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Electronic Supplementary Information

Design, synthesis and biological evaluation of novel ligustrazinylated derivatives as potent cardiovascular agents

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Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

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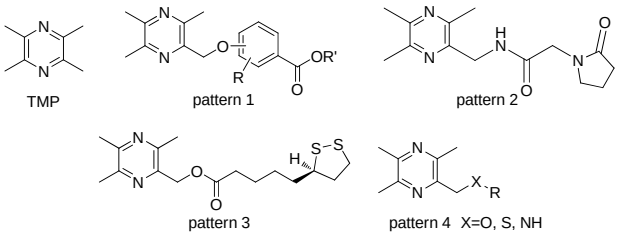


Figure 1: The structure of ligustrazine and newly designed ligustrazinylated compounds.

Biological Activities

Anti-platelet activity

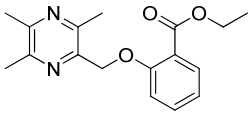
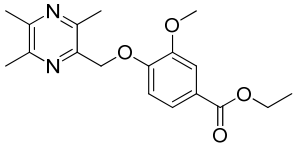
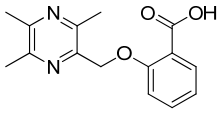
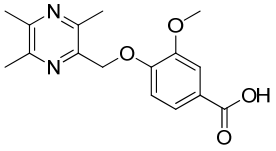
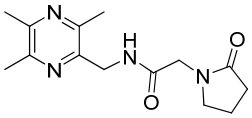
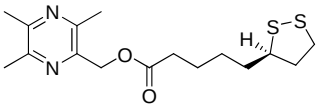
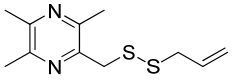
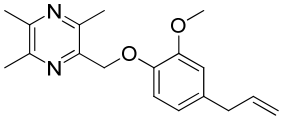
Rabbit blood was obtained and transferred to a test tube containing 3.8% sodium citrate aqueous solution. Platelet-rich plasma (PRP) was obtained following blood sample centrifugation at 1000 rpmin for 5min. The PRP samples were again centrifuged at 3000 rpmin for 15 min to obtain platelet-poor plasma (PPP). The platelets of the PRP were adjusted to 3×10^{11}/L for the aggregation assay. All platelet preparations were conducted at 25 ºC. 90 μL PRP and 5 μL sample solution (final concentration: 400, 200, 100, 50 μM) was added into the microplate and incubated at 37 ºC for 5 min. The microplate was put on the aggregometer and vibrated for 10 min, then monitored by measuring 570 nm transmission (A_0). The monitoring took place every 30 s. After that 5 μL ADP (work concentration: 5μM) was added and 570 nm transmission was monitored every 30 s 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%. The IC_{50} was obtained by linear regression method.

Protective effects on damaged ECV-304 cells

ECV-304 cells were seeded in a 24-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 ºC in a humidified 5% CO_2 atmosphere. 12 hours later, 0.01 mL MTT solution was added to each well and incubated for 4 h. Ligustrazine derivatives were dissolved in DMSO and added into the wells (final concentrations: 400, 200, 100, 50 μM, DMSO ≤ 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 shaked 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 ECV-304 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.

Table 1: Structures of the synthesized compounds

Compd.	Structure	Compd.	Structure
1a		1b	
1c		1d	
2		3	
4a		4b	

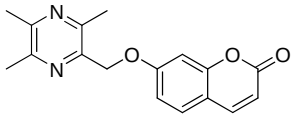
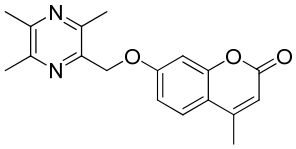
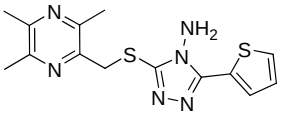
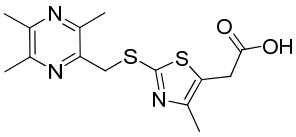
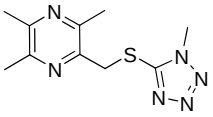
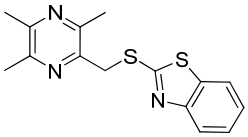
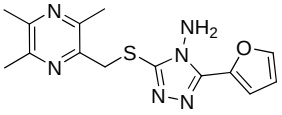
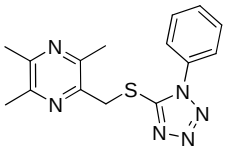
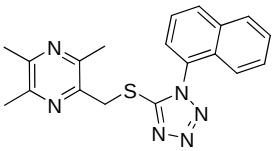
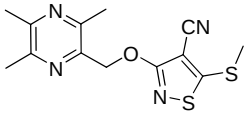
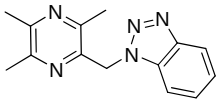
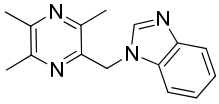
4c		4d	
4e		4f	
4g		4h	
4i		4j	
4k		4l	
4m		4n	

