Supplementary Information

Quinazoline-4(3H)-one-7-carboxamide Derivatives as Human Soluble Epoxide Hydrolase (sEH) Inhibitors with Developable 5-Lipoxygenase Activating Protein (FLAP) Inhibition

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Synthesis of Intermediate Compounds

**Methyl 4-oxo-3-phenyl-2-thioxo-1,2,3,4-tetrahydroquinazoline-7-carboxylate (7)**

To a mixture of dimethylaminoterephthalate (4.78 mmol, 1 eq) in pyridine (8 mL), phenyl isothiocyanate (5.28 mmol, 1.1 eq) was added and stirred for 8 hours at 100 °C. Pyridine was evaporated and reaction mixture was diluted with water. The solid was filtered off under vacuum and dried. The crude product was washed with ethyl acetate. Yield 66%; mp >300 °C (decomp). 1H NMR (400 MHz, DMSO-d$_6$): δH 3.92 (3H, s), 7.29 (2H, d, J = 7.8 Hz), 7.39-7.50 (3H, m), 7.82 (1H, d, J = 7.8 Hz), 8.02-8.07 (2H, m), 13.18 (1H, s); HRMS m/z calculated for C$_{16}$H$_{13}$N$_2$O$_3$S [M+H]$^+$ 313.0647, found: 313.0633. CAS #514857-29-5.

**Methyl 2-((2-chlorobenzyl)thio)-4-oxo-3-phenyl-3,4-dihydroquinazoline-7-carboxylate (8)**

To a solution of Compound 7 (1.2 mmol, 1 eq) in DMF (2 mL), Cs$_2$CO$_3$ (3 mmol, 2.5 eq) and 2-chlorobenzyl bromide (1.2 mmol, 1 eq) were added and the reaction mixture was stirred at room temperature for 3 hours. The mixture was diluted with water and acidified to pH 5 with 2N HCl. The solid was filtered under vacuum. The crude product was used in the next step. Yield 94%; mp 180.5-181.9 °C. 1H NMR (400 MHz, DMSO-d$_6$): δH 3.95 (3H, s), 4.55 (2H, s), 7.28-7.32 (2H, m), 7.43-7.48 (3H, m), 7.55-7.57 (3H, m), 7.66-7.69 (1H, m), 7.97 (1H, dd, J = 8.4, 1.6 Hz), 8.19-8.22 (2H, m); HRMS m/z calculated for C$_{23}$H$_{18}$N$_2$O$_3$SCl [M+H]$^+$ 437.0714, found: 437.0727.

**Methyl 4-oxo-3-phenyl-2-((4-(trifluoromethyl)benzyl)thio)-3,4-dihydroquinazoline-7-carboxylate (9)**

Prepared from Compound 7 and 4-(trifluoromethyl)benzyl bromide under the same conditions that applied to Compound 8. Yield 81%; mp 174.9-176.7 °C. 1H NMR (400 MHz, DMSO-d$_6$): δH 3.95 (3H, s), 5.52 (2H, s), 7.46-7.49 (2H, m), 7.55-7.58 (3H, m), 7.65-7.70 (4H, m), 7.97 (1H, dd, J = 8.4, 1.6 Hz), 8.18-8.20 (2H, m); HRMS m/z calculated for C$_{24}$H$_{18}$N$_2$O$_3$SF$_3$ [M+H]$^+$ 471.0984, found: 471.0990.

**2-Aminoterephthalic acid (15)**

Dimethylaminoterephthalate (9.56 mmol, 1 eq) and LiOH.H$_2$O (23.92 mmol, 2.5 eq) were dissolved in THF:H$_2$O (3:3 mL) and stirred under reflux for 2 hours. THF was evaporated, the reaction mixture diluted with water and the pH was adjusted to 5 with HCl. The solid was filtered under vacuum and dried. The crude product was used in the next step. Yield 95%; mp
To a solution of Compound 15 (9 mmol, 1 eq) in MeOH (4 mL), chloromethylsilane (13.5 mmol, 1.5 eq) was added and refluxed for 4 hours. After cooling at rt, the reaction mixture was concentrated and saturated aqueous solution of K₂CO₃ were added, and the solution was extracted with ethyl acetate. The aqueous layer was acidified at pH 5 with acetic acid and extracted with ethyl acetate. The organic layer was dried, filtered, and evaporated. The crude was used in the next step. Yield 80%; mp 218.2-220.2°C. HRMS m/z calculated for C₉H₁₀NO₄ [M+H]+ 196.0610, found 196.0617. CAS #85743-02-8.

Methyl 4-oxo-2-thioxo-1,2,3,4-tetrahydroquinazoline-7-carboxylate (17)

Step 1: Compound 16 (5.584 mmol, 1 eq) in SOCl₂ (3.5 mL) was refluxed for 3 hours. The reaction mixture was then concentrated in vacuo. The obtained methyl-3-amino-4-(chlorocarbonyl) benzoate was used in the next step. Step 2: Methyl-3-amino-4-(chlorocarbonyl) benzoate (5.584 mmol, 1 eq) was dissolved in acetone (4 mL) and added dropwise to a suspension of NH₄SCN (437.8 mg, 5.752 mmol, 1.03 eq) in acetone (2mL). The reaction mixture was stirred at rt for 2 hours and filtered off under vacuum. This solid was then suspended in an aqueous solution of NaOH (10% w/w, 5 mL), stirred and filtered off under vacuum. Water was added to the residue and mixture was acidified to pH 2 with aqueous 2N HCl, the solid was filtered under vacuum. The compound was used in the next step. Yield 60%; mp 269.2-271.2°C. HRMS m/z calculated for C₁₀H₉N₂O₃S [M+H]+ 237.0334, found: 237.0339. CAS #422277-15-4.
8.58 (1H, d, J = 6.4 Hz), 12.54 (1H, s), 12.80 (1H, s); HRMS m/z calculated for C_{14}H_{18}N_{3}O_{2}S [M+H]^+ 292.1120, found: 292.1132.

