Design of dual inhibitors of soluble epoxide hydrolase and LTA_4 hydrolase

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Contents of SI:

Chemistry materials and general procedures

All used solvents and chemicals were purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany), Acros Organics (Geel, Belgium), Alfa-Aesar GmbH & Co KG (Karlsruhe, Germany), TCI Europe (Zwijndrecht, Belgium), Fluorochem (Derbyshire, England) and Apollo Scientific Ltd. (Manchester, England) and used without further purification. Analytical TLC (thin layer chromatography) was performed with TLC plates F254 from Merck (KGaA, Darmstadt, Germany) and visualized with ultraviolet light (254 and 365 nm). Column chromatography was performed with technical grade solvent mixtures specified in the corresponding experiment with either Fluka silica gel 60 (230–400 mesh ASTM) or an Intelliflash 310 system from Varian Medical Systems Deutschland GmbH (Darmstadt, Germany) with silica columns from Varian (particle size 50 µm). ^{1}H-NMR spectra were recorded on a Bruker DPX 250 (250 MHz), AV300 (300 MHz), AV400 (400 MHz) or AV500 (500 MHz)

spectrometer (Bruker, Karlsruhe, Germany). ^{1}H-NMR data are reported in ppm downfield relative to tetramethylsilane: internal reference non-deuterated solvent. The multiplicities are b, broad; s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet and the approximate coupling constant (J) are reported in hertz (Hz). ^{13}C-NMR spectra were recorded on a Bruker AV300 (75 MHz) or AV500 (125 MHz) spectrometer (Bruker, Karlsruhe, Germany). HPLC analysis were performed by a LCMS 2020 from Shimadzu (Duisburg, Germany) under the use of a column from Phenomenex Luna 10µ C18(2) 100A (250 x 4.60 mm) for analytical purposes and a Luna 10µ C18(2) (250 x 21.20 mm) column from Phenomenex LTD Deutschland (Aschaffenburg, Germany) for preparative purposes. Condition were as followed: acetonitrile/0.1% aqueous formic acid eluent at 1 mL/min (Scout column) or 21 mL/min (semi-preparative) flow rate at room temperature. UV absorption was monitored at 254 and 280 nm. Purity of all compounds were determined by following methods: method A: Gradient of 30% to 90% acetonitrile within 10 min, 90% acetonitrile were hold for another 10 min. method B: 5% acetonitrile for 5 min, afterwards a gradient of 5% to 90% acetonitrile within 10 min, 90% acetonitrile were hold for another 10 min. ESI-MS was performed on a VG Platform II from Fisons Instruments (Glasgow, UK) or on a LCMS-2020 from Shimadzu (Duisburg, Germany). MALDI-HRMS was performed on a MALDI LTQ Orbitrap XL instrument (Thermo Scientific, USA). Purity of all final compounds were 90% or higher determined by HPLC.

Procedure A

Under inert conditions 2.0 eq thionyl chloride were diluted in 5 mL dry chloroform and a cat. amount of DMF. 1.0 eq 4-phenoxyphenylacetic acid was added and the solution stirred 3 h under reflux conditions. After removing all solvents under reduced pressure, the solid was solved in 5 mL dry chloroform. A solution of 1.5 eq DIPEA and 1.0 eq amine was added and the approach stirred overnight at room temperature. The reaction mixture was washed with 2 M aqueous hydrochloric acid (1x), with 1 M aqueous sodium hydroxide solution (1x), with dest. water (3x) and brine (1x). After drying over $MgSO_4$ and filtration the solvent was evaporated under reduced pressure. Flash chromatography (hexane:ethyl acetate 2:1 to 100% ethyl acetate) yielded a yellow – white solid.

Procedure B

The acid derivative was dissolved in 15 mL DCM and 1 mL DMF. To the solution 1.0 eq EDC·HCl and 1.0 eq HOBt·H_2O were added and the approach stirred for 30 minutes at room temperature. Additionally, the corresponding 1.0 eq amine and 1.0 - 2.0 eq DIPEA were added and the reaction stirred overnight. The mixture was washed with 2 M aqueous hydrochloric acid (1x), with 1 M aqueous sodium hydroxide solution (1x), with dest. water (3x) and brine (1x). After drying over $MgSO_4$ and filtration the solvent was removed under reduced pressure. Flash chromatography (hexane:ethyl acetate 2:1 to 100% ethyl acetate) yielded a yellow – white solid

Procedure C

1.0 eq phenyl isothiocyanate derivative, 1.1 eq of the phenol derivative, 0.05 eq CuI, 0.1 eq phenanthroline and 2.0 eq K_2CO_3 were suspended in 7 mL 1,4-dioxane. The approach was heated to 90 °C for 3 days. The suspension was filtered over a Celite® pad with ethyl acetate and the solvent was evaporated. The residue was purified via flash chromatography (hexane:ethyl acetate 2:1, 1:1 to 100% ethyl acetate). A solid was obtained.

Analytical data of the synthesized compounds

2-(4-phenoxyphenyl)-*N*-(4-(trifluoromethoxy)phenethyl)acetamide (**3**): procedure B: educts: 2-(4-phenoxyphenyl)acetic acid, 2-(4-(trifluoromethoxy)phenyl)ethanamine; $C_{23}H_{20}F_3NO_3$; MW: 415.41 g/mol; yield: 96 mg, 0.23 mmol, 77%; ^{1}H-NMR: (500.2 MHz, $CDCl_3$): δ = 7.40-7.32 (m, 2H, H_{ar}), 7.15-7.07 (m, 7H, H_{ar}), 7.03-7.00 (m, 2H, H_{ar}), 6.96 -6.93 (m, 2H, H_{ar}), 5.40 (bs, 1H, NH), 3.50 (s, 2H, CH_2), 3.47 (q, 2H, $^{3}J_{HH}$ = 6.6 Hz, CH_2), 2.76 (t, 2H, $^{3}J_{HH}$ = 6.9 Hz, CH_2) ppm; ^{13}C-NMR: (125.8 MHz, $CDCl_3$): δ =

171.1, 157.0, 156.9, 148.0 (q), 137.6, 130.9, 130.1, 130.0, 129.4, 123.8, 121.3, 119.3, 119.1, 43.2, 40.7, 35.0 ppm; t_R: 12.33 min (method A), purity: 99%, HRMS: measured: 416.14639, calculated: 416.14680, Δm: 1.0 ppm, [M+H$^+$].

2-(4-phenoxyphenyl)-*N*-(4-(trifluoromethyl)phenethyl)acetamide (**5**): procedure B: educts: 2-(4-phenoxyphenyl)acetic acid, 2-(4-(trifluoromethyl)phenyl)ethanamine; $C_{23}H_{20}F_3NO_2$; MW: 399.41 g/mol; yield: 105 mg, 0.26 mmol, 88%; ^{1}H-NMR: (500.2 MHz, $CDCl_3$): δ = 7.51 (d, 2H, $^3J_{HH}$ = 8.0 Hz, H_{ar}), 7.37-7.33 (m, 2H, H_{ar}), 7.19 (d, 2H, $^3J_{HH}$ = 8.0 Hz, H_{ar}), 7.15-7.11 (m, 3H, H_{ar}), 7.03-7.00 (m, 2H, H_{ar}), 6.96-6.93 (m, 2H, H_{ar}), 5.40 (bs, 1H, NH), 3.51-3.47 (m, 4H, CH_2), 2.82 (t, 2H, $^3J_{HH}$ = 6.9 Hz, CH_2) ppm; ^{13}C-NMR: (125.8 MHz, $CDCl_3$): δ = 171.2, 157.0, 156.9, 143.0 (d), 130.9, 130.0, 129.3, 129.2, 128.9, 125.6 (d), 125.4, 123.8, 123.2, 119.3, 119.1, 43.2, 40.5, 35.5 ppm; t_R: 12.06 min (method A), purity: 99%, HRMS: measured: 400.15157, calculated: 400.15189, Δm: 0.8 ppm, [M+H$^+$].

***N*-(4-fluorophenethyl)-2-(4-phenoxyphenyl)acetamide (6)**: procedure A; educts: 2-(4-phenoxyphenyl)acetic acid, 2-(4-fluorophenyl)ethanamine; $C_{22}H_{20}FNO_2$; MW: 349.40 g/mol; Yield: 79 mg, 0.23 mmol, 31%; ^{1}H-NMR: (250.0 MHz, $CDCl_3$): δ = 7.39–7.33 (m, 2H, H_{ar}), 7.14 (m, 3H, H_{ar}), 7.10-6.90 (m, 8H, H_{ar}), 5.34 (bs, 1H, NH), 3.47–3.42 (m, 4H, CH_2), 2.72 (t, 2H, $^3J_{HH}$ = 5.0 Hz, CH_2) ppm; ^{13}C-NMR: (75.4 MHz, $CDCl_3$): δ =171.1, 157.0 (d), 130.9, 130.3, 130.2, 130.0, 129.5, 123.7, 119.2 (d), 115.7, 115.4, 43.2, 40.8, 34.9 ppm; t_R: 11.19 min (method A), purity: 99%, HRMS: measured: 350.15563, calculated: 350.15508, Δm: 1.4 ppm, [M+H$^+$].