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

Compd	AIR(400μM)	AIR(200μM)	AIR(100μM)	AIR(50μM)	IC_{50}[a] (mM)
1a	21.2%	16.8%	10.8%	8.70%	2.0
1b	-3.31%	7.48%	3.10%	9.70%	>2
1c	51.7%	34.6%	32.0%	15.6%	0.40
1d	4.41%	-11.2%	1.01%	11.2%	>2
2	36.0%	19.6%	10.4%	3.23%	1.2
3	27.6%	29.0%	13.4%	18.8%	>2
4a	38.1%	30.4%	18.0%	16.7%	1.3

4b	6.42%	12.3%	1.45%	-4.29%	>2
4c	39.2%	35.1%	25.0%	21.7%	1.3
4d	40.1%	17.2%	8.73%	2.27%	0.92
4e	38.5%	23.2%	13.4%	0.150%	0.81
4f	46.9%	38.3%	35.0%	27.5%	0.62
4g	9.95%	13.5%	4.04%	1.53%	>2
4h	33.4%	20.9%	13.4%	5.12%	1.6
4i	35.6%	20.0%	10.4%	-2.62%	0.94
4j	9.33%	4.93%	12.3%	-4.07%	>2
4k	13.1%	5.75%	10.2%	2.25%	>2
4l	32.2%	22.6%	13.5%	3.36%	1.5
4m	28.3%	16.9%	7.23%	-2.21%	1.8
4n	30.9%	13.1%	11.0%	-0.970%	1.9
ozagrel	55.3%	45.4%	27.3%	23.3%	0.36

[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 ECV-304 cells and *P*% at different concentration of the ligustrazinylated derivatives

Compd.	Proliferation rate (%)				EC_{50} [a] (mM)
	100μM	50μM	25μM	12.5μM	
1a	20%	19.2%	18.1%	17.3%	1.0
1b	16.0%	15.8%	15.5%	14.9%	3.2
1c	20.0%	18.2%	17.4%	14.1%	0.61
1d	19.0%	18.4%	16.4%	15.1%	0.81
2	26.4%	23.4%	14.5%	-35.8%	1.3
3	206%	160%	71.4%	-60.0%	0.0040
4a	51.1%	15.0%	-17.6%	13.0%	1.1
4b	36.0%	30.1%	28.2%	22.0%	0.20
4c	46.6%	44.2%	42.0%	38.8%	0.14
4d	36.6%	25.8%	21.0%	12.0%	0.14
4e	40.2%	38.2%	37.4%	36.2%	0.33
4f	48.1%	47.0%	46.2%	44.9%	0.15
4g	-117%	-77.0%	-180%	-220%	/

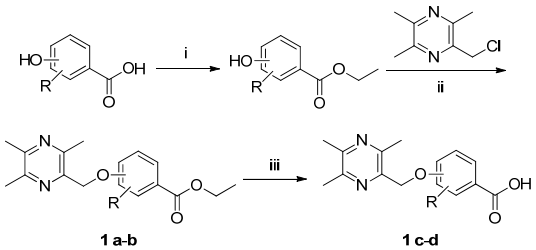
4h	17.2%	9.51%	7.95%	4.33%	0.36
4i	25.8%	23.5%	21.3%	19.0%	0.42
4j	-136%	-78.4%	-119%	-135%	/
4k	-394%	-594%	-393%	-400%	/
4l	-261%	160%	25.2%	-257%	>100
4m	-178%	-96.5%	25.1%	23.2%	>100
4n	-224%	0.250%	-13.8%	-91.8%	/
TMP	42.8%	22.1%	12.7%	10.4%	0.60
lipoic acid	58.1%	48.7%	-12.3%	30.5%	0.068

[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

Melting points were determined on a Gallenkamp capillary apparatus and are uncorrected. ^{1}H-NMR (600 MHz) spectra were obtained on a Bruker Avance-600 instrument in the indicated solvent. Chemical shifts are expressed in δ units with tetramethylsilane as internal reference. Infrared spectra (IR) were recorded with a Nexus 470FT-IR Spectrometer. Mass spectra were recorded on a LC Autosampler Device: Standard G1313A instrument. All compounds were routinely checked by thin-layer chromatography (TLC) on precoated silica gel G plates. Solvents were of reagent grade and, when necessary, were purified and dried by standard methods. Concentration of the reaction solutions involved the use of rotary evaporator under reduced pressure.



