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

Hongfei Chen a, Guoning Li a, Peng Zhan a, Xiuli Guo b, Qian Ding c, Shouxun Wang c, Xinyong Liu a *

a Department of medicinal Chemistry, Key Laboratory of Chemical Biology (Educational Ministry of China), School of Pharmaceutical Sciences, Shandong University, No.44 Wenhuaxi Road, Jinan 250012, China

b Department of Pharmacology, School of Pharmaceutical Sciences, Shandong University, No. 44, Wenhuaxi Road, Jinan 250012, China

c Department of Biological Chemistry and Molecular Biology, School of Medicine, Weifang Medical University, No.7166, Baotongxi Road, Weifang 261053, China

Prof. Xinyong Liu (Corresponding Author)
Professor of Medicinal Chemistry
School of Pharmaceutical Sciences
Shandong University
Tel.: +86 531 88380270; fax: +86 531 88382731.
E-mail address: xinyongl@sdu.edu.cn
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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 rpm/min for 5 min. The PRP samples were again centrifuged at 3000 rpm/min for 15 min to obtain platelet-poor plasma (PPP). The platelets of the PRP were adjusted to $3 \times 10^{11}$/L for the aggregation assay. All platelet preparations were conducted at 25 °C. 90 μL PRP and 5 μL sample solution (final concentration: 400, 200, 100, 50 μM) was added into the microplate and incubated at 37 °C 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/ARcontrol)]×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 $6 \times 10^3$ /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$_2$O$_2$.
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% CO₂ 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 H₂O₂. 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 \[
\frac{\text{OD}_{570 (\text{Compd})}-\text{OD}_{570 (\text{H}_2\text{O}_2)}}{\text{OD}_{570 (\text{Control})}-\text{OD}_{570 (\text{H}_2\text{O}_2)}}\times100% ,
\]
which was then used to obtain EC₅₀ values.

<table>
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<th>Compd.</th>
<th>Structure</th>
<th>Compd.</th>
<th>Structure</th>
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<td><img src="image" alt="Structure" /></td>
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<td><img src="image" alt="Structure" /></td>
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<td>4a</td>
<td><img src="image" alt="Structure" /></td>
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Table 2: The IC\textsubscript{50} for inhibition of platelet aggregation.

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<th>(\text{AIR(100,\mu M)})</th>
<th>(\text{AIR(50,\mu M)})</th>
<th>IC\textsubscript{50}\textsuperscript{a} (mM)</th>
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<tr>
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<td>Compd.</td>
<td>Proliferation rate (%)</td>
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</tr>
<tr>
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<td>20% 19.2% 18.1% 17.3%</td>
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Table 3: The EC$_{50}$ values for protection on damaged ECV-304 cells and $P\%$ at different concentration of the ligustrazinylated derivatives.

$^a$I$_{C50}$: concentration of compound required to achieve 50% inhibition on aggregation of rabbit platelet.
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<td>17.2%</td>
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<td>-393%</td>
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<tr>
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<td>0.36</td>
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<td>/</td>
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<td>4n</td>
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<tr>
<td>lipoic acid</td>
<td>58.1%</td>
<td>48.7%</td>
<td>-12.3%</td>
<td>30.5%</td>
<td>0.068</td>
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EC<sub>50</sub>: concentration of compound required to achieve 50% protection of ECV-304 cell from H<sub>2</sub>O<sub>2</sub> induced cytotoxicity, as determined by the MTT method.

**Synthesis**

**Materials and Methods**

Melting points were determined on a Gallenkamp capillary apparatus and are uncorrected. ¹H-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](image_url)

**Scheme 1**: Reagents and conditions: (i) EtOH, SOCl₂, reflux for 12 h, (ii) K₂CO₃, TBAB, DMF, 60 °C, 6 h, (iii) 20% NaOH, 5 h

**General procedure for the preparation of ligustrazineoxy-benzoic acid esters (1 a-b,
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$_2$CO$_3$ (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$_2$SO$_4$, 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$_3$, $\delta$ 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, CH$_2$OAr), 4.30 (dd, 2H, $J$ = 14.4 Hz, $J$ = 7.2 Hz, 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, 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$_2$O$_3$ 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$_3$, $\delta$ 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 (-OCH$_3$), 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$_2$O$_4$ 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 °C 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 °C. 1H-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, CH2), 2.53-2.52 (m, 9H, CH3×3). IR (KBr, cm⁻¹): 3197.48 (OH), 3074.72 (Ar-H),
2922.26 (CH3), 1712.87 (C=O), 1603.10, 1583.08 (C=N, C=C). ESI-MS: 273.3 (M+H)+, for C15H16N2O3 272.12.