***N*-(4-chlorophenethyl)-2-(4-phenoxyphenyl)acetamide (7)**: procedure A; educts: 2-(4-phenoxyphenyl)acetic acid, 2-(4-chlorophenyl)ethanamine; $C_{22}H_{20}ClNO_2$; MW: 365.85 g/mol; Yield: 60 mg, 0.16 mmol, 31%; ^{1}H-NMR: (250.0 MHz, $CDCl_3$): δ = 7.39–7.33 (m, 2H, H_{ar}), 7.24-7.20 (m, 2H, H_{ar}), 7.17-7.11 (m, 3H, H_{ar}), 7.03-6.94 (m, 6H, H_{ar}), 5.34 (bs, 1H, NH), 3.50 (s, 2H, CH_2), 3.48-3,43(m, 2H, CH_2), 2.72 (t, 2H, $^3J_{HH}$ = 7.1 Hz, CH_2) ppm; ^{13}C-NMR: (75.4 MHz, $CDCl_3$): δ = 170.0, 156.8, 138.5, 131.6, 130.7, 130.6, 130.5, 129.8, 128.7, 123.6, 119.2, 119.0, 43.0, 40.5, 34.9; t_R: 11.87 min (method A), purity: 93%, HRMS: measured: 366.1255, calculated: 366.1258, Δm: 0.8 ppm, [M+H$^+$].

***N*-(4-methylphenethyl)-2-(4-phenoxyphenyl)acetamide (8)**: procedure A; educts: 2-(4-phenoxyphenyl)acetic acid, 2-(*p*-tolyl)ethanamine; $C_{23}H_{23}NO_2$**;** MW: 345.43 g/mol; Yield: 80 mg, 0.23 mmol, 21%; ^{1}H-NMR: (400.0 MHz, $CDCl_3$): δ =7.38–7.33 (m, 2H, H_{ar}), 7.15–7.11 (m, 3H, H_{ar}), 7.07–7.00 (m, 4H, H_{ar}), 6.96–6.92 (m, 4H, H_{ar}), 5.36 (bs, 1H, NH), 3.50 (s, 2H, CH_2), 3.49–3.44 (m, 2H, CH_2), 2.71 (t, 2H, $^3J_{HH}$ = 7.0 Hz, CH_2), 2.31 (s, 3H, CH_3) ppm; ^{13}C-NMR: (75.4 MHz, $CDCl_3$): δ = 170.8, 156.9, 156.6, 136.0, 135.5, 130.8, 129.8, 129.5, 129.3, 128.6, 123.5, 119.1, 119.0, 43.0, 40.7, 35.0, 21.0; t_R 11,34 min (method A), purity: 99%; HRMS: measured: 346.18079, calculated: 346.18016, Δm: 1.7 ppm, [M+H$^+$].

2-(4-fluorophenyl)-*N*-(4-phenoxybenzyl)ethanamine (**9**):
Under an inert atmosphere 0.20 g (1.01 mmol, 1.0 eq) 4-phenoxybenzaldehyde and 0.17 g (1.21 mmol, 1.2 eq) 2-(4-fluorophenyl)ethanamine were dissolved in 3 mL abs. 1,2-dichloroethane. The approach stirred for 4 h and a suspension was formed. 0.26 g (1.21 mmol, 1.2 eq) sodium triacetoxyhydroborate were added and stirred 16 h. After removal of the solvent the residue was diluted with ethyl acetate and washed with saturated sodium bisulfite solution. The formed precipitate was removed, the organic phase was dried over $MgSO_4$ and filtered. The solvent was evaporated and flash chromatography (100% ethyl acetate) yielded a colorless oil.
$C_{21}H_{20}FNO$; MW: 321.39 g/mol; yield: 65 mg, 0.20 mmol, 20%; ^{1}H-NMR: (300.1 MHz, CD_3OD): 7.36-7.26 (m, 4H, H_{ar}), 7.23-7.17 (m, 2H, H_{ar}), 7.12-7.06 (m, 1H, H_{ar}), 7.03-6.91 (m, 6H, H_{ar}), 3.74 (s, 2H, CH_2), 2.80 (s, 4H, CH_2) ppm; ^{13}C-NMR: (75.4 MHz, CD_3OD): δ = 164.6, 161.3, 158.8, 157.9, 137.0 (d), 135.5, 311.1, 130.8, 124.3, 119.8, 116.2, 116.9, 53.7, 51.4, 35.8; t_R: 10.68 min (method B), purity: 98%, HRMS: measured: 322.16039, calculated: 322.16017, Δm: 0.68 ppm, [M+H$^+$].

***N*-(4-fluorophenethyl)-3-(4-hydroxyphenyl)propanamide** (**precursor of 10**): Under an argon atmosphere at 0 °C 0.18 g (0.71 mmol, 1.0 eq) iodine were dissolved in 10 mL abs. DCM and 0.19 g (0.71 mmol, 1.0 eq) PPh_3 were added. After 15 min the lilac color disappeared and 0.99 g (0.71 mmol, 1.0 eq) 2-(4-fluorophenyl)ethanamine, dissolved in 5 mL abs. DCM, were added portionwise. 0.10 g (0.60 mmol, 0.9 eq) 3-(4-hydroxyphenyl)propanoic acid and 0.2 mL (1.20 mmol, 1.7 eq) triethylamine were added and the approach stirred for 19 h at room temperature. The solution was concentrated and purified via column chromatography (hexane: ethyl acetate 1:4).
$C_{17}H_{18}FNO_2$; MW: 287.33 g/mol; yield: 0.05 g, 0.17 mmol, 24%; ^{1}H-NMR: (250.1 MHz, DMSO): 9.11 (s, 1H, OH), 7.82 (t, 1H, $^3J_{HH}$ = 5.6 Hz, NH), 7.19-7.10 (m, 4H, H_{ar}), 6.97-6.94 (m, 2H, H_{ar}), 6.67-6.62 (m, 2H, H_{ar}), 3.26-3.18 (m, 2H, CH_2), 2.69-2.62 (m, 4H, CH_2), 2.30-2.2.24 (m, 2H, CH_2) ppm; LRMS: measured: 287.95, calculated: 287.13, [M+H$^+$].

***N*-(4-fluorophenethyl)-3-(4-(pyridin-4-ylmethoxy)phenyl)propanamide** (**10**):
43 mg (0.15 mmol, 1.0 eq) *N*-(4-fluorophenethyl)-3-(4-hydroxyphenyl)propanamide were dissolved in 5 mL THF and 18 mg (0.45 mmol, 3.0 eq, 60% oil dispersion) NaH were added at 0 °C. The suspension stirred 30 min at 0 °C. 42 mg (0.17 mmol, 1.1 eq) 4-(bromomethyl)pyridine hydrobromide were added portionwise and 1 mL dest. water was added. The reaction stirred for 18 h at room temperature and was quenched with 1 N aqueous hydrochloric acid. The product was extracted with ethyl acetate three times and the combined organic phases were washed with brine. After drying over $MgSO_4$ and filtration the solvent was removed under reduced pressure. The crude product was purified via preparative HPLC (method: gradient of 10% acetonitrile to 90% within 14 min, 90% acetonitrile were hold for 3 minutes).
$C_{23}H_{23}FN_2O_2$; MW: 378.40 g/mol; yield: 65 mg; 0.20 mmol; 20%; ^{1}H-NMR: (250.1 MHz, DMSO): 8.55 (dd, 2H, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.5 Hz, H_{ar}), 7.86 (t, 1H, $^3J_{HH}$ = 6.7 Hz, NH), 7.42-7.40 (m, 2H, H_{ar}), 7.02-7.04 (m, 6H, H_{ar}), 6.94-6.88 (m, 2H, H_{ar}), 5.14 (s, 2H, CH_2), 3.26-3.18 (m, 2H, CH_2), 2.75-2.61 (m, 4H, CH_2), 2.33-2.27 (m, 2H, CH_2) ppm; ^{13}C-NMR: (125.8 MHz, DMSO): δ =171.3, 161.8, 159.9, 156.2, 149.7, 146.4, 135.7 (d), 133.9, 130.4, 129.3, 121.8, 115.0, 114.8, 114.6, 67.4, 372, 34.3, 30.2 ppm; t_R: 7.2 min (method B), purity: 97%, HRMS: measured: 379.18171, calculated: 379.18163, Δm: 0.21 ppm, [M+H$^+$].