Scheme 1: Reagents and conditions: (i) EtOH, $SOCl_2$, reflux for 12 h, (ii) K_2CO_3, TBAB, DMF, 60 ºC, 6 h, (iii) 20% NaOH, 5 h

General procedure for the preparation of ligustrazinyloxy-benzoic acid esters (1 a-b,

as shown in Scheme 1)

To an icy solution of hydroxy benzoic acid (10.00 mmol) in ethanol was added thionyl chloride (0.50 mL) dropwise. The reaction mixture was refluxed for 24 h to give the corresponding crude ethyl benzoate.

K_2CO_3 (2.00 mmol) was added to N,N-dimethyl formamide (DMF) solution of ethyl benzoate (2.00 mmol) at 25 ºC in the presence of catalytic amount of tetra-n-butylammonium bromide (TBAB). When the addition was finished with further stirring for 10 min, 2.00 mmol of 2-chloromethyl-3,5,6-trimethylpyrazine prepared according to our previously reported method [23] in DMF was added to the mixture which was then stirred at 60 ºC for 6 h (checked by TLC). The mixture was diluted with ethyl acetate, washed with water and brine, successively. The organic layer was dried with Na_2SO_4, filtered and concentrated to afford the crude product. The final product was purified by flash column chromatography and recrystallization from *n*-hexane.

Ethyl 2-((3,5,6-trimethylpyrazin-2-yl)methoxy)benzoate (1a)

White needle crystals, yield: 63%, mp: 64-68 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.78-7.76 (m, 1H, Ar-H), 7.44-7.41 (m, 1H, Ar-H), 7.16-7.14 (m, 1H, Ar-H), 7.00-6.97 (m, 1H, Ar-H), 5.24 (s, 2H, $\underline{CH_2}$-OAr), 4.30 (dd, 2H, J = 14.4 Hz, J = 7.2 Hz, $\underline{CH_2}CH_3$), 2.64 (s, 3H, CH_3), 2.51 (s, 6H, CH_3), 1.28-1.26 (t, 3H, J = 7.2 Hz, $\underline{CH_3}$-CH_2). IR (KBr, cm^{-1}): 2981.08, 2923.43 (CH_3), 1697.13 (C=O), 1596.86 (C=N, C=C), 1240.78 (C-O). ESI-MS: 301.6 $(M+H)^+$, for $C_{17}H_{20}N_2O_3$ 300.15.

Ethyl 3-methoxy-4-((3,5,6-trimethylpyrazin-2-yl)methoxy)benzoate (1b)

Yellow needle crystals, yield: 53%, mp: 94-98 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.61 (s, 1H, Ar-H), 7.05-7.27 (m, 2H, Ar-H×2), 5.23 (s, 2H, -CH_2-O-), 4.21-4.25 (m, 2H, -O-CH_2-), 3.87 (s, 3H, -OCH_3), 2.61 (s, 3H, -CH_3), 2.51 (s, 6H, 2-CH_3), 1.31-1.33 (t, 3H, J = 7.2 Hz, -CH_3). IR (KBr, cm^{-1}): 2985.90, 2932.34 (CH_3), 2824.35 (-O$\underline{CH_3}$), 1701.46 (C=O), 1600.71, 1520.86 (C=N, C=C), 1225.53 (C-O). ESI-MS: 331.4 $(M+H)^+$, for $C_{18}H_{22}N_2O_4$ 330.16.

General procedure for the preparation of ligustrazinyloxy-benzoic acids (1 c-d, as shown in Scheme 1)

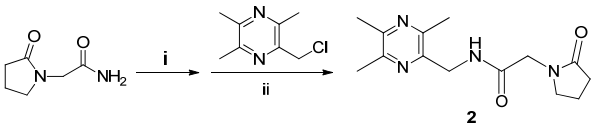
An aqueous solution of NaOH (4.00 mmol) was added to a solution of ligustrazinyloxy-benzoate esters (**1 a-b**, 2.00 mmol) in ethanol. The mixture was stirred at 25 ºC for 24 h. Upon completion, pH was adjusted to 4, and the solution was extracted with ethyl acetate, the product was obtained by removing the solvent.

2-((3,5,6-trimethylpyrazin-2-yl)methoxy)benzoic acid (1c)

White needle crystals, yield: 84%, mp: 150-154 ºC. ^{1}H-NMR (DMSO, δ ppm): 8.12-8.10 (m, 1H, Ar-H), 7.54-7.52 (m, 1H, Ar-H), 7.16-7.12 (m, 2H, Ar-H), 5.40 (s, 2H, CH_2), 2.53-2.52 (m, 9H, CH_3×3). IR (KBr, cm^{-1}): 3197.48 (OH), 3074.72 (Ar-H), 2922.26 (CH_3), 1712.87 (C=O), 1603.10, 1583.08 (C=N, C=C). ESI-MS: 273.3 $(M+H)^+$, for $C_{15}H_{16}N_2O_3$ 272.12.

