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Design, synthesis and biological evaluation of novel trimethylpyrazine-2-carbonyloxy-cinnamic acids as potent cardiovascular agents

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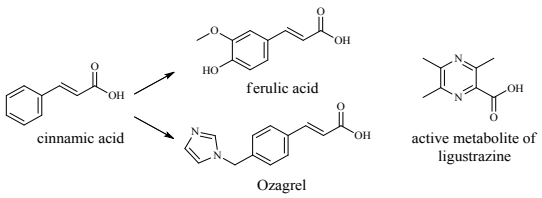

Figure **1**. Structure of cinnamic acid, ferulic acid, ozagrel and the 3,5,6-trimethylpyrazine-2-carboxylic acid.

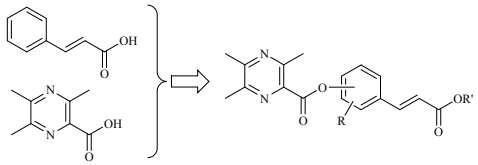

Figure **2**. Design of trimethylpyrazine-2-carbonyloxy-cinnamic acids.

Biological Activities

Anti-platelet activity

Rabbit blood was obtained using the cardiac puncture and transferred to a test tube containing 3.8% sodium citrate aqueous solution. Platelet-rich plasma (PRP) was obtained following blood sample centrifugation at 1000 rpmin for 5min. The PRP samples were again centrifuged at 3000 rpmin for 15 min to obtain platelet-poor plasma (PPP), which was used as a reference solution in aggregation assays. The platelets of the precipitate were adjusted to the proper number (3×10^{11}/L) for the aggregation assay. All platelet preparations were conducted at room temperature. 90 μL PRP and 5 μL sample solution (final concentration: 400, 200, 100, 50 μM) was added into the microplate and incubated on 37ºC for 5min. The microplate was put on the aggregometer and vibrated for 10 min, then monitored by measuring 570nm transmission (A_0). The monitoring took place every 30 s. After that 5 μL ADP (work concentration: 5 μM) was added and 570 nm transmission was monitored every 30s until it became stable (A). The aggregation rate (AR) = (Abs PRP -Abs sample)/(Abs PRP -Abs PPP). The aggregation inhibition rate (AIR) = [1-(ARsample/ ARcontorl)]×100%. Analysis of the derived data using a curve fitting software from OriginLab gave the IC_{50} values.

Protective effects on damaged ECV-304 cells

Ea.hy926 cells were seeded in a 96-well plate at a density of 6×10^3 /well and allowed to grow to the desired confluence. The cells were pretreated with various concentrations of ligustrazine derivatives for 24 h, and then exposed to 150 μM H_2O_2 for another 12 h. Control cells were incubated with a media containing an equivalent solvent amount without the test materials. The plate was incubated at 37 °C in a humidified 5% CO_2 atmosphere. 12 hours later, 0.01 mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL in DMSO) was added to each well and then incubated for 4 h. Ligustrazine derivatives were dissolved in dimethyl sulfoxide (DMSO) and added into the wells (the final concentration of ligustrazine derivatives was to 400, 200, 100, 50 μM, and the DMSO content should never exceed 0.05%) and were incubated with cells for 24 h before the addition of H_2O_2. The supernatant was removed carefully by pipetting from wells without disturbing the attached cells and formazan crystals were solubilized by adding 200 μL of DMSO to each well and shaken for 15 min. The absorbance at 570 nm was measured with a microplate reader, using wells without cells as control. The proliferation rates of damaged Ea.hy926 cells were calculated by [OD570 (Compd)-OD570 (H_2O_2)]/[OD570 (Control)- OD570 (H_2O_2)]×100%, which was then used to obtain EC_{50} values, according to the equation: $-pEC_{50} = \log C_{max} - 2\times(\Sigma P - 0.75 + 0.25P_{max} + 0.25P_{min})$, where C_{max} = maximum concentration, ΣP = sum of proliferation rates, P_{max} = maximum value of proliferation rate and P_{min} = minimum value of proliferation rate.