***N*-(4-fluorophenethyl)-2-(4-hydroxyphenyl)acetamide** (**precursor of 13**): procedure B: educts: 2-(4-hydroxyphenyl)-acetic acid, 2-(4-fluorophenyl)ethanamine; $C_{16}H_{16}FNO_2$; MW: 273.30; yield: 1.47 g, 5.38 mmol, 40%; ^{1}H-NMR: (400.1 MHz, DMSO): δ = 9.20 (s, 1H, OH), 7.92 (t, 1H, $^3J_{HH}$ = 5.4 Hz, NH), 7.20-7.15 (m, 2H, H_{ar}), 7.09-7.03 (m, 2H, H_{ar}), 7.00-6.97 (m, 2H, H_{ar}), 6.68-6.64 (m, 2H, H_{ar}), 3.27-3.22 (m, 4H, CH_2), 2.67 (t, 2H, $^3J_{HH}$ = 7.2 Hz, CH_2) ppm; LRMS: measured: 274.00, calculated: 273.12 [M+H$^+$].

2-(2-fluoro-4-hydroxyphenyl)-*N*-(4-fluorophenethyl)acetamide (precursor of **14**-**18**): procedure B; educts: 2-(2-fluoro-4-hydroxyphenyl)acetic acid, 2-(4-fluorophenyl)ethanamine; $C_{16}H_{15}F_2NO_2$; MW: 291.29 g/mol; yield: 256 mg, 0.88 mmol, 91%; ^{1}H-NMR: (250.1 MHz, $CDCl_3$): δ = 8.58 (s, 1H, OH), 7.05-6.89 (m, 5H, H_{ar}), 6.60–6.55 (m, 2H, H_{ar}), 5.52 (t, 1H, $^3J_{HH}$ = 5.7 Hz, NH), 3.48-3.40 (m, 4H, CH_2), 2.71 (t, 2H, $^3J_{HH}$ = 6.9 Hz, CH_2) ppm; LRMS: measured: 289.80, calculated: 291.11, [M-H$^+$].

2-(2-fluoro-4-hydroxyphenyl)-*N*-(2-fluorophenethyl)acetamide (precursor of **19**): procedure B; educts: 2-(2-fluoro-4-hydroxyphenyl)acetic acid, 2-(2-fluorophenyl)ethanamine; $C_{16}H_{15}F_2NO_2$; MW: 291.29 g/mol; yield: 359 mg, 1.23 mmol, 84%; ^{1}H-NMR: (250.1 MHz, $CDCl_3$): δ = 7.94 (bs, 1H, OH), 7.22-7.14 (m, 1H, H_{ar}), 7.12-6.93 (m, 4H, H_{ar}), 6.56-6.49 (m, 2H, H_{ar}), 5.76 (t, 1H, $^3J_{HH}$ = 5.6 Hz, NH), 3.54-3.46 (m, 4H, CH_2), 2.82 (t, 2H, $^3J_{HH}$ = 7.0 Hz, CH_2) ppm; LRMS: measured: 292.19, calculated: 291.11, [M+H$^+$].

2-(2-fluoro-4-hydroxyphenyl)-*N*-(3-fluorophenethyl)acetamide (precursor of **20**): procedure B, educts: 2-(2-fluoro-4-hydroxyphenyl)acetic acid, 2-(3-fluorophenyl)ethanamine; $C_{16}H_{15}F_2NO_2$; MW: 291.29 g/mol; yield: 370 mg, 1.27 mmol, 86%; ^{1}H-NMR: (300.0 MHz, $CDCl_3$): δ = 7.98 (bs, 1H, OH), 7.25-

7.17 (m, 1H, H_{ar}), 7.00-6.79 (m, 4H, H_{ar}), 6.58-6.52 (m, 2H, H_{ar}), 5.76 (t, 1H, $^3J_{HH}$ = 5.7 Hz, NH), 3.52-3.46 (m, 4H, CH_2), 2.76 (t, 2H, $^3J_{HH}$ = 6.1 Hz, CH_2) ppm; LRMS: measured: 292.19, calculated: 291.11, [M+H$^+$].

methyl 4-((2-(2-fluoro-4-hydroxyphenyl)acetamido)methyl)benzoate (precursor of **21**): procedure B; educts: 2-(2-fluoro-4-hydroxyphenyl)acetic acid, methyl 4-(aminomethyl)benzoate hydrochloride; $C_{17}H_{16}FNO_4$; MW: 317.31 g/mol; yield: 202 mg, 0.64 mmol, 43%; ^{1}H-NMR: (400.1 MHz, DMSO): δ = 9.67 (bs, 1H, OH), 8.50 (t, 1H, $^3J_{HH}$ = 8.8 Hz, NH), 7.91-7.89 (m, 2H, H_{ar}), 7.36 (d, 2H, $^3J_{HH}$ = 6.1 Hz, H_{ar}), 7.09 (t, 1H, $^3J_{HH}$ = 8.6 Hz, H_{ar}), 6.55-6.49 (m, 2H, H_{ar}), 4.35 (d, 2H, $^3J_{HH}$ = 6.0 Hz, CH_2), 3.84 (s, 3H, CH_3), 3.41 (s, 2H, CH_2) ppm; LRMS: measured: 318.18, calculated: 317.11, [M+H$^+$].

***N*-((6-fluoro-1*H*-indol-3-yl)methyl)-2-(2-fluoro-4-hydroxyphenyl)acetamide** (precursor of **22**): procedure B; educts: 2-(2-fluoro-4-hydroxyphenyl)acetic acid, (6-fluoro-1H-indol-3-yl)methanamine; $C_{17}H_{14}F_2N_2O_2$; MW: 316.30 g/mol; yield: 344 mg, 1.09 mmol, 82%; ^{1}H-NMR: (400.1 MHz, DMSO): δ = 10.97 (s, 1H, NH indole), 9.66 (bs, 1H, OH), 8.22 (t, 1H, $^3J_{HH}$ = 10.7 Hz, NH), 7.56-7.48 (m, 1H, H_{ar}), 7.23 (s, 1H, H_{ar}), 7.14-7.06 (m, 2H, H_{ar}), 6.86-6.80 (m, 1H, H_{ar}), 6.54-6.49 (m, 2H, H_{ar}), 4.38 (d, 2H, $^3J_{HH}$ = 6.7 Hz, CH_2), 3.34 (s, 2H, CH_2) ppm, LRMS: measured: 339.04, calculated: 339.09, [M+Na$^+$].

2-(4-(benzo[d]thiazol-2-yloxy)phenyl)-*N*-(4-fluorophenethyl)acetamide (**13**): procedure C: educts: *N*-(4-fluorophenethyl)-2-(4-hydroxyphenyl)acetamide, 1-bromo-3-isothiocyanatobenzene; $C_{23}H_{19}FN_2O_2S$; MW: 406.47 g/mol; yield: 350 mg, 0.86 mmol, 86%; ^{1}H-NMR: (500.2 MHz, DMSO): δ = 8.16 (t, 1H, $^3J_{HH}$ = 5.5 Hz, NH), 7.92 (dd, 1H, $^3J_{HH}$ = 8.0 Hz, $^4J_{HH}$ = 0.8 Hz, H_{ar}), 7.69 (d, 1H, $^3J_{HH}$ = 8.0 Hz, H_{ar}), 7.44-7.40 (m, 1H, H_{ar}), 7.37-7.31 (m, 5H, H_{ar}), 7.22-7.18 (m, 2H, H_{ar}), 7.11-7.07 (m, 2H, H_{ar}), 3.43 (s, 2H, CH_2), 3.29 (q, 2H, $^3J_{HH}$ = 6.7 Hz, CH_2), 2.71 (t, 2H, $^3J_{HH}$ = 7.1 Hz, CH_2) ppm; ^{13}C-NMR: (125.8 MHz, DMSO): δ = 172.0, 169.8, 161.8, 159.9, 152.9, 148.6, 135.6 (d), 134.9, 131.8, 130.7, 130.5, 130.4, 126.5, 124.2, 122.2, 121.2, 120.7, 115.0, 114.9, 41.7, 40.3, 34.1 ppm; t_R: 11.35 min (method A), purity: 98%, HRMS: measured: 429.10447, calculated: 429.10435, Δm: 0.3 ppm, [M+Na$^+$].