3-methoxy-4-((3,5,6-trimethylpyrazin-2-yl)methoxy)benzoic acid (1d)

White needle crystals, yield: 81%, mp: 190-194 ºC. ^{1}H-NMR (DMSO, δ ppm): 12.63 (s, 1H, -COOH), 7.55 (s, 1H, Ar-H), 7.44 (d, 1H, J = 3 Hz, Ar-H), 7.20 (d, 1H, J = 3 Hz, Ar-H), 5.20 (s, 2H, -CH_2-O-), 3.43 (s, 3H, -OCH_3), 2.41 (s, 3H, -CH_3), 2.36 (s, 6H, 2-CH_3). IR (KBr, cm^{-1}): 3484.89 (OH), 2954.67 (CH_3), 2828.27 (-O$\underline{CH_3}$), 2652.17, 1683.24 (C=O), 1598.04, 1523.34 (C=N, C=C), 1298.30, 1281.71 (C-O). ESI-MS: 303.5 $(M+H)^+$, for $C_{16}H_{18}N_2O_4$ 302.13.

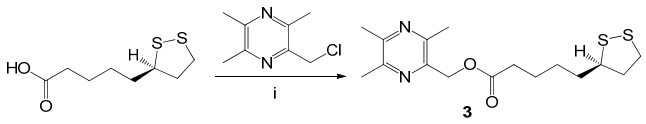


Scheme 2: Reagents and conditions: (i) anhydrous DMF, NaH, N_2, 0 ºC, 1 h, (ii) 60 ºC, 5 h

2-(2-oxopyrrolidin-1-yl)-N-((3,5,6-trimethylpyrazin-2-yl)methyl)acetamide (2, Scheme 2)

A solution of piracetam (2.00 mmol) and NaH (2.20 mmol) in dry DMF was stirred at 25 ºC for 1 h. Then 2-chloromethyl-3,5,6-trimethylpyrazine (2.00 mmol) was added and the mixture was stirred for 6 h. The final product was purified by flash column chromatography and recrystallization from ethanol.

Yellow solid, yield: 53%, mp: 84-88 ºC. ^{1}H-NMR (DMSO, δ ppm): 4.62 (s, 1H, CH_2), 4.60 (s, 1H, CH_2), 4.20 (s, 2H, CH_2), 3.09-3.07 (m, 2H, CH_2), 2.42-2.41 (m, 2H, CH_2), 2.25-2.34 (m, 9H, CH_3×3), 2.24-2.18 (m, 2H, CH_2). IR (KBr, cm^{-1}): 3438.95 (NH), 2975.34, 2938.50 (CH_3) 1696.97 (O=C-N), 1649.14 (C=N, C=C). ESI-MS: 277.4 $(M+H)^+$, for $C_{14}H_{20}N_4O_2$ 276.16.

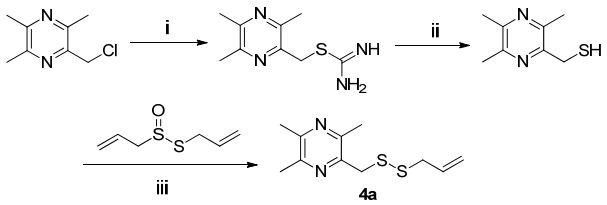


Scheme 3: Reagents and conditions: (i) K_2CO_3, DMF, 60 ºC, 3 h

(R)-(3,5,6-trimethylpyrazin-2-yl)methyl 5-(1,2-dithiolan-3-yl)pentanoate (3, Scheme 3)

K_2CO_3 (2.00 mmol) was added to the DMF solution of lipoic acid (2.00 mmol) at 25 ºC. When the addition was finished with further stirring for 10 min, 2.00 mmol of 2-chloromethyl-3,5,6-trimethylpyrazine was added to the mixture which was then stirred at 60 ºC for 6 h. The final product was purified by flash column chromatography and recrystallization from *n*-hexane.

Yellow oil, yield: 83%. ^{1}H-NMR ($CDCl_3$, *δ* ppm): 5.19 (s, 2H, CH_2-OAr), 3.56-3.54 (m, 1H, CH_2), 3.19-3.09 (m, 2H, CH_2), 2.60-2.48 (m, 9H, CH_2), 2.44-2.37 (m, 4H, CH_2), 1.72-1.60 (m, 4H, CH_2), 1.52-1.42 (m, 2H, CH_2). IR (KBr, cm^{-1}): 2926.57 (CH_3), 2858.63 (-S-CH_2), 1736.89 (C=O), 1548.10 (C=N, C=C). ESI-MS: 341.4 $(M+H)^+$, for $C_{16}H_{24}N_2O_2S_2$ 340.13.