**Method B: General synthesis method for the alkylation of quinazolinone-7-carboxylic acids**

Compound 17 (1.5 mmol, 1 eq) was dissolved in EtOH (3 mL) and 1N NaOH (1.5 mL), appropriate benzyl bromide derivate (1.5 mmol, 1 eq) was added dropwise and refluxed for 2 hours. The mixture was diluted with water and acidified with 2N HCl to pH 3. The solid was filtered under vacuum. The compounds were used in the next step.

2-((2-Chlorobenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (22)

It was prepared according to Method B. Yield 85%; mp 280°C (decomp). 1H NMR (500 MHz, DMSO-d$_6$): δH 4.62 (2H, s), 7.31-7.34 (2H, m), 7.48-7.52 (1H, m), 7.70-7.74 (1H, m), 7.91 (1H, dd, J = 8.3, 1.4 Hz), 8.11-8.13 (2H, m), 12.79 (1H, s), 13.47 (1H, s). 13C-NMR (125 MHz, DMSO-d$_6$): δC 32.10, 123.44, 125.99, 127.14, 127.58, 129.89, 129.93, 132.14, 133.88, 135.22, 136.76, 148.59, 156.46, 161.18, 166.98. HRMS (m/z) [M+H]^+ calculated for C$_{16}$H$_{12}$ClN$_2$O$_3$S [M+H]^+ 347.0257, found: 347.0259.

2-((3-Chlorobenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (23)

Prepared from Compound 17 and 3-chlorobenzyl bromide under the same condition that was used in Method B. Yield 71%; mp > 300.0 °C (decomp.). 1H NMR (400 MHz, DMSO-d$_6$): δH 4.51 (2H, s), 7.30-7.42 (2H, m), 7.47 (1H, d, J = 7.6 Hz) 7.60 (1H, m), 7.91 (1H, dd, J = 8.0, 1.2 Hz), 8.08 (1H, d, J = 1.2 Hz), 8.12 (1H, d, J = 8.0 Hz), 12.90 (1H, s), 13.40 (1H, bs); HRMS m/z calculated for C$_{16}$H$_{12}$ClN$_2$O$_3$S [M+H]^+ 347.0251, found: 347.0257.

2-((4-Chlorobenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (24)

Prepared from Compound 17 and 4-chlorobenzyl bromide under the same condition that was used in Method B. Yield 91%; mp 310.1 °C (decomp.). 1H NMR (400 MHz, DMSO-d$_6$): δH 4.50 (2H, s), 7.38 (2H, d, J = 8.4 Hz), 7.52 (2H, d, J = 8.4 Hz), 7.90 (1H, dd, J = 8.0, 1.6 Hz), 8.07 (1H, d, J = 1.6 Hz), 8.11 (1H, d, J = 8.0 Hz), 12.78 (1H, s); HRMS m/z calculated for C$_{16}$H$_{12}$N$_2$O$_3$S [M+H]^+ 347.0257, found: 347.0248.

4-oxo-2-((2-(trifluoromethyl)benzyl)thio)-3,4-dihydroquinazoline-7-carboxylic acid (25)

Prepared from Compound 17 and 2-(trifluoromethyl)benzyl bromide under the same condition that was used in Method B. Yield 71%; mp 329.1 °C (decomp.). 1H NMR (400 MHz, DMSO-d$_6$): δH 4.72 (2H, s), 7.52 (1H, t, J = 7.8 Hz), 7.66 (1H, t, J = 7.8 Hz), 7.76 (1H, d, J = 7.8 Hz), 7.85 (1H, d, J = 7.8 Hz), 7.91 (1H, dd, J = 8.0, 1.4 Hz), 8.07 (1H, d, J = 1.4 Hz), 8.13 (1H, d, J = 8.0 Hz), 12.79 (1H, s), 13.47 (1H, s). HRMS m/z calculated for C$_{16}$H$_{12}$N$_2$O$_3$S [M+H]^+ 347.0257, found: 347.0257.

2-((2-Chlorobenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (22)

It was prepared according to Method B. Yield 85%; mp 280°C (decomp). 1H NMR (500 MHz, DMSO-d$_6$): δH 4.62 (2H, s), 7.31-7.34 (2H, m), 7.48-7.52 (1H, m), 7.70-7.74 (1H, m), 7.91 (1H, dd, J = 8.3, 1.4 Hz), 8.11-8.13 (2H, m), 12.79 (1H, s), 13.47 (1H, s). 13C-NMR (125 MHz, DMSO-d$_6$): δC 32.10, 123.44, 125.99, 127.14, 127.58, 129.89, 129.93, 132.14, 133.88, 135.22, 136.76, 148.59, 156.46, 161.18, 166.98. HRMS (m/z) [M+H]^+ calculated for C$_{16}$H$_{12}$ClN$_2$O$_3$S [M+H]^+ 347.0257, found: 347.0259.