2-(4-(benzo[*d*]thiazol-2-yloxy)-2-fluorophenyl)-*N*-(4-fluorophenethyl)acetamide (**14**). procedure C; educts: 2-(2-fluoro-4-hydroxyphenyl)-*N*-(4-fluorophenethyl)acetamide, 1-bromo-2-isothiocyanato-benzene; $C_{23}H_{18}F_2N_2O_2S$; MW: 424.46 g/mol; yield: 65 mg, 0.15 mmol, 22%; ^{1}H-NMR: (250.1 MHz, $CDCl_3$): 7.77-7.70 (m, 2H, H_{ar}), 7.45-7.27 (m, 3H, H_{ar}), 7.23-7.15 (m, 2H, H_{ar}), 7.08-6.94 (m, 4H, H_{ar}), 5.50 (s, 1H, NH), 3.54-3.44 (m, 4H, CH_2), 2.77 (t, 2H, $^3J_{HH}$ = 7.4 Hz, CH_2) ppm; ^{13}C-NMR: (75.4 MHz, $CDCl_3$): δ = 169.3, 160.0, 154.4, 148.7, 134.3(d), 130.2, 130.0, 126.4, 124.5, 121.9, 121.4, 119.9, 119.6, 116.6 (d), 115.6, 115.2, 108.8, 108.4, 41.0, 36.7, 34.7 ppm; t_R: 11.81 min (method A), purity: 96%, HRMS: measured: 425.11298, calculated: 425.11287, Δm: 0.21 ppm, [M+H$^+$].

2-(2-fluoro-4-((6-fluorobenzo[*d*]thiazol-2-yl)oxy)phenyl)-*N*-(4-fluorophenethyl)acetamide (**15**): procedure C; educts: 2-(2-fluoro-4-hydroxyphenyl)-*N*-(4-fluorophenethyl)acetamide, 2-bromo-4-fluoro-1-isothiocyanatobenzene; $C_{23}H_{17}F_3N_2O_2S$; MW: 442.45 g/mol; yield: 334 mg, 0.76 mmol, 97%; ^{1}H-NMR: (400.1 MHz, DMSO): 8.17 (t, 1H, $^3J_{HH}$ = 5.5 Hz, NH), 7.90 (dd, 1H, $^3J_{HH}$ = 8.7 Hz, $^4J_{HH}$ = 2.7 Hz, H_{ar}), 7.76-7.73 (m, 1H, H_{ar}), 7.44-7.38 (m, 2H, H_{ar}), 7.32-7.21 (m, 4H, H_{ar}), 7.13-7.08 (m, 2H, H_{ar}), 3.49 (s, 2H, CH_2), 3.30-3.27 (m, 2H, CH_2), 2.72 (2H, t, $^3J_{HH}$ = 7.9 Hz, CH_2) ppm; ^{13}C-NMR: (75.4 MHz, DMSO): δ = 168.7, 145.1, 135.5 (d), 133.0, 132.9, 132.6 (d), 130.4 (d), 122.5, 122.4, 121.8, 121.7, 116.6, 115.0, 114.8. 114.7, 114.4, 109.1, 108.9, 108.7, 108.5, 40.7, 34.9, 34.1 ppm; t_R: 11.3 min (method A), purity: 99%, HRMS: measured: 443.10342, calculated: 443.10356, Δm: 0.32 ppm, [M+H$^+$].

2-(2-fluoro-4-((6-(trifluoromethyl)benzo[*d*]thiazol-2-yl)oxy)phenyl)-*N*-(4-fluorophenethyl)acetamide (**16**); procedure C; educts: 2-(2-fluoro-4-hydroxyphenyl)-*N*-(4-fluorophenethyl)acetamide, 2-bromo-1-isothiocyanato-4-(trifluoromethyl)benzene; $C_{24}H_{17}F_5N_2O_2S$; MW: 492.46 g/mol; yield: 179 mg, 0.36 mmol, 58%; ^{1}H-NMR: (300.1 MHz, DSMO): 8.48 (d, 1H, $^4J_{HH}$ = 1.2 Hz, H_{ar}), 8.18 (t, 1H, $^3J_{HH}$ = 5.5 Hz, NH), 7.90 (d, 1H, $^3J_{HH}$ = 8.5 Hz, H_{ar}), 7.75 (dd, 1H, $^3J_{HH}$ = 8.5 Hz, $^4J_{HH}$ = 1.6 Hz, H_{ar}), 7.48-7.40 (m, 2H, H_{ar}), 7.30-7.7.21 (m, 3H, H_{ar}), 7.13-7.07 (m, 2H, H_{ar}), 3.50 (s, 2H, CH_2), 3.31-3.29 (m, 2H, CH_2),

2.72 (2H, t, $^3J_{HH}$ = 7.9 Hz, CH_2) ppm; ^{13}C-NMR: (100.6 MHz, DMSO): δ = 174.4, 168.7,153.2, 151.3, 135.5, 132.7, 132.5, 130.4 (d), 123.4, 122.9, 122.2, 122.0, 121.8, 120.3 (q), 116.7 (d), 115.5, 114.8, 108.9, 108.7, 40.3, 34.9, 34.1 ppm; t_R: 12.0 min (method A), purity: 97%, HRMS: measured: 493.099762, calculated: 493.10037, Δm: 1.23 ppm, [M+H$^+$].

2-(2-fluoro-4-((5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)oxy)phenyl)-*N*-(4-fluorophenethyl)acetamide (**17**): procedure C; educts: 2-(2-fluoro-4-hydroxyphenyl)-*N*-(4-fluorophenethyl)acetamide, 1-bromo-2-isothiocyanato-4-(trifluoromethyl)benzene; $C_{24}H_{17}F_5N_2O_2S$; MW: 492.46 g/mol; yield: 157 mg, 0.32 mmol, 48%; ^{1}H-NMR: (400.1 MHz, $CDCl_3$): 8.23-8.17 (m, 2H, NH, H_{ar}), 8.10 (bs, 1H, H_{ar}), 7.68 (dd, 1H, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 1.4 Hz, H_{ar}), 7.48-7.40 (m, 2H, H_{ar}), 7.30-7.21 (m, 3H, H_{ar}), 7.13-7.07 (m, 2H, H_{ar}), 3.50 (s, 2H, CH_2), 3.31-3.28 (m, 2H, CH_2), 2.78 (t, 2H, $^3J_{HH}$ = 7.2 Hz, CH_2) ppm; ^{13}C-NMR: (100.6 MHz, DSMO): δ = 173.3, 168.7, 162.0, 161.6, 159.6, 159.2, 153.1, 148.4, 136.2, 135.5, 132.7 (q), 132.5, 130.4 (d), 123.6, 116.7, 115.0, 114.8, 10.9, 108.6, 40.4, 34.9, 34.1 ppm; t_R: 12.0 min (method A), purity: 96%, HRMS: measured: 493.09961, calculated: 493.10037, Δm: 1.54 ppm, [M+H$^+$].

2-(2-fluoro-4-((5-fluorobenzo[*d*]thiazol-2-yl)oxy)phenyl)-*N*-(4-fluorphenethyl)acetamide (**18**): procedure C; educts: 2-(2-fluoro-4-hydroxyphenyl)-*N*-(4-fluorophenethyl)acetamide, 1-bromo-4-fluoro-2-isothiocyanatobenzene; $C_{23}H_{17}F_3N_2O_2S$; MW: 442.45 g/mol; yield: 142 mg, 0.32 mmol, 49%; ^{1}H-NMR: (250.1 MHz, $CDCl_3$): 7.69-7.65 (m, 1H, H_{ar}), 7.44-7.29 (m, 2H, H_{ar}), 7.22-7.13 (m, 3H, H_{ar}), 7.11-6.93 (m, 4H, H_{ar}), 5.49 (bs, 1H, NH), 3.55 (s, 2H, CH_2), 3.51-3.47 (m, 2H, CH_2), 2.77 (t, 2H, $^3J_{HH}$ = 7.3 Hz, CH_2) ppm; ^{13}C-NMR: (75.4 MHz, $CDCl_3$): δ = 169.4, 154.5, 134.4, 132.4 (d), 130.3 (d), 123.0, 122.9, 120.2, 119.9, 116.7 (d), 115.7, 115.5, 115.0, 114.6, 108.9, 108.6, 108.4, 108.3, 108.0, 40.9, 36.6, 34.7 ppm; t_R: 11.3 min (method A), purity: 97%, HRMS: measured: 443.10320, calculated: 443.10356, Δm: 0.81 ppm, [M+H$^+$].

2-(4-(benzo[d]thiazol-2-yloxy)-2-fluorophenyl)-*N*-(2-fluorophenethyl)acetamide (**19**): procedure C; educts: 2-(2-fluoro-4-hydroxyphenyl)-*N*-(2-fluorophenethyl)acetamide, 1-bromo-2-isothiocyanato-benzene; $C_{23}H_{18}F_2N_2O_2S$; MW: 424.46 g/mol; yield: 384 mg, 1.21 mmol, 98%; ^{1}H-NMR: (400.1 MHz, DMSO): 8.23 (t, 1H, $^3J_{HH}$ = 5.6 Hz, NH), 7.95 (dd, 1H, $^3J_{HH}$ = 8.0 Hz, $^4J_{HH}$ = 2.9 Hz, H_{ar}), 7.72 (d, 1H, $^3J_{HH}$ = 8.0 Hz, H_{ar}), 7.46-7.24 (m, 7H, H_{ar}), 7.17-7.00 (m, 2H, H_{ar}), 3.50 (s, 2H, CH_2), 3.31-3.30 (m, 4H, CH_2), 2.78 (t, 2H, $^3J_{HH}$ = 7.0 Hz, CH_2) ppm; ^{13}C-NMR: (100.6 MHz, DMSO): δ = 171.3, 168.7, 153.4, 153.3, 148.4, 131.9, 131.2 (d), 128.3, 128.2, 126.5, 124.3, 122.2, 121.3, 116.6 (d), 115.2, 115.0, 108.7, 108.5, 40.1 , 34.8 , 34.6 ppm; t_R: 11.3 min (method A), purity: 97%, HRMS: measured: 425.11267, calculated: 425.11298, Δm: 0.73 ppm, [M+H$^+$].