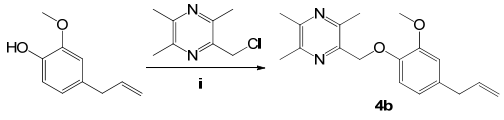


Scheme 4: Reagents and conditions: (i) C_2H_5OH, thiourea, reflux for 3 h, (ii) NaOH aq, reflux for 2 h, (iii) $NaHCO_3$, pH 8.0-8.4, 60 ºC, 10 h

2-((allyldisulfanyl)methyl)-3,5,6-trimethylpyrazine (4a, Scheme 4)

The 2-chloromethyl-3,5,6-trimethylpyrazine (4 mmol) in ethanol solution was added dropwise into the ethanol solution of thiourea (4 mmol). The mixture was refluxed for 3 h to give isothiouronium salt, which underwent hydrolysis in NaOH aqueous solution to yield the ligustrazine-thiol. The ligustrazine-thiol (1.00 mmol) in ethanol solution was added at 25 ºC to allicin (0.55 mmol) in aqueous solution. The pH was adjusted to 8.0-8.4 using solid $NaHCO_3$. Upon the completion of the reaction, the turbid was extracted with ethyl acetate to afford the crude product. The final product was purified by flash column chromatography.

Yellow oil, yield: 49%. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.63 (d, 1H, J=9.6 Hz, =CH_2), 7.37 (d, 1H, J = 8.4 Hz, =CH_2), 6.97-6.92 (m, 2H, -S-$\underline{CH_2}$-CH=), 6.26 (d, 1H, J = 9.6 Hz, =CH-), 5.22 (s, 2H, -S-CH_2-), 2.59 (s, 9H, -CH_3×3). IR (KBr, cm^{-1}): 3080.82 (C=$\underline{C\text{-}H}$), 2978.40, 2947.28, 2919.59 (CH_3), 2856.45 (-S$\underline{CH_2}$), 1634.14 (C=C), 1545.36 (C=N, C=C). ESI-MS: 241.3 $(M+H)^+$, for $C_{11}H_{16}N_2S_3$ 240.08.



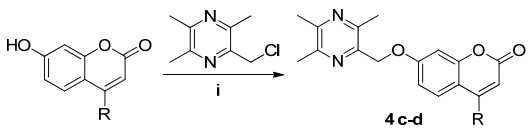
Scheme 5: Reagents and conditions: (i) K_2CO_3, TBAB, DMF, 60 ºC, 6 h

2-((4-allyl-2-methoxyphenoxy)methyl)-3,5,6-trimethylpyrazine (4b, Scheme 5)

K_2CO_3 (2.00 mmol) was added to eugenol (2.00 mmol) in DMF solution at 25 ºC and stirred for 10 min. After that 2.00 mmol of 2-chloromethyl-3,5,6-trimethylpyrazine

was added to the mixture which was then stirred at 60 ºC for 6 h (checked by TLC). The final product was purified by flash column chromatography and recrystallization from *n*-hexane.

White needle crystals, yield: 63%, mp: 76-80 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 6.96 (s, 1H, Ar-H), 6.86 (d, 1H, *J*=1.2 Hz, Ar-H), 6.72 (d, 1H, *J*=1.8 Hz, Ar-H), 5.98-5.93 (m, 1H, -CH=), 5.17 (s, 2H, -CH_2-O-), 5.06 (d, 2H, *J*=1.2 Hz, =CH_2), 3.83 (s, 3H, -OCH_3), 3.33 (s, 2H, Ar-CH_2-), 2.62 (s, 3H, -CH_3), 2.50 (s, 6H, 2-CH_3). IR (KBr, cm^{-1}): 3078.44 (C=C-H), 2999.77, 2933.39 (CH_3), 2897.59, 2828.00 (-OCH_3), 1638.85 (C=C), 1591.29, 1513.41 (C=N, C=C). ESI-MS: 299.7 $(M+H)^+$, for $C_{18}H_{22}N_2O_2$ 298.17.



Scheme 6: Reagents and conditions: (i) K_2CO_3, TBAB, DMF, 60 ºC, 6 h

General procedure for the preparation of (E)-ethyl 3-(substituted-2-yl) methoxy) phenyl) acrylate (4 c-d, Scheme 6)

K_2CO_3 (2.00 mmol) and TBAB was added to coumarin (2.00 mmol) in DMF at 25 ºC and the mixture was stirred for 10 min. After that 2.00 mmol of 2-chloromethyl-3,5,6-trimethylpyrazine was added to the mixture which was then stirred at 60 ºC for 6 h. The final product was purified by flash column chromatography and recrystallization from *n*-hexane.