2-((3-Chlorobenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (23)

Prepared from Compound 17 and 3-chlorobenzyl bromide under the same condition that was used in Method B. Yield 71%; mp > 300.0 °C (decomp.). 1H NMR (400 MHz, DMSO-d$_6$): δH 4.51 (2H, s), 7.30-7.42 (2H, m), 7.47 (1H, d, J = 7.6 Hz) 7.60 (1H, m), 7.91 (1H, dd, J = 8.0, 1.2 Hz), 8.08 (1H, d, J = 1.2 Hz), 8.12 (1H, d, J = 8.0 Hz), 12.90 (1H, s), 13.40 (1H, bs); HRMS m/z calculated for C$_{16}$H$_{12}$ClN$_2$O$_3$S [M+H]^+ 347.0251, found: 347.0257.

2-((4-Chlorobenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (24)

Prepared from Compound 17 and 4-chlorobenzyl bromide under the same condition that was used in Method B. Yield 91%; mp 310.1 °C (decomp.). 1H NMR (400 MHz, DMSO-d$_6$): δH 4.50 (2H, s), 7.38 (2H, d, J = 8.4 Hz), 7.52 (2H, d, J = 8.4 Hz), 7.90 (1H, dd, J = 8.0, 1.6 Hz), 8.07 (1H, d, J = 1.6 Hz), 8.11 (1H, d, J = 8.0 Hz), 12.78 (1H, s); HRMS m/z calculated for C$_{16}$H$_{12}$ClN$_2$O$_3$S [M+H]^+ 347.0257, found: 347.0248.

4-oxo-2-([2-(trifluoromethyl)benzyl]thio)-3,4-dihydroquinazoline-7-carboxylic acid (25)

Prepared from Compound 17 and 2-(trifluoromethyl)benzyl bromide under the same condition that was used in Method B. Yield 71%; mp 329.1 °C (decomp.). 1H NMR (400 MHz, DMSO-d$_6$): δH 4.72 (2H, s), 7.52 (1H, t, J = 7.8 Hz), 7.66 (1H, t, J = 7.8 Hz), 7.76 (1H, d, J = 7.8 Hz), 7.85 (1H, d, J = 7.8 Hz), 7.91 (1H, dd, J = 8.0, 1.4 Hz), 8.07 (1H, d, J = 1.4 Hz), 8.13 (1H, d, J
= 8.0 Hz), 12.83 (1H, s); HRMS m/z calculated for C$_{17}$H$_{12}$N$_2$O$_3$SF$_3$ [M+H]$^+$ 381.0521, found: 381.0506.

2-((3,4-dichlorobenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (26)

Prepared from Compound 17 and 3,4-dichlorobenzyl chloride under the same condition that was used in Method B. Yield 75%; mp 258.1 °C (decomp.). $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$H 4.50 (2H, s), 7.51 (1H, dd, $J$ = 8.4, 2.0 Hz), 7.57 (1H, d, $J$ = 8.4 Hz), 7.81 (1H, d, $J$ = 2.0 Hz), 7.90 (1H, dd, $J$ = 8.4, 1.4 Hz), 8.09 (1H, d, $J$ = 1.4 Hz), 8.11 (1H, d, $J$ = 8.4 Hz), 12.79 (1H, s); HRMS m/z calculated for C$_{18}$H$_{12}$N$_2$O$_3$SCl$_2$ [M+H]$^+$ 380.9667, found: 380.9662.

2-((2-fluorobenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (27)

Prepared from Compound 17 and 2-fluorobenzyl bromide under the same condition that was used in Method B. Yield 74%; mp 210.0 °C (decomp.). $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$H 4.53 (2H, s), 7.12-7.36 (4H, m), 7.60 (1H, td, $J$ = 7.7, 1.8 Hz), 7.88 (1H, dd, $J$ = 8.2, 1.6 Hz), 8.07 (1H, d, $J$ = 1.6 Hz), 8.09 (1H, d, $J$ = 8.2 Hz), 12.76 (1H, s); HRMS m/z calculated for C$_{18}$H$_{12}$N$_2$O$_3$SF [M+H]$^+$ 335.0553, found: 335.0538.

2-((2-cyanobenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (28)

Prepared from Compound 17 and 2-cyanobenzyl bromide under the same condition that was used in Method B. Yield 87%; mp 257.0 °C (decomp). HRMS m/z calculated for C$_{17}$H$_{12}$N$_3$O$_3$S [M+H]$^+$ 338.0599, found: 338.0607.

2-((2-methylbenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (29)

Prepared from Compound 17 and 2-methylbenzyl bromide under the same condition that was used in Method B. Yield 84%; mp 252.5 °C (decomp). HRMS m/z calculated for C$_{17}$H$_{15}$N$_2$O$_3$S [M+H]$^+$ 327.0803, found: 327.0798.

2-((2-methoxybenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (30)

Prepared from Compound 17 and 2-methoxybenzyl chloride under the same condition that was used in Method B. Yield 61%; mp 230.0 °C (decomp.). $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$H 3.84 (3H, s), 4.54 (2H, s), 7.14-7.24 (2H, m), 7.30-7.35 (1H, m), 7.62 (1H, td, $J$ = 7.6, 1.6 Hz), 7.90 (1H, dd, $J$ = 8.0, 1.2 Hz), 8.09 (1H, d, $J$ = 1.2 Hz), 8.11 (1H, d, $J$ = 8.0 Hz), 12.78 (1H, s); HRMS m/z calculated for C$_{18}$H$_{13}$N$_2$O$_3$S [M+H]$^+$ 343.0753, found: 343.0751.