2-(4-(benzo[d]thiazol-2-yloxy)-2-fluorophenyl)-*N*-(3-fluorophenethyl)acetamide (**20**): procedure C, educts: 2-(2-fluoro-4-hydroxyphenyl)-*N*-(3-fluorophenethyl)acetamide, 1-bromo-2-isothiocyanato-benzene; $C_{23}H_{18}F_2N_2O_2S$; MW: 424.46 g/mol; yield: 228 mg, 0.54 mmol, 77%; ^{1}H-NMR: (250.1 MHz, $CDCl_3$): 7.76-7.70 (m, 2H, H_{ar}), 7.45-7.14 (m, 6H, H_{ar}), 6.94-6.81 (m, 3H, H_{ar}), 5.62 (bs, 1H, NH), 3.56-3.47 (m, 4H, CH_2), 2.79 (2H, $^3J_{HH}$ = 6.8 Hz, CH_2) ppm; ^{13}C-NMR: (75.4 MHz, $CDCl_3$): δ = 170.9, 169.5, 132.4, 132.3, 130.3, 130.2, 126.6, 124.6, 124.5 (d), 122.0, 121.5, 116.7 (d), 115.9, 115.6, 113.8, 113.5, 109.0, 108.7, 40.8, 36.6, 35.4 ppm; t_R: 11.3 min (method A), purity: 97%, HRMS: measured: 425.11260, calculated: 425.11298, Δm: 0.89 ppm, [M+H$^+$].

methyl 4-((2-(4-(benzo[*d*]thiazol-2-yloxy)-2-fluorphenyl)acetamido) methyl)benzoate (**21**): procedure C, educts: methyl 4-((2-(2-fluoro-4-hydroxyphenyl)acetamido)methyl)benzoate, 1-bromo-2-isothiocyanatobenzene; $C_{24}H_{19}FN_2O_4S$; MW: 450.48 g/mol; yield: 127 mg, 0.28 mmol, 51%; ^{1}H-NMR: (400.1 MHz, $CDCl_3$): 8.01-7.97 (m, 2H, H_{ar}), 7.75-7.69 (m, 2H, H_{ar}), 7.44-7.27 (m, 6H, H_{ar}), 7.24-7.17 (m, 1H, H_{ar}), 5.99-5.94 (m, 1H, NH), 4.51 (d, 2H, $^3J_{HH}$ = 6.0 Hz, CH_2), 3.83 (s, 3H, CH_3), 3.60 (d, 2H, $^4J_{HH}$ = 1.1 Hz, CH_2); ^{13}C-NMR: (75.4 MHz, $CDCl_3$): δ = 169.6, 154.7, 154.6, 148.9, 143.1, 132.5, 132.4, 130.2, 127.5,

126.6, 124.6, 122.03, 121.5, 116.6 (d), 109.0, 108.9, 52.2, 42.9, 36.2 ppm; t_R: 10.6 min (method A), purity: 97%, HRMS: measured: 451.11201, calculated: 451.11223, Δm: 0.49 ppm, [M+H$^+$].

2-(4-(benzo[*d*]thiazol-2-yloxy)-2-fluorophenyl)-*N*-((6-fluoro-1H-indol-3-yl)methyl)acetamide (**22**): procedure C, educts: *N*-((6-fluoro-1*H*-indol-3-yl)methyl)-2-(2-fluoro-4-hydroxyphenyl)acetamide, 1-bromo-2-isothiocyanatobenzene; $C_{24}H_{17}F_2N_3O_2S$; MW: 449.47 g/mol; yield: 265 mg, 0.59 mmol, 66%; ^{1}H-NMR: (400.1 MHz, DMSO): 11.00 (s, 1H, NH), 8.43 (t, 1H, $^3J_{HH}$ = 5.4 Hz, NH), 7.95 (dd, 1H, $^3J_{HH}$ = 7.9 Hz, $^4J_{HH}$ = 2.9 Hz, H_{ar}), 7.75 (dd, 1H, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 2.9 Hz, H_{ar}), 7.55-7.49 (m, 4H, H_{ar}), 7.36-7.32 (m, 1H, H_{ar}), 7.28-7.25 (m, 2H, H_{ar}), 7.15 (dd, 1H, $^3J_{HH}$ = 10.2 Hz, $^4J_{HH}$ = 4.1 Hz, H_{ar}), 6.88-6.83 (m, 1H, H_{ar}), 4.44 (d, 2H, $^3J_{HH}$ = 5.6 Hz, CH_2), 3.58 (s, 2H, CH_2); ^{13}C-NMR: (100.6 MHz, DMSO): δ =171.3, 168.5, 148.4, 136.3, 136.1, 132.6 (d), 131.9, 126.5, 124.4, 123.3, 122.2, 121.3, 119.8, 119.7, 116.6 (d), 112.5, 108.8, 108.5, 107.1, 106,9, 97.5, 97.2, 34.8, 34.2 ppm; t_R: 10.55 min (method A), purity: 90%, HRMS: measured: 488.06406, calculated: 488.06411, Δm: 0.10 ppm, [M+K$^+$].

LTA_4H expression and purification

The cloning, expression and purification of the recombinant human leukotriene A4 hydrolase (LTA_4H) with a hexahistidin-tag was described by Moser et. al.[1] The published expression and purification protocol was slightly modified in the performed experiments. In brief, LTA_4H was overexpressed in E. coli BL21(DE3)RIPL-Codon Plus cells (Invitrogen).Cells were grown in 1L culture of Miller's LB Broth Base™ (Invitrogen) at 37 °C and 180 rpm till an OD_{600} of approximately 0.8 before protein expression was induced by the addition of 400 µM IPTG (Isopropyl-β-D-thiogalactopyranosid, AppliChem). Temperature was reduced to 21 °C and cultures were harvested the next day by centrifugation. Generated cell pellets were suspended in LTA_4H buffer A (50 mM Tris, 500 mM NaCl, and 20 mM imidazole, HCl pH 8) supplemented with approximately 0.5 g DNAse (AppliChem) and an EDTA-free protease inhibitor complete tablet (Roche) before lysis. The cell debris were removed by consecutive centrifugation. Supernatant was sterile filtered (0.45 µM syringe filter) before further purification by immobilized metal ion affinity chromatography on a His-Trap HP column (GE Healthcare) using a step gradient protocol occurred. As running buffer buffer A was used, while LTA_4H buffer B (identical to buffer A with an imidazole concentration of 400 mM) was used as elution buffer. Protein was eluted at 100% LTA_4H buffer B. Fractions containing the protein were further purified by a Superdex200 column (GE Healthcare). As running buffer LT_4H buffer C (50 mM Tris, 500 mM NaCl, HCl pH 8) was used. The purity of the fractions containing LTA_4H was analyzed by SDS-PAGE. Pure fractions were pooled and the concentration determined by Nanodrop (Implen, Muenchen, Germany). Aliquots of the protein were flash frozen in liquid nitrogen and stored at -80 °C.

LTA_4H activity assay with L-arginine-7-amido-4-methylcoumarine

The fluorescence-based LTA_4H activity assay was performed according the protocol published by Wittmann et.al.[2] using the non-fluorescent L-arginine-7-amino-4-methylcoumarine (abcr, Karlsruhe, Germany) as substrate which is cleaved by the LTA_4H in L-arginine as well as the fluorescent amino-4-methylcoumarine. The assay was performed in black polystyrol 96-well plates in a final volume of 100 µL. LTA_4H buffer C supplemented with 0.01 % Triton-X 100 was used and resulting from the addition of the ligands the final samples contained 1% DMSO. For determination of IC_{50} values 0.25 µM protein were incubated with different concentrations of inhibitor for 30 minutes. Buffer containing DMSO and Triton-X 100 but no protein was used for negative control, while a sample with protein but without compound (with pure DMSO) was used as positive control. The substrate was added (200 µM final concentration) after the incubation and change in the fluorescence intensity was measured at Tecan Infinite F200 Pro Plate Reader (λ_{em} = 360 nm, λ_{ex} = 465 nm) for 30 minutes (one point every minute) at room temperature. Percent inhibition was calculated by referencing the slope in the linear

phase of the reactions to the slopes of negative and positive controls in MS Excel and fitting the obtained data with a sigmoidal dose response curve fit (variable slope with 4 parameters) in Graph Pad Prism 5.3 to determine IC_{50} values.

sEH expression and purification

The sEH full length (sEH-FL; aa1-aa555) for activity assays as well as the sEH-Hydrolase domain (aa222–aa555) (sEH-Hyd) used in the crystallization experiments were expressed and purified as published previously by Klingler et. al.[3] and Lukin et. al.[4] The same expression constructs were used for the expression sEH hydrolase and sEH-FL described in both publications. In brief, both proteins were expressed in *E. coli* BL21-(DE3) cells grown in ZYP5052 autoinduction media with kanamycin as selection marker. Cultures were incubated at 37 °C and 180 rpm for 2 h, before transferred to a 16 °C shaker. The cells were harvested after 36 h by centrifugation. Cell pellets were stored at -20 °C.