Table 1: Structures of the synthesized compounds

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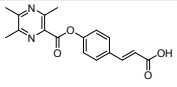
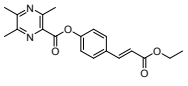
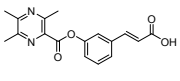
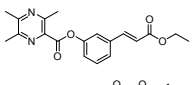
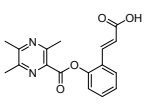
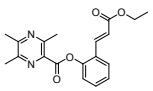
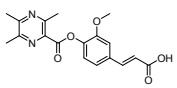
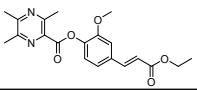
F1		F'1	
F2		F'2	
F3		F'3	
F4		F'4	

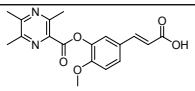
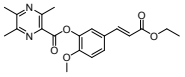
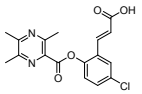
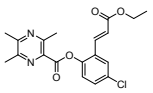
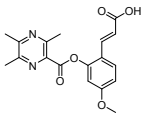
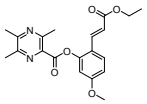
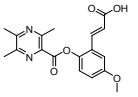
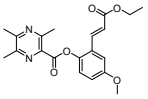
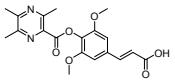
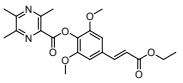
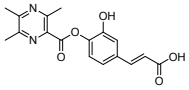
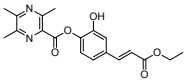
F5		F'5	
F6		F'6	
F7		F'7	
F8		F'8	
F9		F'9	
F10		F'10	

Table 2: The IC_{50} for inhibition of platelet aggregation.

Compd	AIR 100μM	AIR 50μM	AIR 25μM	AIR 12.5μM	IC_{50}[a] (μM)
F1	25.3%	41.7%	53.3%	64.3%	37.8
F′1	20.5%	32.2%	39.1%	68.0%	24.4
F2	8.16%	28.0%	38.5%	73.4%	26.4
F′2	69.4%	59.6%	62.2%	25.1%	35.8
F3	17.2%	53.8%	54.8%	32.9%	9.59
F′3	42.4%	39.0%	16.6%	11.8%	110
F4	73.2%	46.2%	22.8%	60.6%	44.6
F′4	64.4%	49.9%	37.2%	31.0%	58.4
F5	75.2%	59.8%	53.5%	21.6%	41.8
F′5	39.7%	32.2%	20.6%	22.3%	143
F6	74.5%	37.0%	38.1%	30.0%	57.2
F′6	61.7%	44.4%	36.9%	26.3%	67.1
F7	40.9%	34.1%	35.1%	16.5%	134
F′7	47.0%	18.7%	32.6%	84.2%	28.2
F8	59.8%	48.8%	46.5%	43.4%	49.0
F′8	60.7%	72.9%	73.9%	70.5%	196
F9	52.1%	51.2%	17.4%	12.0%	82.3
F′9	59.5%	55.9%	47.2%	44.0%	37.5

F10	53.6%	44.5%	25.0%	32.1%	84.6
F′10	58.4%	37.9%	36.4%	22.0%	77.4
ozagrel	36.8%	25.0%	4.81%	6.45%	144
clopidogrel	94.5%	92.3%	77.6%	86.9%	7.57

[a]IC_{50}: concentration of compound required to achieve 50% inhibition on aggregation of rabbit platelet.

Table 3: The EC_{50} values for protection on damaged Ea.hy926 cells and *P*% at different concentration of the trimethylpyrazine-2-carbonyloxy-cinnamic acids.

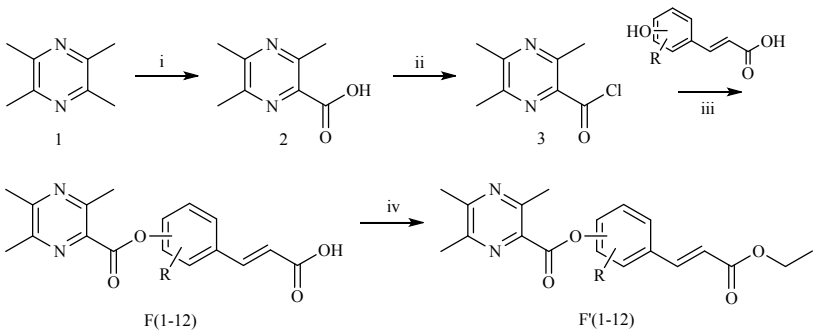
Compd	P%(100μM)	P%(50μM)	P%(25μM)	P%(12.5μM)	EC_{50}[a] (mM)
F1	93.2%	74.1%	46.6%	34.2%	38.0
F'1	96. 1%	62.6%	58.8%	42.7%	34.9
F2	103%	75.3%	64.3%	61.6%	24.0
F'2	47.0%	39.5%	34.2%	27.9%	92.6
F3	80.1%	64.9%	61.8%	45.7%	36.7
F'3	40.9%	36.9%	17.8%	9.57%	108
F4	55.4%	49.1%	40.3%	38.7%	69.4
F'4	71.6%	69.7%	53.3%	52.4%	38.6
F5	216%	160%	128%	41.3%	8.84
F'5	117%	89.0%	60.1%	26.4%	29.9
F6	52.2%	23.7%	26.0%	6.85%	95.2
F'6	11.0%	33.7%	62.5%	64.9%	36.1
F7	18.0%	31.6%	-52.1%	39.7%	115
F'7	39.9%	20.7%	16.9%	-57.6%	138
F8	79.2%	62.9%	45.0%	21.7%	48.6
F'8	46.2%	27.8%	23.4%	15.9%	105
F9	163%	96.6%	24.0%	5.70%	34.7
F'9	-47.0%	-13.6%	20.9%	96.6%	21.4
F10	601%	285%	271%	92.8%	2.23
F'10	375%	211%	207%	75.7%	1.71
lipoic acid	58.1%	48.7%	-12.3%	30.5%	68.0
BHA	43.4%	28.0%	22.6%	17.2%	111