4-oxo-2-((2-(trifluoromethoxy)benzyl)thio)-3,4-dihydroquinazoline-7-carboxylic acid (31)

Prepared from Compound 17 and 2-(trifluoromethoxy)benzyl bromide under the same condition that was used in Method B. Yield 90%; mp 287.0 °C (decomp.). $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$H 4.60 (2H, s), 7.34-7.45 (3H, m), 7.72-7.74 (1H, m), 7.90 (1H, dd, $J$ = 8.0, 1.2 Hz),
8.08 (1H, d, J = 1.2 Hz), 8.12 (1H, d, J = 8.0 Hz), 12.90 (1H, bs); HRMS m/z calculated for C_{17}H_{12}O_{4}SF_{3}Cl [M+H]^+ 397.0470, found: 397.0473.

2-(((2-methylpyridin-3-yl)methyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (32)

Prepared from Compound 17 and 3-(chloromethyl)-2-methylpyridine under the same condition that was used in Method B. Yield 61%; mp 280.0 °C (decomp.). $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$H 2.60 (3H, s), 4.55 (2H, s), 7.18 (1H, dd, $J = 7.8, 1.2$ Hz), 7.86-7.91 (2H, m), 8.08 (1H, d, J = 1.2 Hz), 8.11 (1H, d, J = 8.0 Hz), 8.33 (1H, dd, J = 5.2, 1.2 Hz), 12.95 (1H, bs); HRMS m/z calculated for C_{16}H_{12}O_{4}SF [M+H]^+ 331.0553, found: 331.0538.

4-oxo-2-((4-(trifluoromethyl)benzyl)thio)-3,4-dihydroquinazoline-7-carboxylic acid (33)

Prepared from Compound 17 and 4-(trifluoromethyl)benzyl bromide under the same condition that was used in Method B. Yield 95%; mp 273.0 °C (decomp.). HRMS m/z calculated for C_{17}H_{12}O_{3}SF [M+H]^+ 381.0521, found 381.0520.

Tert-buty 4-((2-chlorobenzyl)thio)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (47)

Prepared from Compound 22 and N-Boc-piperazine under the same condition that was used in Method A. Yield 35%; mp 139.8-141.2 °C. $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$H 1.41 (9H, s), 3.30 (2H, bs), 3.35 (2H, bs), 3.45 (2H, bs), 3.64 (2H, bs), 4.59 (2H, s), 7.29-7.35 (2H, m), 7.41 (1H, dd, J = 8.0, 1.6 Hz), 7.48-7.50 (1H, m), 7.64 (1H, s), 7.73-7.75 (1H, m), 8.07 (1H, d, J = 8.0 Hz), 12.73 (1H, s). HRMS (m/z) calcld for C_{25}H_{28}ClN_{4}O_{5}S [M+H]^+: 515.1520, found: 515.1540.

Methyl 2,4-dioxo-1,2,3,4-tetrahydroquinazoline-7-carboxylate (51)

Compound 16 (1.64 mmol, 1 eq) was taken in acetic acid (5 mL), urea (16.4 mmol, 10 eq) was added, and refluxed for 5 hours. The reaction mixture was taken into water and the solid was filtered off. The crude was used in the next step. Yield 65%; mp > 300.0 °C. HRMS m/z calculated for C_{10}H_{9}N_{4}O_{4} [M+H]^+: 221.0562, found 221.0551. CAS # 174074-88-5.

Methyl 2,4-dichloroquinazoline-7-carboxylate (52)

Compound 51 (1.07 mmol, 1 eq) was taken into toluene (5mL) and DIEA (3.2 mmol, 3 eq) and POCl$_3$ (3 mL) were added to it and stirred at 90 °C for 3 hours. At the end of the period, the reaction flask was cooled with the help of an ice bath, water was added dropwise and extracted with ethyl acetate. The extract was re-extracted with the prepared NaHCO$_3$ solution. The organic layer was dried, filtered, and evaporated. The crude was used in the next step. Yield 61%; mp 129.0-131.0 °C. HRMS m/z calculated for C_{10}H_{7}Cl_{2}N_{2}O_{2} [M+H]^+, 256.9885, found 256.9876 . CAS #174074-89-6.
2-Chloro-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (53)

Compound 52 (1 mmol, 1 eq) was heated with 1N NaOH solution (4 mL) under reflux for 2 hours. The reaction mixture was diluted with water and acidified with acetic acid, the solid was filtered and dried. The compound was used in the next step. Yield 83%; mp 247.0 °C (decomp.). HRMS m/z calculated for C_{9}H_{6}ClN_{2}O_{3} [M+H]^+ 225.0067, found 225.0073. CAS #1594503-33-9

2-((2-Chlorobenzyl)amino)-4-oxo-3,4-dihydroquinazoline-7-carboxylic acid (54)

To the ethanol solution of compound 53 (0.8 mmol, 1 eq), DIEA (1.2 mmol, 1.5 eq) and 2-chlorobenzylamine (0.96 mmol, 1.2 eq) were added and refluxed for 3 hours. At the end of the time, the reaction mixture was cooled, and water was added, the solid was filtered and dried. Yield 79%; mp 288.0 °C (decomp). 1H NMR (500 MHz, DMSO-d_6): δ_H 4.65 (2H, s), 6.94 (1H, bs), 7.28-7.37 (2H, m), 7.47-7.49 (2H, m), 7.06 (1H, d, J = 8.0 Hz), 7.75 (1H, s), 7.98 (1H, d, J = 8.0 Hz), 11.23 (1H, bs), 13.24 (1H, bs); HRMS m/z calculated for C_{16}H_{13}ClN_{3}O_{3} [M+H]^+ 330.0645, found 330.0645.