Bacterial pellets were thawed and re-suspended in sEH buffer A (50 mM Tris/HCl pH 8, 500 mM NaCl, 70 mM imidazole/ HCl pH 7) with one tablet of Complete EDTA free protease inhibitor mix (Roche, Basel, Switzerland) and a trace amount of DNAse I (Applichem, Darmstadt, Germany). The re-suspended cells were lysed and cell debris were removed by centrifugation. The supernatant was purified by nickel affinity chromatography using a step gradient protocol. As running buffer A was used, while sEH buffer B (identical to sEH buffer A with an imidazole concentration of 400 mM) was used as elution buffer. The fractions containing the target protein were pooled. The sEH-FL was dialysed for 18-24 hours at 4°C against 100fold excess of sEH buffer C (50 mM Tris, 50 mM NaCl, HCl pH 8) for activity assays using a 3500 kDa membrane. The dialysis buffer was exchanged twice during the dialysis period. The protein samples intended for the use in assays were mix with glycerol to a final concentration of 20 % (v/v), before the concentration was determined by Nanodrop and aliquots were flash frozen in liquid nitrogen and stored at -80 °C. The sEH-Hyd was concentrated 3fold by ultrafiltration to a final volume of 5 mL through 3000 Da cut-off membrane. The concentrated protein was applied onto the with sEH buffer D (50 mM Tris/HCl pH 8, 500 mM NaCl) pre-equilibrated Superdex 75 HiLoad 16/600 column (GE Healthcare, Germany) and separated at the flow of 1 mL/min in the sEH buffer D. sEH-Hyd in sEH buffer D was dialyzed in sEH buffer E (50 mM NaCl, 50 mM sodium phosphate, 10 % (v/v) glycerol (98%), 2 mM DTT, pH 7.4) before concentrated by ultrafiltration (3000 Da cut-off membrane) to 5-10 mg/mL and flash frozen in liquid nitrogen. Frozen protein samples were stored at -80 °C.

sEH-H activity assay with PHOME

The fluorescence based activity assay was performed according to a modified version of the protocol published by Lukin et.al.[4] and Hahn et. al.[5] In brief, the assay was performed in black flat bottom 96-well plates with a final assay volume of 100 µM and the non-fluorescent PHOME (3-phenyl-cyano-(6-methoxy-2-naphthalenyl)methyl ester-2-oxirane-acetic acid) as the substrate. The substrate can be hydrolyzed by the sEH to the fluorescent 6-methoxynaphtaldehyde[6] which is monitored over the course of the reaction at λ_{em} = 330 nm and λ_{ex} = 465 nm by a Tecan Infinite F200 Pro plate reader. For the assay recombinant human full length sEH (3 nM c_{final}) was dissolved in pH 7 Bis-Tris buffer with 0.1 mg/ml BSA as well as Triton-X 100 in a final concentration of 0.01% (w/v). 89 µl of protein mix were incubated for 30 min at room temperature with 1 µL of different concentrations of compounds in DMSO (final DMSO concentration 1%). The reaction was started by the addition of 10 µl substrate solution (to the final concentration 50 µM). The reaction was monitored for 30 mins (one point every minute). A blank control (no protein and no compound, but DMSO) as well as a positive control (no

compound, but DMSO) was carried out as well. All measurements were performed in triplicates. Data analysis was performed according to protocol of the LTA_4H activity assay with 7-amido-4-methylcoumarine described above.

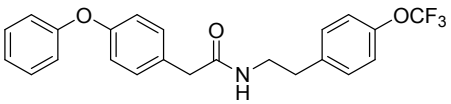
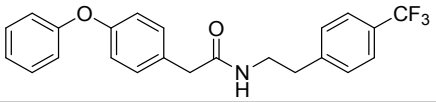
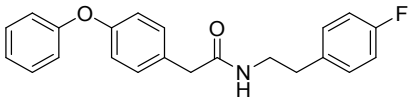
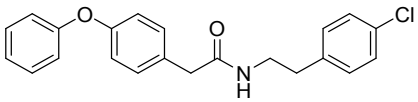
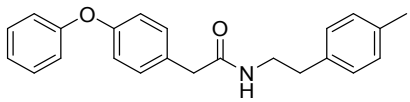
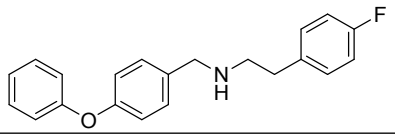
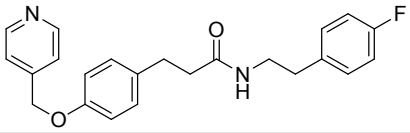
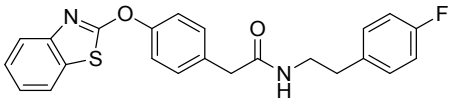
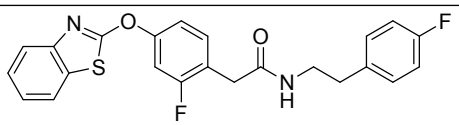
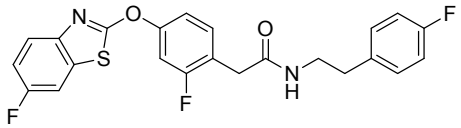
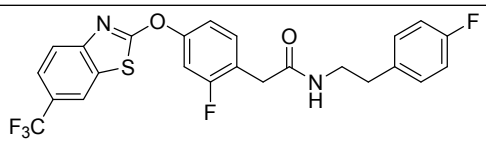
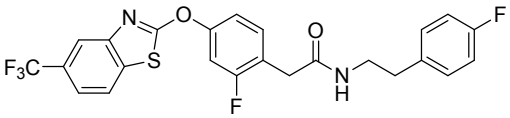
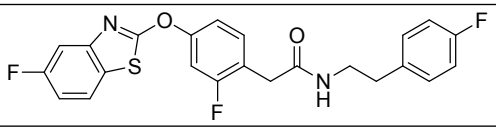
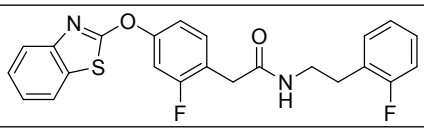
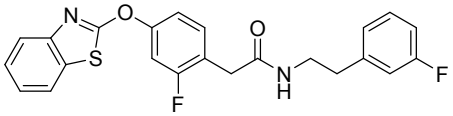
Water solubility assay

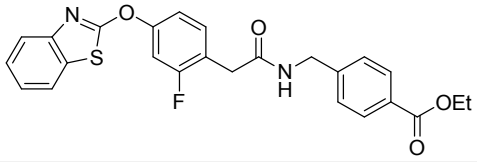
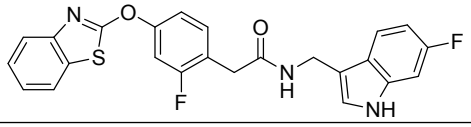
To determine the water solubility limit dilution series of the corresponding compounds in DMSO were performed. 1 µL of the DMSO solution was placed into clear polystyrene microplates and diluted with 99 µL DPBS buffer (pH 7.4) with 0.01% (w/v) Triton X-100. As reference three wells with 1 µL pure DMSO in 99 µL buffer was used. The absorption of the wells was measured by a Tecan infinite M200 at 650 nm. After determination of the means of each triplicate the values were compared to the mean of the reference. If the absorption values of the compound wells are higher than the reference the compound is not solved completely. The change of absorptions compared to the reference marks the solubility limit.

Table S 1: results of the water solubility assay.