[a]EC_{50}: concentration of compound required to achieve 50% protection of ECV-304 cell from H_2O_2 induced cytotoxicity, as determined by the MTT method.

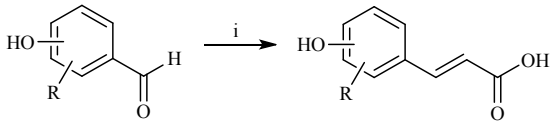
Synthesis

Materials and Methods

The melting points of the compounds were metered on a micromelting point apparatus. Infrared spectra were recorded with a Nexus 470 FT-IR Spectrometer. ^{1}HNMR spectra were determined by a Bruker Avance (400 MHz) NMR-spectrometer in the indicated solvents. Chemical shifts are expressed in δ units and TMS as internal reference. Mass spectra were recorded with an LC Autosampler Device: Standard G1313A instrument. TLC was performed on silica gel GF254 for TLC (Merck) and spots were visualized by iodine vapors or by irradiation with UV light (254 nm). Flash column chromatography was performed on column packed with silica gel 60 (230–400 mesh). Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of the reaction solutions involved the use of rotary evaporator at reduced pressure. The yields were calculated by the last step reaction.



Scheme 1. Reagents and conditions: (i) $KMnO_4$, H_2O, 35 ºC, 24 h, (ii) oxalyl chloride, anhydrous DCM, ice bath, 10 min, (iii) Et_3N, anhydrous DCM, r.t., 1 h, (iv) EtOH, $SOCl_2$, reflux, 12 h.



Scheme 2. Preparation of the hydroxyl cinnamic acids. Reagents and conditions: (i) malonic acid, pyridine, cat. piperidine, 120 ºC, 1.5 h

General procedure for the preparation of the Compounds F(1-10)

To aqueous solution of 2,3,5,6-tetramethylpyrazine (0.1 mol) was added potassium permanganate (0.4 mol). The reaction mixture was stirred at 35 ºC for 24 h to give the intermediate 3,5,6-trimethylpyrazine-2-carboxylic acid. Oxalyl chloride (2 mmol) was added to the dichloromethane (10 mL, anhydrous) solution of 3,5,6-trimethylpyrazine-2-carboxylic acid (2 mmol) in ice bath. When the addition was finished with further stirring for 10 min, the solvent and redundant oxalyl chloride were removed under reduced pressure to obtain the

3,5,6-trimethylpyrazine-2-carbonyl chloride.

Cinnamic acids with different substitutents at phenyl were prepared according to the Knovenagel condensation. To the solution of hydroxybenzaldehyde (2.0 mmol) in pyridine was added malonic acid (2.4 mmol) and piperidine. The mixture was refluxed at 120 °C for 1.5 h. Upon completion of the reaction, the mixture was diluted with ethyl acetate (100 mL) and washed with 0.1 N HCl solution. The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford cinnamic acids.

Cinnamic acid (2 mmol) was added into the dichloromethane solution of 3,5,6-trimethylpyrazine-2-carbonyl chloride (2 mmol) obtained in the above step at room temperature, triethylamine (4 mmol) was added to the mixture which was then stirred at 25 ºC for 1 h (checked by TLC). The solvent was removed and the residue was dissolved in ethyl acetate (100 mL), washed with water and brine, successively. The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford crude product. The final product was purified by flash column chromatography and recrystallization from ethyl acetate.