Table S1. Physicochemical properties and predicted ADME properties of compounds 5, 35 and 37. Given values are calculated with QikProp. ¹

| Properties                                      | Compound 5 | Compound 35 | Compound 37 | Range for 95% of Drugs or Recommended Values |
|------------------------------------------------|------------|-------------|-------------|---------------------------------------------|
| Molecular Weight (mol_MW)                      | 471.357    | 415.937     | 449.49      | 130.0 – 725.0                               |
| Predicted octanol/water partition coefficient (QPlogPo/w) | 5.61       | 4.679       | 4.938       | -2.0 – 6.5                                  |
| Number of Hydrogen Bond Donors (donorHB)       | 1          | 2           | 2           | 0.0 – 6.0                                   |
| Number of Hydrogen Bond Acceptors (acptHB)     | 6          | 6           | 6           | 2.0 – 20.0                                  |
| Rotable Bonds (#rotor)                         | 6          | 6           | 6           | 0-15                                        |
| Predicted Central Nervous System Activity (CNS) | -2         | -1          | 0           | -2 (inactive), +2 (active)                  |
| Computed dipole moment (dipole)                | 6.417      | 4.047       | 5.639       | 1.0 – 12.5                                  |
| Total solvent accessible surface area (SASA)   | 748.315    | 757.062     | 733.08      | 300.0 – 1000.0                              |
| Hydrophobic component of the SASA (FOSA)       | 58.991     | 259.869     | 253.993     | 0.0 – 750.0                                 |
| Hydrophilic component of the SASA (FISA)       | 143.4      | 124.021     | 104.306     | 7.0 – 330.0                                 |
| π (carbon and attached hydrogen) component of the SASA (PISA) | 404.977 | 266.19 | 245.477 | 0.0 – 450.0 |
| Weakly polar component of the SASA (WPSA)      | 140.948    | 106.982     | 129.304     | 0.0 – 175.0                                 |
| Total solvent-accessible volume (volume)       | 1325.18    | 1310.161    | 1316.98     | 500.0 – 2000.0                              |
| Predicted apparent Caco-2 cell permeability in nm/sec (QPPCaco) | 109.556 | 660.409 | 1015.697 | <25 poor, >500 great |
| Predicted brain/blood partition coefficient (QPlogBB) | -1.025 | -0.949 | -0.588 | -3.0 – 1.2 |
| Predicted apparent MDCK cell permeability in nm/sec (QPPMDCK) | 341.083 | 1217.971 | 2570.323 | <25 poor, >500 great |
|---------------------------------------------------------------|---------|-----------|-----------|---------------------|
| Predicted skin permeability, logKo (QPlogKp)                  | -2.16   | -2.291    | -2.001    | -8.0 – -1.0         |
| PM3 calculated ionization potential (negative of HOMO energy) (IP(eV)) | 9.334   | 9.118    | 9.173    | 7.9 – 10.5         |
| PM3 calculated electron affinity (negative of LUMO energy) (EA(eV)) | 1.258    | 1.059    | 1.124    | -0.9 – 1.7         |
| Prediction of binding to human serum albumin (QPlogKh)        | 0.607   | 0.678    | 0.714    | -1.5 – 1.5         |
| Predicted human oral absorption on 0 to 100% scale (PercentHumanOralAbsorption) | 83.337 | 100 | 100 | >80% is high, <25% is poor |
| Van der Waals surface area of polar atoms and carbonyl carbon atoms (PSA) | 85.651 | 85.344 | 82.867 | 7.0 – 200.0 |

**Figure S1.** Sequence alignment of soluble epoxide hydrolase amino acid sequences of human (UniProt ID: P34913), mouse (UniProt ID: P34914), rat(UniProt ID: P80299), and pig (UniProt ID: Q6Q2C2) recorded in SwissProt database. Alignment is done with T-Coffee by using JalView.  

![Sequence alignment of soluble epoxide hydrolase amino acid sequences](image)

![Graphs showing sEH activity](image)
Figure S2. Representative bioactivity results of the selected final compounds.

Figure S3. RMSD values of compounds A) 35 and B) 37 simulated with sEH. Both figures were plotted after fitting each frame against the first one.

Figure S4. Protein-ligand interactions of A) compound 35 and B) compound 37 at FLAP binding site with their occupancy values calculated during the simulation time of 200 ns.
Figure S5. RMSD values of compounds A) 35 and B) 37 simulated with FLAP. Both figures were plotted after fitting each frame against the first one.
Figure S6. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 10
Sample Name: SMT317
Data Collected on: mercury400-mercury400
Archive directory: /home/vmmcl/vmmcl/vnmrj/data
Sample directory: SMT317 20190928 01
PfdFile: PROTON_01

Pulse Sequence: PROTON e2pae
Solvent: ddeo
Data collected on: Sep 28 2019

Temp. 25.0 C / 298.1 K
Operator: vmmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Avg. time 2.559 sec
Width 6400.0 Hz
8 repetitions

OBSERVE H1, 400.1759761 MHz
DATA PROCESSING
PT size 32768
Total time 0 min 31 sec

ppm
Figure S7. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 11

S11

Sample Name: SNT321
Data Collected on: Mercury400-mercury400
Archive directory: /home/vnmrl/vnmrlsoft/data
Sample Directory: SNT321_20190929_01
PID File: PROTON_02

Pulse Sequence: PROTON (a2pul)
Solvent: dmeo
Data collected on: Sep 27 2019

Temp. 25.0 C / 298.1 K
Operator: vnmrl

Relax. delay 1.000 sec
Pulse 95.0 degrees
Acq. time 2.559 sec
Width 6402.0 Hz
8 repetitions

OBSERVE H1, 400.1754673 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 31 sec