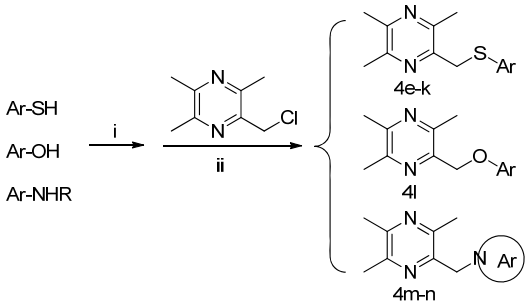
7-((3,5,6-trimethylpyrazin-2-yl)methoxy)-2H-chromen-2-one (4c)

White solid, yield: 53%, mp: 135-140 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.63 (d, 1H, *J*=9.6 Hz, C=CH=Ar), 7.38-7.37 (m, 1H, Ar-H), 6.98-6.97 (m, 1H, Ar-H), 6.94-6.92 (m, 1H, Ar-H), 6.26 (d, 1H, *J* = 9.6 Hz, C=CH-C=O), 5.22 (s, 2H, CH_2), 2.59 (s, 3H, CH_3), 2.53 (s, 6H, CH_3×2). IR (KBr, cm^{-1}): 3065.88 (C=C-H), 2951.77, 2914.56

(CH_3), 1721.57 (C=O), 1616.63 (C=C), 1559.49, 1511.01 (C=N, C=C). ESI-MS: 297.6 $(M+H)^+$, for $C_{17}H_{16}N_2O_3$ 296.12.

4-methyl-7-((3,5,6-trimethylpyrazin-2-yl)methoxy)-2H-chromen-2-one (4d)

Yellow solid, yield: 54%, mp: 140-144 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.51-7.49 (m, 1H, Ar-H), 6.97-6.94 (m, 2H, Ar-H), 6.15-6.14 (m, 1H, C=CH-C=O), 5.22 (s, 1H, CH_2), 2.59-2.57 (m, 3H, CH_3), 2.53-2.50 (m, 6H, CH_3×2), 2.40 (s, 3H, CH_3-C=CH). IR (KBr, cm^{-1}): 3053.11 (C=C-H), 2971.73, 2922.30 (CH_3), 1723.02 (C=O), 1613.46 (C=C), 1561.98, 1510.57 (C=N, C=C). ESI-MS: 311.5 $(M+H)^+$, for $C_{18}H_{18}N_2O_3$ 310.13.



Scheme 7: Reagents and conditions: (i) NaOH aq, C_2H_5OH, r.t., 5 min, (ii) r.t., 4 h

General procedure for the preparation of ligustrazinyl heterocycle derivatives (4 e-n)

To the ethanol solution of heterocyclic mercaptan or heterocyclic alcohol or heterocyclic amine (6 mmo1) was added NaOH aqueous solution (3 mL, 2 mo1/L) dropwise, the reaction mixture was stirred for 5 min. 2-chloromethyl-3,5,6-trimethylpyrazine (6 mmol) in ethanol was then added into the mixture dropwise, and the reaction mixture was stirred at 25 ºC for 4 h. The final product was purified by recrystallization from ethanol.

3-(thiophen-2-yl)-5-((3,5,6-trimethylpyrazin-2-yl)methylthio)-4H-1,2,4-triazol-4-amine (4e)

White needle crystals, yield: 54%, mp: 139-142 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.92 (1H, thiophene-H), 7.34 (1H, thiophene-H), 7.22 (1H, thiophene-H), 6.24 (s, 2H, NH_2), 4.51 (s, 2H, -CH_2-), 2.49 (s, 3H, -CH_3), 2.41 (s, 3H, -CH_3), 2.37 (s, 3H, -CH_3). ^{13}C-NMR ($CDCl_3$, δ ppm): 152.27, 150.59 (triazole-C), 149.99, 148.51, 148.11, 145.97 (pyrazine-C), 128.70, 127.83, 127.80, 127.68 (thiophene-C), 35.45 (-CH_2-), 21.26 (-CH_3), 21.04 (-CH_3), 20.66 (-CH_3). IR (KBI, cm^{-1}): 2948, 2918 (NH_2), 1443, 1416 (C=N). ESI-MS: m/z 333.5 (M+1), for $C_{14}H_{16}N_6S_2$ 332.09.