(E)-3-(4-((3,5,6-trimethylpyrazine-2-carbonyl)oxy)phenyl)acrylic acid (F1)

White needle crystals, yield: 63%, mp: 212-214 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 12.46 (s, 1H, COOH), 7.82 (d, 2H, Ar-H, J = 8.4 Hz), 7.66 (d, 1H, Ar-CH=C, J = 16.0 Hz), 7.36 (d, 2H, Ar-H, J = 8.4 Hz), 6.58 (d, 1H, C=CH-C=O, J = 16.0 Hz), 2.72 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.55 (s, 3H, CH_3). IR(KBr, cm^{-1}): 3400 (OH), 2925 (CH_3), 1743, 1693 (C=O), 1628 (C=C), 1599, 1583, 1506 (C=N, C=C), 1164 (C-O). ESI-MS: 313.3 $(M+H)^+$, calcd. for $C_{17}H_{16}N_2O_4$ 312.32.

(E)-3-(3-((3,5,6-trimethylpyrazine-2-carbonyl)oxy)phenyl)acrylic acid (F2)

Yellow powders, yield: 54%, mp: 208-210 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 12.48 (s, 1H, COOH), 7.68-7.60 (m, 3H, Ar-H, Ar-CH=C), 7.54 (t, 1H, Ar-H, J = 7.8 Hz), 7.36 (dd, 1H, Ar-H, J_1 = 1.5 Hz, J_2 = 7.9 Hz), 6.64 (d, 1H, C=CH-C=O, J = 16.0 Hz), 2.73 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.55 (s, 3H, CH_3). IR(KBr, cm^{-1}): 3450 (OH), 2966, 2852 (CH_3), 1736, 1692 (C=O), 1631 (C=C), 1580, 1539 (C=N, C=C), 1172 (C-O). ESI-MS: 313.4 $(M+H)^+$, calcd. for $C_{17}H_{16}N_2O_4$ 312.32.

(E)-3-(2-((3,5,6-trimethylpyrazine-2-carbonyl)oxy)phenyl)acrylic acid (F3)

Light yellow needle crystals, yield: 57%, mp: 204-206 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 12.48 (s, 1H, COOH), 7.94 (d, 1H, Ar-H, J = 7.8 Hz), 7.67 (d, 1H, Ar-CH=C, J = 15.6 Hz), 7.56-7.53 (m, 1H, Ar-H), 7.42-7.38 (m, 2H, Ar-H), 6.75 (d, 1H, C=CH-C=O, J = 15.6 Hz), 2.71 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 2.50 (s, 3H, CH_3). IR(KBr, cm^{-1}): 3450 (OH), 2980, 2929 (CH_3), 1733, 1692 (C=O), 1627 (C=C), 1577, 1543 (C=N, C=C), 1166 (C-O). ESI-MS: 313.4 $(M+H)^+$, calcd. for $C_{17}H_{16}N_2O_4$ 312.32.

(E)-3-(3-methoxy-4-((3,5,6-trimethylpyrazine-2-carbonyl)oxy)phenyl) acrylic acid (F4)

White needle crystals, yield: 61%, mp: 214-218 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 12.44 (s, 1H, COOH), 7.63 (d, 1H, Ar-CH=C, *J* = 15.6 Hz), 7.56 (s, 1H, Ar-H), 7.34 (d, 1H, Ar-H, *J* = 7.8 Hz), 7.30 (d, 1H, Ar-H, *J* = 7.8 Hz), 6.65 (d, 1H, C=CH-C=O, *J* = 16.2 Hz), 3.85 (s, 3H, OCH_3), 2.70 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.51 (s, 3H, CH_3). IR(KBr, cm^{-1}): 3422 (OH), 2924, 2852 (CH_3), 1745, 1696 (C=O), 1630 (C=C), 1591 (C=N, C=C), 1152 (C-O). ESI-MS: 343.4 $(M+H)^+$, calcd. for $C_{18}H_{18}N_2O_5$ 342.35.

