’), 116.5 (C-8),
3.2.2. 3-Hydroxyphenyl-3-(3,4,5-trimethoxyphenyl)acrylate (4b):
Yield : 56%, mp: 118-20 °C; IR (CDCl$_3$) $\nu \text{max} \text{cm}^{-1}$: 3432, 1722, 1582, 1458, 1243, 1129; NMR $\delta_H$ (400 MHz, CDCl$_3$): 3.90 (9H, s, 3 x Ar-OCH$_3$), 6.50 (1H, d, J = 15.6 Hz, H-3), 6.68 (1H, t, J = 2.4 Hz, H-2), 6.73 (2H, m, H-4''6''), 6.81 (2H, s, H-2',6'), 7.25 (1H, t, J = 8.0 Hz, H-5), 7.78 (1H, d, J = 15.6 Hz, H-2); 13C NMR (100 MHz, CDCl$_3$): $\delta$ 165.7 (C-9), 157.1 (C-3'), 153.4 (C-5), 151.6 (C-1'), 146.8 (C-7), 140.7 (C-4), 130.0 (C-5'), 129.6 (C-1), 116.4 (C-8), 113.3 (C-4'), 113.2 (C-6'), 109.2 (C-2'), 105.8 (C-2,6), 61.0 (C-Ar-OCH$_3$), 56.2 (C-2 x Ar-OCH$_3$); LCMS (Negative Mode): 329 [M-H]$^+$.

3.2.3. m-tolyl 3-(3,4,5-trimethoxyphenyl)acrylate (4c):
Yield : 62%, mp: 132-133 °C; IR (CHCl$_3$) $\nu \text{max} \text{cm}^{-1}$: 1745, 1640, 1570, 1420, 1350, 1120; NMR $\delta_H$ (400 MHz, CDCl$_3$): 1.25 (3H, s, Ar-CH$_3$), 3.80-3.92 (9H, s, Ar-OCH$_3$), 6.52 (1H, d, J = 15.6 Hz, H-3), 6.71 (1H, t, J = 2.4 Hz, H-2), 6.79 (2H, m, H-4''6''), 6.83 (2H, s, H-2',6'), 7.32 (1H, t, J = 8.0 Hz, H-5), 7.77 (1H, d, J = 15.6 Hz, H-2); 13C NMR (100 MHz, CDCl$_3$): $\delta$ 166.2 (C-9), 163.6 (C-3'), 152.4 (3C-3,4,5), 150.5 (C-1'), 145.1 (C-7), 126.4 (C-1), 125.5 (C-5'), 114.2 (C-8), 113.5 (C-6'), 111.2 (C-4'), 107.7 (C-2'), 105.8 (C-2,6), 61.0 (C-Ar-OCH$_3$), 56.2 (C-2 x Ar-OCH$_3$); LCMS (Positive Mode): 306 [M+Na]$^+$, 329 [M+K]$^+$.

3.2.4. 2-Hydroxyethyl -3-(3,4,5-trimethoxyphenyl)propionate (5)
Title compound was prepared by adopting the same procedure as described for 1, starting from 2 (0.5 g, 2.1 mmol) and ethylene glycol (130 mg, 2.1 mmol) as colourless solid (266 mg, 45%); mp: 94-96 °C; IR (CDCl$_3$) $\nu \text{max} \text{cm}^{-1}$: 3492, 1681, 1626, 1585, 1419, 1284, 1123; NMR $\delta_H$ (400 MHz, CDCl$_3$): 3.89 (9H, s, 3 x Ar-OCH$_3$), 3.91 (2H, m, H-1'), 4.35 (2H, m, H-2'), 6.39 (1H, d, J = 15.6 Hz, H-3), 6.76 (2H, s, H-2',6'); 13C NMR (100 MHz, CDCl$_3$): $\delta$ 167.2 (C-9), 153.4 (C-3,5), 145.3 (C-7), 140.5 (C-4), 129.7 (C-1), 116.8 (C-8), 105.6 (C-2,6), 66.1 (C-1'), 61.2 (C-2'), 60.9 (C-Ar-OCH$_3$), 56.2 (C-2 x Ar-OCH$_3$); LCMS (Positive Mode): 283 [M+H]$^+$, 305 [M+Na]$^+$.

3.2.5. 2-Acetylethyl-3-(3,4,5-trimethoxyphenyl)propionate (6)
This compound was prepared by the acylation of 5 by adopting standard literature procedure of acetylation using acetic anhydride and pyridine [15] as a colourless solid (73%); mp: 94-96 °C; IR (CDCl$_3$) $\nu \text{max} \text{cm}^{-1}$: 1739, 1690, 1503, 1244, 1120; NMR $\delta_H$ (400 MHz, CDCl$_3$): 3.88 (9H, s, 3 x Ar-OCH$_3$), 3.91 (2H, m, H-1'), 4.35 (2H, m, H-2'), 6.39 (1H, d, J = 15.6 Hz, H-3), 6.76 (2H, s, H-2',6'); 13C NMR (100 MHz, CDCl$_3$): $\delta$ 170.7 (C-OAc), 166.5 (C-9), 153.4 (C-3,5), 145.3 (C-7), 140.5 (C-4), 129.7 (C-1), 116.7 (C-8), 105.4 (C-2,6), 62.2 (C-1',2'), 60.9 (C-Ar-OCH$_3$), 56.1 (C-2 x Ar-OCH$_3$); 20.8 (C-OCH$_3$); LCMS (Positive Mode): 283 [M+H]$^+$, 305 [M+Na]$^+$.

3.2.6. 2-Hydroxyethyl-3-(3,5-dimethoxy-4-hydroxyphenyl)propionate (7)
This compound was prepared by the monodemethylation of 5 using standard literature procedure using AlCl$_3$[17] as a half-white solid (48%); mp: 98-100 °C; IR (CDCl$_3$) $\nu \text{max} \text{cm}^{-1}$: 1701, 1516, 1336, 1119; NMR $\delta_H$ (400 MHz, CDCl$_3$): 3.92 (8H, s, 2 x Ar-OH and H-2' both are merged), 4.35 (2H, m, H-1'), 5.78 (1H, brs, H-OH), 6.34 (1H, d, J = 15.6 Hz, H-3), 6.78 (2H, s, H-
2''',6''''), 7.63 (1H, d, J = 15.6 Hz, H-2); 13C NMR (100 MHz, CDCl3): δ 167.4 (C-9), 147.3 (C-3,5), 145.7 (C-7), 137.5 (C-4), 125.8 (C-1), 115.2 (C-8), 105.3 (C-2,6), 66.1 (C-1'), 61.4 (C-2'), 56.4 (C-2 x Ar-OCH3); LCMS (Negative Mode): 267 [M-H].

Biological Activity
The antioxidative activity of the synthesized esters in both the NBT and DPPH free radical-scavenging mechanisms, was determined according to the procedure described in our previous communication [16] and 5-lipoxygenases, Tyrosine inhibitory activity are determined according to the literature procedure [18]. Cytotoxic effect of the compound 7 was carried out by adopting literature procedure against Human colorectal carcinoma, HT-29, breast carcinoma, MDA-MB-231, pancreas carcinoma, MIA-PaCa2, prostate adenocarcinoma, DU-145 and oral carcinoma, KB cell lines [19].

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REFERENCES
[15]. Acetylation