3-methoxy-4-((3,5,6-trimethylpyrazin-2-yl)methoxy)benzoic acid (1d)
White needle crystals, yield: 81%, mp: 190-194 °C. 1H-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, -CH2-O-), 3.43 (s, 3H, -OCH3), 2.41 (s, 3H, -CH3), 2.36 (s, 6H, 2-CH3). IR (KBr, cm⁻¹): 3484.89 (OH), 2954.67 (CH3), 2828.27 (-OCH3), 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 C16H18N2O4 302.13.

Scheme 2: Reagents and conditions: (i) anhydrous DMF, NaH, N2, 0 °C, 1 h, (ii) 60 °C, 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 °C 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, $\delta$ 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$$\times$3), 2.24-2.18 (m, 2H, CH$_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}$N$_4$O$_2$ 276.16.

Scheme 3: Reagents and conditions: (i) K$_2$CO$_3$, DMF, 60 ºC, 3 h

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

K$_2$CO$_3$ (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$_3$, $\delta$ 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$_2$O$_2$S$_2$ 340.13.
Scheme 4: Reagents and conditions: (i) C₂H₅OH, thiourea, reflux for 3 h, (ii) NaOH aq, reflux for 2 h, (iii) NaHCO₃, 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 NaHCO₃. 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%. ¹H-NMR (CDCl₃, δ ppm): 7.63 (d, 1H, J=9.6 Hz, =CH₂), 7.37 (d, 1H, J = 8.4 Hz, =CH₂), 6.97-6.92 (m, 2H, -S-CH₂-CH=), 6.26 (d, 1H, J = 9.6 Hz, =CH-), 5.22 (s, 2H, -S-CH₂-), 2.59 (s, 9H, -CH₃×3). IR (KBr, cm⁻¹): 3080.82 (C=C-H), 2978.40, 2947.28, 2919.59 (CH₃), 2856.45 (-SCH₂), 1634.14 (C=C), 1545.36 (C=N, C=C). ESI-MS: 241.3 (M+H)+, for C₁₁H₁₆N₂S₃ 240.08.

Scheme 5: Reagents and conditions: (i) K₂CO₃, TBAB, DMF, 60 ºC, 6 h

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

K₂CO₃ (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 (CDCl₃, 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, -CH₂-O-), 5.06 (d, 2H, J=1.2 Hz, =CH₂), 3.83 (s, 3H, -OCH₃), 3.33 (s, 2H, Ar-CH₂-), 2.62 (s, 3H, -CH₃), 2.50 (s, 6H, 2-CH₃). IR (KBr, cm⁻¹): 3078.44 (C=C-H), 2999.77, 2933.39 (CH₃), 2897.59, 2828.00 (-OCH₃), 1638.85 (C=C), 1591.29, 1513.41 (C=N, C=C). ESI-MS: 299.7 (M+H)⁺, for C₁₈H₂₂N₂O₂ 298.17.

![Scheme 6: Reagents and conditions: (i) K₂CO₃, TBAB, DMF, 60 ºC, 6 h](image)

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

K₂CO₃ (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 (CDCl₃, 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, CH₂), 2.59 (s, 3H, CH₃), 2.53 (s, 6H, CH₃×2). IR (KBr, cm⁻¹): 3065.88 (C=C-H), 2951.77, 2914.56
(CH₃), 1721.57 (C=O), 1616.63 (C=C), 1559.49, 1511.01 (C=N, C=C). ESI-MS: 297.6 (M+H)⁺, for C₁₇H₁₆N₂O₃ 296.12.