(E)-3-(4-methoxy-3-((3,5,6-trimethylpyrazine-2-carbonyl)oxy)phenyl)acrylic acid (F5)

White needle crystals, yield: 64%, mp: 202-204 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 12.36 (s, 1H, COOH), 7.68-7.61 (m, 3H, Ar-H, Ar-CH=C), 7.24 (d, 1H, Ar-H, *J* =8.4 Hz), 6.48 (d, 1H, C=CH-C=O, *J* = 16.2 Hz), 3.84 (s, 3H, OCH_3), 2.71 (s, 3H, CH_3), 2.57-2.49 (m, 6H, CH_3×2). IR(KBr, cm^{-1}): 3488 (OH), 2957, 2927, 2848 (CH_3), 1753, 1720 (C=O), 1636 (C=C), 1610 (C=N, C=C), 1161 (C-O). ESI-MS: 343.5 $(M+H)^+$, calcd. for $C_{18}H_{18}N_2O_5$ 342.35.

(E)-3-(5-chloro-2-((3,5,6-trimethylpyrazine-2-carbonyl)oxy)phenyl)acrylic acid (F6)

White needle crystals, yield: 89%, mp: 229-233 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 12.79 (s, 1H, COOH), 8.06 (d, 1H, Ar-CH=C, *J* = 2 Hz), 7.63-7.58 (m, 2H, Ar-H), 7.50 (d, 1H, Ar-H, *J* = 8.7 Hz), 6.88 (d, 1H, C=CH-C=O, *J* = 16.0 Hz), 2.72 (s, 3H, CH_3), 2.62-2.48 (m, 6H, CH_3×2). IR(KBr, cm^{-1}): 3429 (OH), 2960, 2925, 2854 (CH_3), 1739, 1696 (C=O), 1629 (C=C), 1571, 1541 (C=N, C=C), 1159 (C-O). ESI-MS: 347.3 $(M+H)^+$, calcd. for $C_{17}H_{15}ClN_2O_4$ 346.76.

(E)-3-(4-methoxy-2-((3,5,6-trimethylpyrazine-2-carbonyl)oxy)phenyl) acrylic acid (F7)

White needle crystals, yield: 84%，mp: 210-212 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 12.32 (s, 1H, COOH), 7.88 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.59 (s, 1H, Ar-CH=C, *J* = 16.0 Hz), 7.04 (d, 1H, Ar-H, *J* = 2.5 Hz), 6.99 (dd, 1H, Ar-H, J_1 = 2.6 Hz, J_2 = 8.8 Hz), 6.61 (d, 1H, C=CH-C=O, *J* = 16.0 Hz), 3.83 (s, 3H, OCH_3), 2.72 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 2.51 (s, 3H, CH_3). IR(KBr, cm^{-1}): 3478 (OH), 2956, 2927 (CH_3), 1761, 1720 (C=O), 1636 (C=C), 1610 (C=N, C=C), 1161 (C-O). ESI-MS: 343.4 $(M+H)^+$, calcd. for $C_{18}H_{18}N_2O_5$ 342.35.

(E)-3-(5-methoxy-2-((3,5,6-trimethylpyrazine-2-carbonyl)oxy)phenyl)acrylic acid (F8)

White powders, yield: 81%，mp: 213-215 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 12.48 (s, 1H, COOH), 7.61 (d, 1H, Ar-CH=C, *J* = 16.2 Hz), 7.44 (s, 1H, Ar-H), 7.33 (d, 1H, Ar-H, *J* = 9 Hz), 7.10 (m, 1H, Ar-H), 6.80 (d, 1H, C=CH-C=O, *J* = 16.2 Hz), 3.84 (s, 3H, OCH_3), 2.70 (s, 3H, CH_3), 2.57-2.44 (m, 6H, CH_3×2). IR(KBr, cm^{-1}): 3442 (OH), 2977, 2924, 2836 (CH_3), 1732, 1693 (C=O), 1632 (C=C), 1582, 1541 (C=N, C=C), 1161 (C-O). ESI-MS: 343.4 $(M+H)^+$, calcd. for $C_{18}H_{18}N_2O_5$ 342.35.

(E)-3-(3,5-dimethoxy-4-((3,5,6-trimethylpyrazine-2-carbonyl)oxy)phenyl)acrylic acid (F9)

White needle crystals, yield: 82%, mp: 228-232 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 12.44 (s, 1H, COOH), 7.62 (d, 1H, Ar-CH=C, *J* = 16.0 Hz), 7.19 (s, 2H, Ar-H), 6.70 (d, 1H, C=CH-C=O, *J* = 16.0 Hz), 3.83 (s, 6H, OCH_3×2), 2.70 (s, 3H, CH_3), 2.56-2.50 (m, 6H, CH_3×2). IR(KBr, cm^{-1}): 3429 (OH), 2941, 2847 (CH_3), 1755, 1706 (C=O), 1641 (C=C), 1595, 1541 (C=N, C=C), 1167 (C-O). ESI-MS: 373.3 $(M+H)^+$, calcd. for $C_{19}H_{20}N_2O_6$ 372.37.