2-(4-methyl-2-((3,5,6-trimethylpyrazin-2-yl)methylthio)thiazol-5-yl)acetic acid (4f)

White needle crystals, yield: 30%, mp: 144-147 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.26 (s, 1H, -COOH), 6.24 (s, 2H, NH_2), 5.70 (s, 2H, -CH_2-COOH), 4.42 (s, 2H, -S-CH_2), 2.64 (s, 3H, -CH_3), 2.55 (s, 3H, -CH_3), 2.53 (s, 3H, -CH_3), 2.20 (s, 3H, -CH_3). ^{13}C-NMR ($CDCl_3$, δ ppm): 152.27, 150.59 (triazole-C), 149.99, 148.51, 148.11, 145.97 (pyrazine-C), 128.70, 127.83, 127.80, 127.68 (thiophene-C), 35.45 (-CH_2-), 21.26 (-CH_3), 21.04 (-CH_3), 20.66 (-CH_3). IR (KBr, cm^{-1}): 2920 (CH_2), 1705 (C=O), 1443, 1416 (C=N). ESI-MS: m/z 324.5 (M+1), for $C_{14}H_{17}N_3O_2S_2$ 323.43.

2,3,5-trimethyl-6-((1-methyl-1H-tetrazol-5-ylthio)methyl)pyrazine (4g)

Brownish yellow crystals, yield: 34%, mp: 111-113 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 4.71 (s, 2H, -S-CH_2-), 3.94 (s, 3H, -CH_3), 2.59 (s, 3H, -CH_3), 2.49 (s, 3H, -CH_3), 2.47 (s, 3H, -CH3). ^{13}C-NMR ($CDCl_3$, δ ppm): 153.95 (tetrazole), 150.90, 149.18, 144.33 (pyrazine-C), 37.03 (-CH_3), 33.52 (-CH_2-), 21.57 (-CH_3), 21.32 (-CH_3), 20.90 (-CH_3). IR (KBr, cm^{-1}): 2945, 2909 (CH_2), 1456, 1416 (C=N). ESI-MS: m/z 251.5 (M+1) , for $C_{10}H_{14}N_6S$ 250.32.

2-((3,5,6-trimethylpyrazin-2-yl)methylthio)benzo[d]thiazole (4h)

Brownish yellow crystals, yield: 30%, mp: 154-155 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.90 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.77 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.43 (t, 1H, *J* = 8.2 Hz, Ar-H), 7.31 (t, 1H, *J* = 7.6 Hz, Ar-H), 4.77 (s, 2H, -CH_2-), 2.65 (s, 3H, -CH_3), 2.50 (s, 6H, -CH_3). ^{13}C-NMR ($CDCl_3$, δ ppm): 166.10 (-N=C-S-), 150.59, 149.18, 148.56, 145.50 (pyrazine-C), 153.10, 135.47, 126.17, 124.51, 121.56, 121.10 (benzene-C), 36.83 (-CH_2-), 21.63 (-CH_3), 21.41 (-CH_3), 21.14 (-CH_3). IR (KBr, cm^{-1}):

2986, 2909 (CH_2), 1627, 1557, 1459 (C=C), 1432, 1412 (C=N). ESI-MS: m/z 302.5 (M+1), for $C_{15}H_{15}N_3S_2$ 301.43.

3-(furan-2-yl)-5-((3,5,6-trimethylpyrazin-2-yl)methylthio)-4H-1,2,4-triazol-4-amine (4i)

Light yellow crystals, yield: 13%, mp: 166-169 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.60 (1H, furane), 7.25 (1H, furane), 6.58 (1H, furane), 5.23 (s, 2H, -NH_2-), 4.52 (s, 2H, -CH_2-), 2.59 (s, 3H, -CH_3), 2.47 (s, 3H, -CH_3), 2.42 (s, 3H, -CH_3). ^{13}C-NMR ($CDCl_3$, δ ppm): 151.63, 150.71, 148.78, 148.53 (pyrazine-C), 147.68, 145.24, 144.04, 141.55 (furane-C), 112.07, 111.65 (triazole-C), 36.42 (-CH_2-), 21.54 (-CH_3), 21.15 (-CH_3), 20.98 (-CH_3). IR (KBr, cm^{-1}): 3136 (=CH), 2950, 2918 (CH_2), 1642, 1517 (C=C), 1416 (C=N). ESI-MS: m/z 317.4 (M+1), for $C_{14}H_{16}N_6OS$ 316.38.

2,3,5-trimethyl-6-((1-phenyl-1H-tetrazol-5-ylthio)methyl)pyrazine (4j)

Soil-yellow needle crystals, yield: 48%, mp: 131-135 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.61-7.54 (m, 4H, benzene-H), 4.80 (s, 2H, -CH_2-), 2.62 (s, 3H, -CH_3), 2.50 (s, 3H, -CH_3), 2.46 (s, 3H, -CH_3). ^{13}C-NMR ($CDCl_3$, δ ppm): 154.35 (tetrazole-C), 151.16, 149.44, 148.52, 144.45 (pyrazine-C), 133.89, 130.44, 130.31, 130.18, 130.10, 124.11 (benzene-C), 37.28 (-CH_2-), 21.85 (-CH_3), 21.59 (-CH_3), 21.25 (-CH_3). IR (KBr, cm^{-1}): 3059 (=CH_2), 2954, 2918 (CH_2), 1595, 1501, 1448 (C=C), 1415 (C=N). ESI-MS: m/z 313.4 (M+1), for $C_{15}H_{16}N_6S$ 312.39.