HO
\begin{center}
\includegraphics[width=0.5\textwidth]{spectrum.png}
\end{center}
Figure S8. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 12
Sample Name: SNY440
Data Collected on: mercury400-mercury100
Archive directory: /home/vmnr1/vnmrsys/data
Sample directory: SNY440_20210315_01
PfidFile: CARBON_01

Pulse Sequence: CARBON (z2pul)
Solvent: dmoa
Data collected on: Mar 15 2021

Temp. 25.0 C / 298.1 K
Operator: vmnr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.550 sec
Width 21741.6 Hz
2000 repetitions
OBSERVE C13, 100.6243742 MHz
DECOUPLE H1, 400.1779555 MHz
Power 30 dB
continuously on
WALTZ-16 modulated

DATA PROCESSING
Line broadening 0.5 Hz
PT size 65536
Total time 1 hr, 28 min
**Single Mass Analysis**

Tolerance = 5.0 PPM

| Mass     | Calc. (Mass) | m/z | PPM | DEE | Formula |
|----------|--------------|-----|-----|-----|---------|
| 478.182  | 478.186      | 9.5 | 1.6 | 155 | C20 H25 N2 O2 S O |

**Elements Used**

| C   | H  | N  | O  | S  |
|-----|----|----|----|----|
| 20  | 25 | 2  | 1  |

**Results**

- TIC
  - 3.55e+09
  - 3.90

**Peaks**

- 478.1361
- 461.1335
- 451.1444
- 650.2793
- 650.2855
- 650.2791

**Diode Array**

- Range: 4.426e-1
Figure S9. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 13
Sample Name: SMT314
Data Collected on: mercury400-mercury400
Archive directory: /home/vmnr1/vnaraya/data
Sample directory: SMT314_20190927_01
FidFile: current

Pulse Sequence: CAEBOW (s2pul)
Solvent: dmoa
Data collected on: Sep 27 2019

Temp. 28.0 C / 108.1 K
Operator: vnmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.550 sec
Width 2141.6 Hz
64 repetitions

OBSERVE Cl3, 100.6241836 MHz
DECouple H1, 400.1773555 MHz
Power 18 dB
continuously on

DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 3 hr, 40 min
Figure S10. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 14
Sample Name: SMT325
Data Collected on: mercury400-mercury400
Archive directory:/home/vnnri/vnnrsv/Data
Sample directory: SMT325_20190225_01
PfdFile: CARBON_01

Pulse Sequence: CARBON (x2pul)
Solvent: dmeo
Data collected on: Sep 25 2010

Temp. 25.0 C / 288.1 K
Operator: vnnri

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.304 sec
Width 25125.6 Hz
5000 repetitions

OBSERVE C13, 100.6243002 MHz
DISCOURIS BL, 400.1779555 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA 200000000000
Line broadening 0.5 Hz
PT w1w = 65536
Total time 3 hr, 19 min
Figure S11. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 20
Sample Name: SMT427
Data Collected on: mercury400-mercury400
Archive directory: /home/vnmr/vnmrj/vnmrj400/data
Sample directory: SMT427_20210316.01
PidFile: current

Pulse Sequence: CARBON (o2pul)
Solvent: dmeo
Data collected on: Mar 16 2021

Temp. 25.0 C / 298.1 K
Operator: vnari

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.650 sec
Width 21141.6 Hz
960 repetitions

OBSERVE C13, 100.624736 MHz
DECouple H1, 400.1779555 MHz

Power 38 dB
continuously on
WALES-16 modulated

DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 2 hr, 3 min

S27
**Figure S12.** $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 21
Sample Name: SNV441
Data Collected on: mercury400-mercury400
 Archive directory: /home/vnmri/vnmri/py/data
Sample directory: SNV441_20210316_01
PdFile: CARBON_01
Pulse Sequence: CARRON (e2pul)
Solvent: dmso
Data collected on: Mar 16 2021

Temp. 25.0 C / 298.1 K
Operator: vnmri

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.000 sec
Width 2141.6 Hz
3000 repetitions

C13, 100.6243292 MHz
DECOUPLE W1, 400.1779585 MHz
Power 30 dB
continuously on
WALTZ-16 modulated

DATA PROCESSING
Line broadening 0.5 Hz
FT size 45536
Total time 2 hr, 12 min
Figure S13. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 34
Figure S14. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 35
Sample Name: SMY350

Data Collected on: mercury400-mercury400
Archive directory: /home/vnmr1/vnmrsys/data
Sample directory: SMY350 20200725 01
FidFile: CARBON_01

Pulse Sequence: CARBON (a2ps1)
Solvent: dmo
Data collected on: Jul 25 2020

Temp. 25.0 C / 298.1 K
Operator: vnmrl

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.478 sec
Width 22172.0 Hz
2000 repetitions

Observ. C13, 100.6243851 MHz
Decoupled H1, 400.1779555 MHz
Power 30 dB
continuously on

WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
PT size 65536
Total time 1 hr, 25 min
Figure S15. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 36
Sample Name: SNT504
Data Collected on: mercury400-mercury600
Archive directory: /home/vnmr1/vnmrsys/data
Sample directory: SNT504_20220420_01
FidFile: CARBON_02
Pulse Sequence: CARBON (e2pul)
Solvent: dmso
Data collected on: Apr 30 2022

Temp. 35.0 C / 308.1 K
Operator: vnmrl

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.550 sec
Width 21411.6 Hz
6000 repetitions
OBSERVE C13, 100.6246846 MHz
DECouple B1, 400.1779555 MHz
Power 38 dB
continuously on
HALEZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 2 hr, 56 min
Figure S16. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 37
Figure S17. 1H-NMR, 13C-NMR and HRMS spectrums of 38
**Single Mass Analysis**