(E)-3-(3-hydroxy-4-((3,5,6-trimethylpyrazine-2-carbonyl)oxy)phenyl)acrylic acid (F10)

White needle crystals, yield: 84%, mp: 220-222 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 12.45 (s, 1H, COOH), 7.62 (d, 1H, Ar-CH=C, *J* = 16.2 Hz), 7.19 (s, 2H, Ar-H), 6.69 (d, 1H, C=CH-C=O, *J* = 15.6 Hz), 5.35 (s, 2H, OH), 2.71 (s, 3H, CH_3), 2.56-2.50 (m, 6H, CH_3×2). IR(KBr, cm^{-1}): 3470 (OH), 2966, 2852 (CH_3), 1736, 1692 (C=O), 1631 (C=C), 1580, 1539 (C=N, C=C), 1172 (C-O). ESI-MS: 345.6 $(M+H)^+$, calcd. for $C_{17}H_{16}N_2O_6$ 344.32.

General Procedure for the Preparation of the Compounds F'(1-10)

To an icy solution of the ligustrazinacyl-cinnamic acids (10 mmol) in enthanol (30 mL) was added thionyl chloride (0.5 mL) dropwise. The reaction mixture was refluxed for 24 h to give the corresponding crude ethyl cinnamate. Upon completion of the reaction, the solvent was removed and the residue was diluted with ethyl acetate, and washed with water and brine, successively. The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford crude product. The final product was purified by flash column chromatography and recrystallization from *n*-hexane.

(E)-4-(3-ethoxy-3-oxoprop-1-en-1-yl)phenyl-3,5,6-trimethylpyrazine-2-carboxylate (F'1)

White needle crystals, yield: 81%, mp: 77-80 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 7.86 (d, 2H, Ar-H, *J* = 8.6 Hz), 7.72 (d, 1H, Ar-CH=C, *J* = 16.0 Hz), 7.37 (d, 2H, Ar-H, *J* = 8.6 Hz), 6.68 (d, 1H, C=CH-C=O, *J* = 16.0 Hz), 4.24 (q, 2H, OCH_2, *J* = 7.0 Hz), 2.72 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 1.30 (t, 3H, CH_3, *J* = 7.1 Hz). IR(KBr, cm^{-1}): 3063 (C=C-H), 2988, 2929 (CH_3), 1747, 1712 (C=O), 1642 (C=C), 1601, 1541, 1509 (C=N, C=C), 1169 (C-O). ESI-MS: 341.4 $(M+H)^+$, calcd. for $C_{19}H_{20}N_2O_4$ 340.37.

(E)-3-(3-ethoxy-3-oxoprop-1-en-1-yl)phenyl-3,5,6-trimethylpyrazine-2-carboxylate (F'2)

White needle crystals, yield: 81%, mp: 87-91 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 7.72-7.66 (m, 3H, Ar-H, Ar-CH=C), 7.55 (t, 1H, Ar-H, *J* = 7.9 Hz), 7.37 (dd, 1H, Ar-H, J_1 = 1.6 Hz, J_2 = 7.9 Hz), 6.74 (d, 1H, C=CH-C=O, *J* = 16.0 Hz), 4.23 (q, 2H, OCH_2, *J* = 7.1 Hz), 2.73 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 1.30 (t, 3H, CH_3, *J* = 7.1 Hz). IR(KBr, cm^{-1}): 3061 (C=C-H), 2977, 2927 (CH_3), 1731, 1716 (C=O), 1637 (C=C), 1604, 1583, 1541 (C=N, C=C), 1169 (C-O). ESI-MS: 341.4 $(M+H)^+$, calcd. for $C_{19}H_{20}N_2O_4$ 340.37.