2,3,5-trimethyl-6-((1-(naphthalen-1-yl)-1H-tetrazol-5-ylthio)methyl)pyrazine (4k)

Brownish yellow crystals, yield: 60%, mp: 166-169 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 8.10 (d, 1H, J = 8.2 Hz, phenanthrene-H), 7.99 (d, 1H, J = 8.2 Hz, phenanthrene-H), 7.61 (t, 2H, J = 7.4 Hz, phenanthrene-H), 7.56 (t, 2H, J = 6.4 Hz, phenanthrene-H), 7.32 (d, 1H, J = 8.4 Hz, phenanthrene-H), 4.79 (s, 2H, -CH_2-), 2.59 (s, 3H, -CH_3), 2.46 (s, 3H, -CH_3), 2.39 (s, 3H, -CH_3). ^{13}C-NMR ($CDCl_3$, δ ppm): 156.53 (tetrazole-C), 150.81, 149.07, 148.18, 144.18 (pyrazine-C), 134.28, 131.73, 129.25, 128.71, 128.46, 128.26, 127.42, 125.06, 125.01 (phenanthrene-C), 36.81 (-CH_2-), 21.56 (-CH_3), 21.24 (-CH_3), 21.00 (-CH_3). IR (KBr, cm^{-1}): 3059 (=CH), 2985, 2912 (CH_2), 1597, 1511, 1469 (C=C), 1446, 1419 (C=N). ESI-MS: m/z 363.5 (M+1), for $C_{19}H_{18}N_6S$ 362.45.

5-(methylthio)-3-((3,5,6-trimethylpyrazin-2-yl)methoxy)isothiazole-4-carbonitrile (4l)

Yellowish-white crystals, yield: 16%, mp: 151-153 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 5.50 (2H, s, -CH_2-), 2.67 (3H, s, -S-CH_3), 2.60 (3H, s, -CH_3), 2.54 (3H, s, -CH_3), 2.52 (3H, s, -CH_3). ^{13}C-NMR ($CDCl_3$, δ ppm): 175.93, 167.15, 93.56 (isothiazole-C), 151.90, 149.78, 149.03, 144.14 (pyrazine-C), 111.23 (-CN), 70.42 (-CH_2-), 21.79 (-CH_3), 21.44 (-CH_3), 20.69 (-CH_3). IR (KBr, cm^{-1}): 2989, 2916 (CH_2), 2224 (CN), 1445, 1413 (C=N). ESI-MS: m/z 307.4 (M+1), for $C_{13}H_{14}N_4OS_2$ 306.41.

1-((3,5,6-trimethylpyrazin-2-yl)methyl)-1H-benzo[d][1,2,3]triazole (4m)

Brownish yellow crystals, yield: 33%, mp: 158-161 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 8.04 (d, 1H, *J*=9.1Hz, Ar-H), 7.66 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.43 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.34 (t, 1H, *J* = 8.17 Hz, Ar-H), 5.94 (s, 2H, -CH_2-), 2.57 (s, 3H, -CH_3), 2.50 (s, 3H, -CH_3), 2.48 (s, 3H, -CH_3). ^{13}C-NMR ($CDCl_3$, δ ppm): 151.79, 149.26, 146.16, 143.79 (pyrazine-C), 149.15, 133.20, 127.35, 123.96, 119.87, 110.62 (Ar-C), 52.27 (-CH_2-), 21.68 (-CH_3), 21.51 (-CH_3), 20.83 (-CH_3). IR (KBr, cm^{-1}): 3057 (=CH), 2997, 2942, 2915 (CH_2), 1607, 1496, 1459 (C=C), 1439, 1414 (C=N). ESI-MS: m/z 254.4 (M+1), for $C_{14}H_{15}N_5$ 253.30.

1-((3,5,6-trimethylpyrazin-2-yl)methyl)-1H-benzo[d]imidazole (4n)

Light yellow needle crystals, yield: 55%, mp: 100-102 ºC. ^{1}H-NMR ($CDCl_3$, δ ppm): 8.02 (s, 1H, N=CH), 7.81 (m, 1H, Ar-H), 7.46 (m, 1H, Ar-H), 7.28 (m, 2H, Ar-H), 5.43 (s, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.47 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3075.96 (Ar-H), 2944.53 (CH), 1614.19 (C=C). ESI-MS: 253.3 (M+1), for $C_{15}H_{16}N_4$ 252.31.