- **Tolerance = 5.0 PPM / DDE: min = -1.5, max = 50.0**
- **Elemental prediction: Off**
- **Number of isotope peaks used for i-IT-ToF = 3**
- **Monoisotopic Mass. Even Electron Ion**
- **50 formulas evaluated with 1 results within limits (up to 50 closest results for each mass)**

**Elements Used**

| Mass | Calc./Molar | i-De/PPM | DE | Formula | i-IT | i-IT (iPeak) | C | H | N | O | S | Cl |
|------|-------------|----------|----|---------|------|--------------|---|---|---|---|---|----|
| 450.0794 | 450.0789 | -5.8 | -58 | C21 H10 O2 S Cl | 20 | 20 | 3 | 2 | 1 | 2 |

**SMY356_final 321 (4.606) Cm (321:331)**

- **TOP MS ES+**

| Mass | Calc./Molar | i-De/PPM | DE | Formula | i-IT | i-IT (iPeak) | C | H | N | O | S | Cl |
|------|-------------|----------|----|---------|------|--------------|---|---|---|---|---|----|
| 450.0794 | 450.0789 | -5.8 | -58 | C21 H10 O2 S Cl | 20 | 20 | 3 | 2 | 1 | 2 |
Figure S18. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 39
Figure S19. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 40
Figure S20. $^{1}$H-NMR, $^{13}$C-NMR and HRMS spectrums of 41
Figure S21. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 42
Sample Name: SMY419
Data Collected on: mercury400-mercury400
Archive directory: /home/vmxml/vmxmls/data
Sample directory: SMY419_20210315_01
File: C13N01

Pulse Sequence: C13N01
Solvent: dsmo
Data collected on: Mar 15 2021

Temp. 298.1 K
Operator: vmxml

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.550 sec
Width 21141.6 Hz
1532 repetitions

Carboxylic C13, 100.6245742 MHz
Decoupling R1, 400.1779555 MHz
Power 30 dB
Continuously on

WALTZ-16 modulated

Data Processing
Line broadening 0.5 Hz
Pt size 65536
Total time 1 hr, 6 min
### Single Mass Analysis

Tolerance = 0.0397%  
DDE: min = 1.5, max = 10.3  
Element prediction: OK  
Number of isotope peaks used for iFit = 3  
Monoisotopic Mass: Even-Clathrate Ion  
18 formulas evaluated with 1 results within limits (up to 50 closest results for each mass)

#### Elements Used

| Mass     | Calc. Mass | Delta | PPM | DBE | Formula      | i-RT | i-RT (Mean) | C  | H | N | O | S |
|----------|------------|-------|-----|-----|--------------|------|-------------|----|---|---|---|---|
| 412.1668 | 412.1665   | -0.1  | 3.3 | 31.3| C12H20N8O5S | 6.7  | 0.0          | 22 | 28| 3 | 3 | 1 |

### Mass Spectrum

- m/z values: 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975, 1000.
Figure S22. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 43
### Single Mass Analysis

Tolerance = 5.0 ppm / V ONE: min = 1.5, max = 50.0

- **Elemental prediction:** C/H/N
- **Number of isotopic peaks used for I-PT = 3**
- **Monoisotopic Mass, Even-Number only**

| Mass   | Calc. Mass | Ret. | Formula | I-PT | I-PT Ret. | C | H | N | O | S |
|--------|------------|------|---------|------|-----------|---|---|---|---|---|
| 456.1644 | 456.1462   | 0.2  | C20H12N2O3 F3 | 0.22 | 20 3 3 3 1 |
| 155.2690 | 155.2659   | 1.0  | C12H7O  | 0.17 | 10 2 1 |

---

### Table 2

| Mass   | Calc. Mass | Ret. | Formula | I-PT | I-PT Ret. |
|--------|------------|------|---------|------|-----------|
| 456.1414 |            |      |         |      |           |
| 457.1421 |            |      |         |      |           |
| 468.1097 |            |      |         |      |           |
| 528.3205 |            |      |         |      |           |
| 592.2842 |            |      |         |      |           |
| 633.2093 |            |      |         |      |           |

---

### Table 3

| Mass   | Calc. Mass | Ret. | Formula | I-PT | I-PT Ret. |
|--------|------------|------|---------|------|-----------|
| 222.9932 |            |      |         |      |           |
| 289.9296 |            |      |         |      |           |
| 356.8814 |            |      |         |      |           |
| 414.8241 |            |      |         |      |           |
| 494.8710 |            |      |         |      |           |
| 575.8350 |            |      |         |      |           |
| 631.2010 |            |      |         |      |           |
| 696.1690 |            |      |         |      |           |
| 761.1370 |            |      |         |      |           |
| 826.1050 |            |      |         |      |           |
| 891.0730 |            |      |         |      |           |
| 956.0410 |            |      |         |      |           |
| 1021.0090|            |      |         |      |           |
Figure S23. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 44
Single Mass Analysis

Tolerance = ±5 ppm; DBE max = 1.5, max = 10.6
Element prediction Off
Number of isotopes used for nRT = 3

Monoisotopic ions, Even Electron loss
25 transitions evaluated with 1 transition within limits (up to 5 closest results for each mass)