(E)-2-(3-ethoxy-3-oxoprop-1-en-1-yl)phenyl-3,5,6-trimethylpyrazine-2-carboxylate (F'3)

White needle crystals, yield: 81%, mp: 77-79 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 7.97 (d, 1H, Ar-H, *J* = 7.8 Hz), 7.75 (d, 1H, Ar-CH=C, *J* = 16.2 Hz), 7.61-7.54 (m, 1H, Ar-H), 7.45-7.38 (m, 2H, Ar-H), 6.93 (d, 1H, C=CH-C=O, *J* = 16.1 Hz), 4.23 (q, 2H, OCH_2, *J* = 7.2 Hz), 2.73 (s, 3H, CH_3), 2.57-2.49 (m, 6H, CH_3×2), 1.30 (t, 3H, CH_3, *J* = 7.2 Hz). IR(KBr, cm^{-1}): 3080 (C=C-H), 2988, 2957 (CH_3), 1745, 1705 (C=O), 1629 (C=C), 1601, 1577, 1541 (C=N, C=C), 1151 (C-O). ESI-MS: 341.4 $(M+H)^+$, calcd. for $C_{19}H_{20}N_2O_4$ 340.37.

(E)-4-(3-ethoxy-3-oxoprop-1-en-1-yl)-2-methoxyphenyl-3,5,6-trimethylpyrazine-2-carboxylate (F'4)

White needle crystals, yield: 81%, mp: 107-110 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 7.70 (d, 1H, Ar-CH=C, *J* = 16.0 Hz), 7.61 (s, 1H, Ar-H), 7.38 (d, 1H, Ar-H, *J* = 8.2 Hz), 7.31 (d, 1H, Ar-H, *J* = 8.2 Hz), 6.77 (d, 1H, C=CH-C=O, *J* = 16.0 Hz), 4.23 (q, 2H, OCH_2, *J* = 7.1 Hz), 3.85 (s, 3H, OCH_3), 2.70 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 1.30 (t, 3H, CH_3, *J* = 7.1 Hz). IR(KBr, cm^{-1}): 3064 (C=C-H), 2983, 2928 (CH_3), 1737, 1703 (C=O), 1676 (C=C), 1601, 1510 (C=N, C=C), 1158 (C-O). ESI-MS: 371.4 $(M+H)^+$, calcd. for $C_{20}H_{22}N_2O_5$ 370.40.

(E)-5-(3-ethoxy-3-oxoprop-1-en-1-yl)-2-methoxyphenyl-3,5,6-trimethylpyrazine-2-carboxylate (F'5)

White needle crystals, yield: 54%, mp: 119-122 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 7.74-7.61 (m, 3H, Ar-H, Ar-CH=C), 7.24 (d, 1H, Ar-H, *J* =8.6 Hz), 6.59 (d, 1H, C=CH-C=O, *J* = 16.0 Hz), 4.23 (q, 2H, OCH_2, *J* = 7.0 Hz), 3.84 (s, 3H, OCH_3), 2.71 (s, 3H, CH_3), 2.57-2.50 (m, 6H, CH_3×2), 1.30 (t, 3H, CH_3, *J* = 7.1 Hz). IR(KBr, cm^{-1}): 3064 (C=C-H), 2976, 2930 (CH_3), 1741, 1705 (C=O), 1636 (C=C), 1611, 1512 (C=N, C=C), 1155 (C-O). ESI-MS: 371.5 $(M+H)^+$, calcd. for $C_{20}H_{22}N_2O_5$ 370.40.

(E)-4-chloro-2-(3-ethoxy-3-oxoprop-1-en-1-yl)phenyl-3,5,6-trimethylpyrazine-2-carboxylate (F'6)

White needle crystals, yield: 75%, mp: 96-99 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 8.09 (d, 1H, Ar-CH=C, *J* = 2.5 Hz), 7.70-7.60 (m, 2H, Ar-H), 7.52 (d, 1H, Ar-H, *J* = 8.7 Hz), 7.04 (d, 1H, C=CH-C=O, *J* = 16.2 Hz), 4.18 (q, 2H, OCH_2, *J* = 7.1 Hz), 2.71 (s, 3H, CH_3), 2.60-2.50 (m, 6H, CH_3×2), 1.30 (t, 3H, CH_3, *J* = 7.1 Hz). IR(KBr, cm^{-1}): 3088 (C=C-H), 2994, 2981 (CH_3), 1735, 1717 (C=O), 1634 (C=C), 1570, 1542 (C=N, C=C), 1180 (C-O). ESI-MS: 375.4 $(M+H)^+$, calcd. for $C_{19}H_{19}ClN_2O_4$ 374.82.