Compound	**solubility limit (DBPS-buffer, pH 7.4)**
3	between 10 and 30 µM
5	between 10 and 30 µM
6	between 10 and 30 µM
7	between 10 and 30 µM
8	between 10 and 30 µM
9	between 10 and 30 µM
10	between 30 and 100 µM
13	between 10 and 30 µM
14	between 10 and 30 µM
15	between 3 and 10 µM
16	between 10 and 30 µM
17	between 10 and 30 µM
18	between 3 and 10 µM
19	between 10 and 30 µM
20	between 10 and 30 µM
21	between 10 and 30 µM
22	between 3 and 10 µM

Determination of cLogP values occurred via a web-based tool.[7]

compound	structure	clogP
3		5.30
5		5.12
6		4.49
7		5.19
8		4.90
9		4.93
10		4.11
13		5.28
14		5.17
15		5.27
16		5.67
17		5.72
18		5.28
19		5.13
20		5.15

21		5.01
22		5.04

Co-crystallization of 9 with the C-terminal domain of sEH

The protocol for the crystallization of the C-terminal domain is biased of the original publication of Xing et al.[8] and the modified protocol used for the co-crystallization of compound **7** is identical to the protocols published by Lukin et.al.[4] as well as Hiesinger and Kramer et.al.[9] and is referred in the following. The C-terminal sEH domain was crystallized over the course of a month at 277 K by sitting-drop vapor diffusion. 1 µL of the protein solution (5-10 mg/mL, 50 mM NaCl, 50 mM sodium phosphate, 10% (v/v) glycerol (98%), 2 mM DTT at pH 7.4 was mixed 1:1 with precipitant mixture (23 %-28 % (w/v) polyethylenglycol (PEG) 6000, 70 mM ammonium acetate, 200 mM magnesium acetate, 100 mM sodium cacodylate at pH 6.1-6.5). For soaking of the crystals, a saturated DMSO inhibitor solution of **9** was diluted 1:100 in PEG 400, before a second 1:9 dilution step in PEG 400/ precipitant mixture (3:7). Protein crystals where transferred into the soaking solution for 24 h, before the crystals were picked and flash frozen in the liquid nitrogen.

X-ray diffraction data of a single crystal was collected at the beamline station ID29 at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. All diffraction data was obtained from a single crystal. The data was processed with XDS software package[10]. The initial structure was obtained using the Phaser program[11] within PHENIX software package[12] where polypeptide model from the PDB record 4JNC where coordinates for heteroatoms (water and ligands) were excluded from the starting model. After several iterative rounds of model building with Coot[13] and the model refinement using the PHENIX software package[14] a value of 0.18 for R_{work} and a value of 0.24 for R_{free} were reached after the final round of refinement. Cif file the compound used during the refinement was generated using the elbow tool[15]. The overall electron density of the ligand was relatively low. In addition, density indicated that one additional binding conformation of the ligand might be present in lower abundance in the binding pocket, resulting in a relatively low RSCC score for the modeled main binding conformation. The graphical representations were made using MOE. Statistics of data collection and structural refinement are summarized in Table S1 which was generated using the PHENIX table one tool. The coordinates and structure-factor amplitudes of the structure have been deposited in the Protein Data Bank as entry 6HGW (9).

Table S1. Data collection and refinement statistics of sEH-H in complex with compound **9.** Statistics for the highest-resolution shell (10 % of reflections) are shown in parentheses.

Wavelength	1.072
Resolution range	53.26 - 2.407 (2.493 - 2.407)
Space group	I 2 2 2

Unit cell	80.18 92.42 106.53 90 90 90
Total reflections	203034 (19680)
Unique reflections	15703 (1526)
Multiplicity	12.9 (12.9)
Completeness (%)	99.92 (100.00)
Mean I/sigma(I)	15.34 (2.08)
Wilson B-factor	52.99
R-merge	0.1115 (1.331)
R-meas	0.1162 (1.386)
R-pim	0.03234 (0.3835)
CC1/2	0.998 (0.728)
CC*	1 (0.918)
Reflections used in refinement	15694 (1526)
Reflections used for R-free	1569 (153)
R-work	0.1804 (0.2909)
R-free	0.2387 (0.3532)
CC(work)	0.962 (0.863)
CC(free)	0.940 (0.754)
Number of non-hydrogen atoms	2623
macromolecules	2571
ligands	24
solvent	28
Protein residues	319

RMS(bonds)	0.005
RMS(angles)	0.74
Ramachandran favored (%)	96.53
Ramachandran allowed (%)	3.15
Ramachandran outliers (%)	0.32
Rotamer outliers (%)	0.00
Clashscore	4.32
Average B-factor	62.97
macromolecules	62.72
ligands	102.72
solvent	52.07
Number of TLS groups	5

Proposed binding mode of 13 in the binding site of LTA_4H

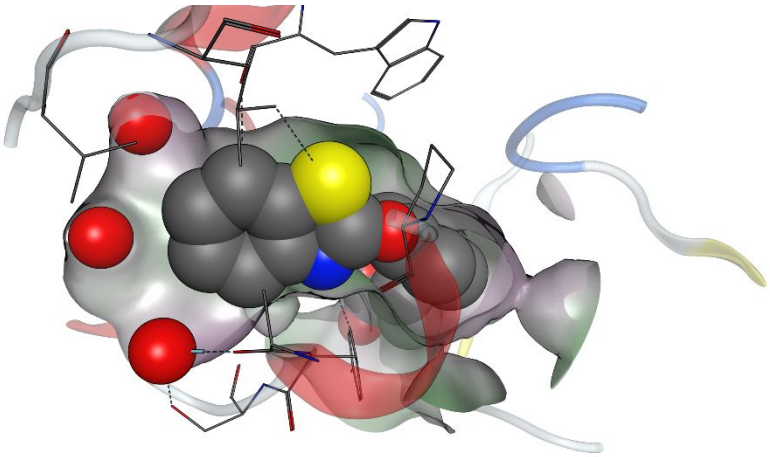


Figure S1: A benzthiazole-based inhibitor in the binding pocket of LTA_4H(PDB code 6ENC)[16] revealed a water cluster (red balls), which might be disturbed by substituents in the 5- and 6-position of the benzothiazole moiety.

Cellular assays in macrophages

Cells and cell isolation

Human peripheral blood (University Hospital Jena, Germany) was withdrawn from fasted (12 h) healthy adult donors (18-65 years) that had not taken anti-inflammatory drugs during the last 10 days, by venipuncture in heparinized tubes (16 IE heparin/ml blood), with written informed consent. The experimental protocol was approved by the ethical committee of the University Hospital Jena. All methods were performed in accordance with the relevant guidelines and regulations. In brief, the blood was centrifuged at 4000 × g for 20 min at 20 °C for preparation of leukocyte concentrates. Leukocyte concentrates were then subjected to dextran sedimentation and centrifugation on Ficoll-Histopaque 1077-1 (Sigma-Aldrich) cushions. Monocytes were isolated from peripheral blood mononuclear cell fraction that was obtained after Ficoll-Histopaque 1077-1 centrifugation of leukocyte concentrates and adherence for 1.5 h at 37 °C to culture flasks (Greiner, Nuertingen, Germany). The monocyte purity was > 85%, defined by forward- and side-light scatter properties and detection of the CD14 surface molecule by flow cytometry (BD FACS Calibur, Heidelberg, Germany).

Differentiation of monocytes to macrophages and macrophage polarization

The differentiation of monocytes to macrophages and polarization towards M1 and M2 was performed as described.[17] M1-macrophages were obtained by incubating monocytes with 20 ng/ml GM-CSF (Peprotech, Hamburg, Germany) for 6 days in RPMI 1640 supplemented with 10% FCS, 2 mmol/L L-glutamine, penicillin (100 U/mL) and streptomycin (100 µg/mL), followed by 100 ng/ml LPS and 20 ng/ml INF-γ (Peprotech) treatment for another 48 h. M2 were obtained by incubating monocytes with 20 ng/ml M-CSF (Peprotech) for 6 days and subsequent treatment with 20 ng/ml IL-4 (Peprotech) for additional 48 h. Correct polarization and purity of macrophages was routinely checked by flow cytometry (FACS Canto Plus flow cytometer, BD Bioscience) as reported[18] using the following antibodies: FITC anti-human CD14 (2 µg/test, clone M5E2, BD Bioscience), PE anti-human CD54 (1 µg/test, clone HA58, BD Bioscience), APC-H7 anti-human CD80 (0.25 µg/test, clone L307.4, BD Bioscience), PE-Cy7 anti-human CD163 (2 µg/test, clone RM3/1, Biolegend, San Diego, CA), PerCP-eFluor710 anti-human CD206 (0.06 µg/test, clone 19.2, Biosciences, San Diego, CA).