Elements: [C, H, N, O, S]

| Mass (m/z) | Calc. Mass (m/z) | m/z | DBE | Formula | IRT | IRT (Recal) | C | H | N | O | S |
|-----------|------------------|-----|-----|---------|-----|-------------|---|---|---|---|---|
| 100.0548  | 100.0548         | -0.0| 0   | 100 N  | 0   | 0           | 0 | 0 | 0 | 0 | 0 |
| 101.0548  | 101.0548         | -0.0| 0   | 101 N  | 0   | 0           | 0 | 0 | 0 | 0 | 0 |
| 102.0548  | 102.0548         | -0.0| 0   | 102 N  | 0   | 0           | 0 | 0 | 0 | 0 | 0 |
| 103.0548  | 103.0548         | -0.0| 0   | 103 N  | 0   | 0           | 0 | 0 | 0 | 0 | 0 |

[Graph showing mass spectrometry data with m/z values and relative intensities]
Figure S24. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 45
Figure S25. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 46
Sample Name: AGU293
Data Collected on: mercury400-mercury400
Archive directory: /home/vnmrl/vnmrjsys/data
Sample directory: AGU293_20191001_01
FigFile: CARBON_01

Pulse Sequence: CARBON (e2pul)
Solvent: DMSO
Data collected on: Oct 1 2019

Temp. 25.0 C / 298.1 K
Operator: vnmrl

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.550 sec
Width 21141.6 Hz
6000 repetitions
OBSERVE 1H, 190.624768 MHz
DECOUPLER 81, 690.1773555 MHz
Power 38 dB
continuously on
WAVE-le modulated
DATA PROCESSING
Line broadening 0.5 Hz
PT size 65536
Total time 3 hr. 40 min
Figure S26. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 48
Single Mass Analysis

- Tolerance: ± 5.0 ppm
- DEE: min = -1.6, max = 10.0
- Elements: C, H
- Number of isotopes used: 3
- Formula: $[\text{mass} \pm \text{error}]$ evaluated with 1 result within limits (up to 50 closest results for each mass)

Elements Used:

| Mass   | Cnd. Mass | mDa | PPM | DEE | Formula | $[\text{mass} \pm \text{error}]$ | C | H | N | O | S | Cl |
|--------|-----------|-----|-----|-----|---------|---------------------------------|---|---|---|---|---|----|
| 415.0085 | 415.0086  | 0.9 | 2.2 | 11.3 | C20 H10 N H2 O S Cl | 3.2 | 7.2 | 0 | 2 | 4 | 1 |

**Results:**

- Masses: 458.0271, 417.0984, 436.1270, 459.1220, 456.1224, 480.3072, 605.3851, 693.2820, 845.2007, 829.1952, 633.1794, 584.2224, 513.0729, 805.3851, 927.1767, 1006.7007, 845.2007, 829.1952, 633.1794, 584.2224, 513.0729, 805.3851, 927.1767, 1006.7007.
Figure S27. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 49
Figure S28. $^1$H-NMR, $^{13}$C-NMR and HRMS spectrums of 50
Sample Name: SMY409
Data Collected on: mercury400-mercury400
Archive directory: /homa/vnmrj/vnmreys/data
Sample directory: SMY409_20210413_02
FidFile: CARRON_01

Pulse Sequence: CARRON (a2pul)
Solvent: dmso
Data collected on: Jan 13 2021

Temp. 37.0 C / 310.1 K
Operator: vnmrl

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.550 sec
Width 31141.6 Hz
4990 repetitions

OBSERVE: C13, 100.6243842 MHz
DECOUPLB RL, 400.1779555 MHz
Power 35 dB
continuously on

WALTZ-16 modulated

DATA PROCESSING
Line broadening 0.5 Hz

FT size 65536
Total time 2 hr, 56 min
**Single Mass Analysis**

- Tolerance = 5.0 ppm
- DDE: min = -1.5, max = 50.0
- Number of isotope peaks used for IRT = 3
- Monoisotopic Mass, Exact Electron loss

21 formulae included with 1 results within limits (up to 50 dose results for each mass)

| Mass | Cal. Mass | m/z | PPM | DBE | Formula | IRT  | IRT (Norm) | C  | H  | N  | O  | S  | Cl  |
|------|-----------|-----|-----|-----|---------|------|------------|----|----|----|----|----|-----|
| 465.0941 | 465.0940 | 0.5 | 1.2 | 22.5 | C20 H29 N3 O3 S O | 536.6 | 6.0 | 20 | 19 | 3  | 3  | 1  | 1   |

**SMY409_final 3: Diode Array**

Range: 3.966e-13.13

**SMY409_final 1: TOF MS ES+**

TIC 4.92e5

8.62

8.31

6.46

4.89

**SMY409_final 225 (3.229) Cm (225:244)**

TIC 3.23e5

416.0841 416.0842 343.2942 271.1866 249.2041 199.1780 130.1586 144.9816 288.2859

392.1584 357.1470 418.0812 831.1594 419.0839 450.0403 491.0659 528.1583 598.0579 565.1999 806.3594 792.3273 664.2979 835.1702 867.1107 901.0829 931.1338 951.1372
Figure S29. $\text{^1H-NMR, ^13C-NMR and HRMS spectrums of 55}$
References

(1) Schrödinger Release 2022-2: QikProp, Schrödinger, LLC, New York, NY, 2022.
(2) Bairoch, A.; Apweiler, R. The SWISS-PROT protein sequence database and its supplement TrEMBL in 2000. *Nucleic Acids Res* **2000**, **28** (1), 45-48. DOI: 10.1093/nar/28.1.45  From NLM Medline.
(3) Notredame, C.; Higgins, D. G.; Heringa, J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol* **2000**, **302** (1), 205-217. DOI: 10.1006/jmbi.2000.4042.
(4) Waterhouse, A. M.; Procter, J. B.; Martin, D. M.; Clamp, M.; Barton, G. J. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **2009**, **25** (9), 1189-1191. DOI: 10.1093/bioinformatics/btp033.