(E)-2-(3-ethoxy-3-oxoprop-1-en-1-yl)-5-methoxyphenyl-3,5,6-trimethylpyrazine-2-carboxylate (F'7)

White needle crystals, yield: 80%, mp: 87-89 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 7.70 (d, 1H, Ar-CH=C, *J* = 16.2 Hz), 7.61 (s, 1H, Ar-H), 7.38 (d, 1H, Ar-H, *J* = 7.8 Hz), 7.31 (d, 1H, Ar-H, *J* = 8.4 Hz), 6.77 (d, 1H, C=CH-C=O, *J* = 15.6 Hz), 4.23 (q, 2H, OCH_2, *J* = 7.1 Hz), 3.86 (s, 3H, OCH_3), 2.70 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 1.30 (t, 3H, CH_3, *J* = 7.1 Hz). IR(KBr, cm^{-1}): 3064 (C=C-H), 2976, 2930 (CH_3), 1741, 1705 (C=O), 1636 (C=C), 1611, 1512 (C=N, C=C), 1155 (C-O). ESI-MS: 371.5 $(M+H)^+$, calcd. for $C_{20}H_{22}N_2O_5$ 370.40.

(E)-2-(3-ethoxy-3-oxoprop-1-en-1-yl)-4-methoxyphenyl-3,5,6-trimethylpyrazine-2-carboxylate (F'8)

White needle crystals, yield: 80%, mp: 78-80 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 7.69 (d, 1H, Ar-CH=C, *J* = 16.1 Hz), 7.48 (s, 1H, Ar-H), 7.35 (d, 1H, Ar-H, *J* = 8.9 Hz), 7.11 (dd, 1H, Ar-H, J_1 = 3.0 Hz, J_2 = 8.9 Hz), 6.97 (d, 1H, C=CH-C=O, *J* = 16.1 Hz), 4.18 (q, 2H, OCH_2, *J* = 7.1 Hz), 3.84 (s, 3H, OCH_3), 2.70 (s, 3H, CH_3), 2.59-2.50 (m, 6H, CH_3×2), 1.33 (t, 3H, CH_3, *J* = 7.1 Hz). IR(KBr, cm^{-1}): 3064 (C=C-H), 2981, 2963 (CH_3), 1740, 1704 (C=O), 1635 (C=C), 1581, 1543 (C=N, C=C), 1157 (C-O). ESI-MS: 371.4 $(M+H)^+$, calcd. for $C_{20}H_{22}N_2O_5$ 370.40.

(E)-4-(3-ethoxy-3-oxoprop-1-en-1-yl)-2,6-dimethoxyphenyl-3,5,6-trimethylpyrazine-2-carboxylate (F'9)

White needle crystals, yield: 75%, mp: 83-85 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 7.68 (d, 1H, Ar-CH=C, *J* = 16.0 Hz), 7.23 (s, 2H, Ar-H), 6.82 (d, 1H, C=CH-C=O, *J* = 16.0 Hz), 4.23 (q, 2H, OCH_2, *J* = 7.1 Hz), 3.85 (s, 6H, OCH_3×2), 2.70 (s, 3H, CH_3), 2.56-2.50 (m, 6H, CH_3×2), 1.30 (t, 3H, CH_3, *J* = 7.1 Hz). IR(KBr, cm^{-1}): 3075 (C=C-H), 2977, 2925 (CH_3), 1691 (C=O), 1633 (C=C), 1600, 1515 (C=N, C=C), 1156 (C-O). ESI-MS: 401.4 $(M+H)^+$, calcd. for $C_{21}H_{24}N_2O_6$ 400.43.

(E)-4-(3-ethoxy-3-oxoprop-1-en-1-yl)-2-hydroxyphenyl-3,5,6-trimethylpyrazine-2-carboxylate (F'10)

White needle crystals, yield: 64%, mp: 90-92 ºC. ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 7.62 (d, 1H, Ar-CH=C, *J* = 16.2 Hz), 7.19 (s, 2H, Ar-H), 6.69 (d, 1H, C=CH-C=O, *J* = 15.6 Hz), 5.35 (s, 2H, OH), 4.23 (q, 2H, OCH_2, *J* = 7.1 Hz), 2.71 (s, 3H, CH_3), 2.56-2.50 (m, 6H, CH_3×2), 1.30 (t, 3H, CH_3, *J* = 7.1 Hz). IR(KBr, cm^{-1}): 3384.13 (OH), 3064 (C=C-H), 2976, 2930 (CH_3), 1741, 1705 (C=O), 1636 (C=C), 1611, 1512 (C=N, C=C), 1155 (C-O). ESI-MS: 373.5 $(M+H)^+$, calcd. for $C_{19}H_{20}N_2O_6$ 372.37.