LM metabololipidomics in human macrophages

Macrophages (2 × 10^6/ml) were stimulated in PBS containing 1 mM $CaCl_2$. Compounds or vehicle control (0.1% DMSO) were added 10 min prior stimulation with *E. coli* (serotype O6:K2:H1, ratio 1:50, M1/M2:*E. coli*) for 180 min at 37 °C. Supernatants were transferred to 2 ml of ice-cold methanol containing 10 µl of deuterium-labeled internal standards (200 nM d_8-5S-HETE, d_4-LTB_4, d_5-LXA_4, d_5-RvD2, d_4-PGE_2 and 10 µM d_8-AA; Cayman Chemical/Biomol GmbH, Hamburg, Germany) to facilitate quantification. Sample preparation was conducted as described previously.[18] In brief, samples were kept at -20 °C for 60 min to allow protein precipitation. After centrifugation (1200 g, 4 °C, 10 min) 8 ml acidified H_2O (pH 3.5) was added and subjected to solid phase extraction. Solid phase cartridges (Sep-Pak® Vac 6cc 500 mg/ 6 ml C18; Waters, Milford, MA) were equilibrated with 6 ml methanol and 2 ml H_2O before samples were loaded onto columns. After washing with 6 ml H_2O and additional 6 ml *n*-hexane, LM were eluted with 6 ml methyl formiate. Finally, the samples were brought to dryness using an evaporation system (TurboVap LV, Biotage, Uppsala, Sweden) and resuspended in 100 µl methanol-water (50/50, v/v) for UPLC-MS-MS automated injections. LM profiling was analyzed with an Acquity™ UPLC system (Waters, Milford, MA) and a QTRAP 5500 mass spectrometer (AB Sciex, Darmstadt,

Germany) equipped with a Turbo V™ Source and electrospray ionization (ESI). LM were eluted using an ACQUITY UPLC® BEH C18 column (1.7 µm, 2.1 × 100 mm; Waters, Eschborn, Germany) as reported before.[18] The QTRAP 5500 mass spectrometer was operated in negative ionization mode using scheduled multiple reaction monitoring (MRM) coupled with information-dependent acquisition. The scheduled MRM window was 60 sec, optimized LM parameters were adopted, and the curtain gas pressure was set to 35 psi. The retention time and at least six diagnostic ions for each LM were confirmed by means of an external standard (Cayman Chemicals). Quantification was achieved by calibration curves for each LM. Linear calibration curves were obtained for each LM and gave r^2 values of 0.998 or higher (for fatty acids 0.95 or higher).

References

(1) Moser, D.; Wittmann, S. K.; Kramer, J.; Blöcher, R.; Achenbach, J.; Pogoryelov, D.; Proschak, E. PENG: A Neural Gas-Based Approach for Pharmacophore Elucidation. Method Design, Validation, and Virtual Screening for Novel Ligands of LTA4H. *J Chem Inf Model* **2015**, *55* (2), 284–293. https://doi.org/10.1021/ci500618u.

(2) Wittmann, S. K.; Kalinowsky, L.; Kramer, J. S.; Bloecher, R.; Knapp, S.; Steinhilber, D.; Pogoryelov, D.; Proschak, E.; Heering, J. Thermodynamic Properties of Leukotriene A4 Hydrolase Inhibitors. *Bioorg. Med. Chem.* **2016**, *24* (21), 5243–5248. https://doi.org/10.1016/j.bmc.2016.08.047.

(3) Klingler, F.-M.; Wolf, M.; Wittmann, S.; Gribbon, P.; Proschak, E. Bacterial Expression and HTS Assessment of Soluble Epoxide Hydrolase Phosphatase. *J Biomol Screen* **2016**, *21* (7), 689–694. https://doi.org/10.1177/1087057116637609.

(4) Lukin, A.; Kramer, J.; Hartmann, M.; Weizel, L.; Hernandez-Olmos, V.; Falahati, K.; Burghardt, I.; Kalinchenkova, N.; Bagnyukova, D.; Zhurilo, N.; et al. Discovery of Polar Spirocyclic Orally Bioavailable Urea Inhibitors of Soluble Epoxide Hydrolase. *Bioorg. Chem.* **2018**, *80*, 655–667. https://doi.org/10.1016/j.bioorg.2018.07.014.

(5) Hahn, S.; Achenbach, J.; Buscató, E.; Klingler, F.-M.; Schroeder, M.; Meirer, K.; Hieke, M.; Heering, J.; Barbosa-Sicard, E.; Loehr, F.; et al. Complementary Screening Techniques Yielded Fragments That Inhibit the Phosphatase Activity of Soluble Epoxide Hydrolase. *ChemMedChem* **2011**, *6* (12), 2146–2149. https://doi.org/10.1002/cmdc.201100433.

(6) Wolf, N. M.; Morisseau, C.; Jones, P. D.; Hock, B.; Hammock, B. D. Development of a High-Throughput Screen for Soluble Epoxide Hydrolase Inhibition. *Anal. Biochem.* **2006**, *355* (1), 71–80. https://doi.org/10.1016/j.ab.2006.04.045.

(7) Tetko, I. V.; Gasteiger, J.; Todeschini, R.; Mauri, A.; Livingstone, D.; Ertl, P.; Palyulin, V. A.; Radchenko, E. V.; Zefirov, N. S.; Makarenko, A. S.; et al. Virtual Computational Chemistry Laboratory--Design and Description. *J. Comput. Aided Mol. Des.* **2005**, *19* (6), 453–463. https://doi.org/10.1007/s10822-005-8694-y.

(8) Xing, L.; McDonald, J. J.; Kolodziej, S. A.; Kurumbail, R. G.; Williams, J. M.; Warren, C. J.; O'Neal, J. M.; Skepner, J. E.; Roberds, S. L. Discovery of Potent Inhibitors of Soluble Epoxide Hydrolase by Combinatorial Library Design and Structure-Based Virtual Screening. *J. Med. Chem.* **2011**, *54* (5), 1211–1222. https://doi.org/10.1021/jm101382t.

(9) Hiesinger, K.; Kramer, J. S.; Achenbach, J.; Moser, D.; Weber, J.; Wittmann, S. K.; Morisseau, C.; Angioni, C.; Geisslinger, G.; Kahnt, A. S.; et al. Computer-Aided Selective Optimization of Side Activities of Talinolol. *ACS Med Chem Lett* **2019**, *10* (6), 899–903. https://doi.org/10.1021/acsmedchemlett.9b00075.

(10) Kabsch, W. XDS. *Acta Crystallogr D Biol Crystallogr* **2010**, *66* (Pt 2), 125–132. https://doi.org/10.1107/S0907444909047337.

(11) McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. Phaser Crystallographic Software. *J Appl Cryst* **2007**, *40* (4), 658–674. https://doi.org/10.1107/S0021889807021206.

(12) Adams, P. D.; Afonine, P. V.; Bunkóczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L.-W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; et al. PHENIX: A Comprehensive Python-Based

System for Macromolecular Structure Solution. *Acta Cryst D* **2010**, *66* (2), 213–221. https://doi.org/10.1107/S0907444909052925.
(13) Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. Features and Development of Coot. *Acta Cryst D* **2010**, *66* (4), 486–501. https://doi.org/10.1107/S0907444910007493.
(14) Afonine, P. V.; Grosse-Kunstleve, R. W.; Echols, N.; Headd, J. J.; Moriarty, N. W.; Mustyakimov, M.; Terwilliger, T. C.; Urzhumtsev, A.; Zwart, P. H.; Adams, P. D. Towards Automated Crystallographic Structure Refinement with Phenix.Refine. *Acta Cryst D* **2012**, *68* (4), 352–367. https://doi.org/10.1107/S0907444912001308.
(15) Moriarty, N. W.; Grosse-Kunstleve, R. W.; Adams, P. D. Electronic Ligand Builder and Optimization Workbench (ELBOW): A Tool for Ligand Coordinate and Restraint Generation. *Acta Cryst D* **2009**, *65* (10), 1074–1080. https://doi.org/10.1107/S0907444909029436.
(16) Numao, S.; Hasler, F.; Laguerre, C.; Srinivas, H.; Wack, N.; Jäger, P.; Schmid, A.; Osmont, A.; Röthlisberger, P.; Houguenade, J.; et al. Feasibility and Physiological Relevance of Designing Highly Potent Aminopeptidase-Sparing Leukotriene A4 Hydrolase Inhibitors. *Sci Rep* **2017**, *7* (1), 1–19. https://doi.org/10.1038/s41598-017-13490-1.
(17) Werz, O.; Gerstmeier, J.; Libreros, S.; De la Rosa, X.; Werner, M.; Norris, P. C.; Chiang, N.; Serhan, C. N. Human Macrophages Differentially Produce Specific Resolvin or Leukotriene Signals That Depend on Bacterial Pathogenicity. *Nat Commun* **2018**, *9* (1), 59. https://doi.org/10.1038/s41467-017-02538-5.
(18) Werner, M.; Jordan, P. M.; Romp, E.; Czapka, A.; Rao, Z.; Kretzer, C.; Koeberle, A.; Garscha, U.; Pace, S.; Claesson, H.-E.; et al. Targeting Biosynthetic Networks of the Proinflammatory and Proresolving Lipid Metabolome. *FASEB J.* **2019**, *33* (5), 6140–6153. https://doi.org/10.1096/fj.201802509R.