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

Yellow solid, yield: 54%, mp: 140–144 °C. ¹H-NMR (CDCl₃, 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, CH₂), 2.59–2.57 (m, 3H, CH₃), 2.53–2.50 (m, 6H, CH₃×2), 2.40 (s, 3H, CH₃-C=CH). IR (KBr, cm⁻¹): 3053.11 (C=C-H), 2971.73, 2922.30 (CH₃), 1723.02 (C=O), 1613.46 (C=C), 1561.98, 1510.57 (C=N, C=C). ESI-MS: 311.5 (M+H)⁺, for C₁₈H₁₈N₂O₃ 310.13.

**Scheme 7:** Reagents and conditions: (i) NaOH aq, C₂H₅OH, 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 mmol) was added NaOH aqueous solution (3 mL, 2 mol/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 °C 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 °C. $^1$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 (KBr, cm$^{-1}$): 2948, 2918 (NH$_2$), 1443, 1416 (C=N). ESI-MS: m/z 333.5 (M+1), for C$_{14}$H$_{16}$N$_6$S$_2$ 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 °C. $^1$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$_3$O$_2$S$_2$ 323.43.

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

Brownish yellow crystals, yield: 34%, mp: 111-113 °C. $^1$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, -CH$_3$). $^{13}$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$_6$S 250.32.

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

Brownish yellow crystals, yield: 30%, mp: 154-155 °C. $^1$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$^{-1}$):
3-(furan-2-yl)-5-((3,5,6-trimethylpyrazin-2-yl)methylthio)-4H-1,2,4-triazol-4-am
ine (4i)
Light yellow crystals, yield: 13%, mp: 166-169 ºC. 1H-NMR (CDCl3, δ ppm): 7.60
(1H, furane), 7.25 (1H, furane), 6.58 (1H, furane), 5.23 (s, 2H, -NH2-), 4.52 (s, 2H,
-CH2-), 2.59 (s, 3H, -CH3), 2.47 (s, 3H, -CH3), 2.42 (s, 3H, -CH3). 13C-NMR (CDCl3,
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 (-CH2-), 21.54 (-CH3), 21.15 (-CH3),
20.98 (-CH3). IR (KBr, cm-1): 3136 (=CH), 2950, 2918 (CH2), 1642, 1517 (C=N). ESI-MS: m/z 317.4 (M+1), for C14H16N6OS 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. 1H-NMR (CDCl3, δ ppm):
7.61-7.54 (m, 4H, benzene-H), 4.80 (s, 2H, -CH2-), 2.62 (s, 3H, -CH3), 2.50 (s, 3H,
-CH3), 2.46 (s, 3H, -CH3). 13C-NMR (CDCl3, 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 (-CH2-), 21.85 (-CH3), 21.59 (-CH3), 21.25 (-CH3). IR (KBr, cm-1):
3059 (=CH2), 2954, 2918 (CH2), 1595, 1501, 1448 (C=C), 1415 (C=N). ESI-MS: m/z 313.4 (M+1), for C14H16N6OS 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. 1H-NMR (CDCl3, 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, -CH2-), 2.59 (s, 3H, -CH3),
2.46 (s, 3H, -CH3), 2.39 (s, 3H, -CH3). 13C-NMR (CDCl3, 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 (-CH2-),
21.56 (-CH3), 21.24 (-CH3), 21.00 (-CH3). IR (KBr, cm-1): 3059 (=CH2), 2985, 2912
(CH2), 1597, 1511, 1469 (C=C), 1446, 1419 (C=N). ESI-MS: m/z 363.5 (M+1), for
C19H18N6OS 362.45.
5-(methylthio)-3-((3,5,6-trimethylpyrazin-2-yl)methoxy)isothiazole-4-carbonitrile (4l)

Yellowish-white crystals, yield: 16%, mp: 151-153 °C. $^1$H-NMR (CDCl$_3$, $\delta$ 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$_3$, $\delta$ 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$_4$O$_2$ 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 °C. $^1$H-NMR (CDCl$_3$, $\delta$ 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$_3$, $\delta$ 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 °C. $^1$H-NMR (CDCl$_3$, $\delta$ 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$_{18}$H$_{16}$N$_4$ 252.31.