Computer-aided fragment growing strategies to design dual inhibitors of soluble epoxide hydrolase and LTA4 hydrolase.

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Chemistry materials and general procedures

All used solvents and chemicals were purchased from Acros Organics (Geel, Belgium), Alfa-Aesar GmbH & Co KG (Karlsruhe, Germany), BLD Pharmatec Ltd (Shanghai, China), TCI Europe (Zwijndrecht, Belgium), Fluorochem (Derbyshire, United Kingdom) and used without further purification. All fragments for amide coupling were commercially available from Fluorochem (Derbyshire, United Kingdom), Enamine (Riga, Latvia), and Carbosynth (Newbury, United Kingdom). Microwave-assisted reactions were performed in a device Biotage Initiator 2.0 from Biotage (Uppsala, Sweden). For analytical thin layer chromatography (TLC) TLC plates F254 from Merck (KGaA, Darmstadt, Germany) were used and visualized with ultraviolet light (254 and 365 nm). Silica gel (particle size 0.063-0.200 nm) was purchased from Merck (Darmstadt, Germany) and used for column chromatography with technical grade solvent mixtures specified in the corresponding experiment. NMR spectra (^{1}H; ^{13}C) were recorded on a Bruker DPX 250 (250 MHz),

AV300 (300 MHz; 75 MHz), AV400 (400 MHz; 100 MHz) or AV500 (500 MHz; 125 MHz) spectrometer (Bruker, Karlsruhe, Germany). The multiplicities are b, broad; s, singlet; d, doublet; dd, double doublet; t, triplet; q quartett; m, multiplet and the approximate coupling constant (J) are reported in hertz (Hz). NMR data are given in ppm downfield relative to tetramethylsilane: internal reference non-deuterated solvent. HPLC spectra were recorded on a LCMS 2020 from Shimadzu (Duisburg, Germany). The used columns Luna 10µ C18(2) 100A (250 x 4.60 mm) for analytical purposes and Luna 10µ C18(2) (250 x 21.20 mm) column from Phenomenex LTD Deutschland (Aschaffenburg, Germany) for preparative purposes were run with acetonitrile (HPLC grade) and ultrapure water with 0.1% formic acid. Conditions were as followed: flowrate was 1 mL/min (Scout column) or 21 mL/min (semi-preparative) at room temperature with UV absorption at 254 and 280 nm. Determination of the purity of all compounds and preparative purification of several compounds was done by following method: 2 min 5% acetonitrile, afterwards a gradient from 5% to 90% acetonitrile within 10 min, 90% acetonitrile were hold for another 10 min. ESI-MS was performed on a LCMS-2020 from Shimadzu (Duisburg, Germany) or on a VG Platform II from Fisons Instruments (Glasgow, UK). MALDI-HRMS was performed on a MALDI LTQ Orbitrap XL instrument (Thermo Scientific, USA). Purity of all final compounds were 95% or higher determined by HPLC.

Preparation of 3-(4-(benzyloxy)phenyl)propionic acid (5):

Methyl 3-(4-hydroxyphenyl)propanoate: 3-(4-hydroxyphenyl)propanoic acid (10 g, 60 mmol, 1 eq) was dissolved in 150 mL methanol and a cat. amount of conc. sulfuric acid was added. The solution was heated under reflux conditions for 24 hours. The solvent was evaporated, and the oily residue was carefully neutralized with saturated $NaHCO_3$-solution (pH: 8 was adjusted). The aqueous phase was extracted with ethyl acetate and dried over $MgSO_4$. A brownish solid was obtained after filtration and evaporation. Yield: 8.4 g (47 mmol, 85%); $C_{10}H_{12}O_3$, MW: 180.20 g/mol; ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.07 - 7.04 (m, 2H), 6.76 - 6.73 (m, 2H), 5.08 (bs, 1H), 3.67 (s, 3H), 2.91 - 2.85 (m, 2H), 2.63 - 2.57 (m, 2H) ppm; LRMS (ESI) *m/z* calculated for [$C_{10}H_{12}NaO_3^+$] 203.07, found: 203.09.

Methyl 3-(4-(benzyloxy)phenyl)propanoate: methyl 3-(4-hydroxyphenyl)propanoate (8.4 g, 47 mmol, 1.0 eq) was dissolved in 150 mL acetone. K_2CO_3 (12.9 g, 93 mmol, 2.0 eq.), KI (0.78 g, 5 mmol, 0.1 eq) and benzyl bromide (5.7 mL, 47 mmol, 1.0 eq.) were added. The suspension was heated under reflux conditions for 19 hours. After cooling to room temperature acetone was removed under reduced pressure, the residue was dissolved in ethyl acetate and washed with water (3x). The organic layer was dried over $MgSO_4$, filtered and concentrated. Further purification with column chromatography (hexane/ ethyl acetate 10:1 to 5:1) yielded a white solid. Yield: 8.4 g (31 mmol, 71%); $C_{17}H_{18}O_3$, MW: 270.32 g/mol; ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.48-7.28 (m, 5H), 7.18-7.14 (m, 2H), 6.95-6.91 (m, 2H), 5.09 (s, 2H), 3.60 (s, 3H), 2.88-2.79 (m, 2H), 2.60-2.54 (m, 2H) ppm; LRMS (ESI) *m/z* calculated for [$C_{17}H_{19}O_3^+$] 271.13, found: 270.90.

3-(4-(Benzyloxy)phenyl)propanoic acid (**5**): For alkaline hydrolysis methyl 3-(4-(benzyloxy)phenyl)propanoate (8.4 g, 31 mmol, 1 eq.) was suspended in 200 mL THF/water/methanol (1:2:1). To this suspension KOH (10.3 g, 155 mmol, 5 eq.) was added and heated under reflux conditions for 21 hours. After cooling to room temperature the solvent was evaporated. The residue was suspended in dichloromethane and washed with 2 M hydrochloric acid (3x). The organic layer was dried over $MgSO_4$ and filtered. A white solid was obtained. Yield: 6.7 g (26 mmol, 83%); $C_{16}H_{16}O_3$, MW: 256.30 g/mol; ^{1}H-NMR (250 MHz, acetone-d_6) δ : 10.52 (bs, 1H), 7.49-7.45 (m, 2H), 7.42-7.28 (m, 3H), 7.20-7.16 (m, 2H), 6.95-6.91 (m, 2H), 5.09 (s, 2H), 2.88-2.82 (m, 2H), 2.61-2.54 (m, 2H) ppm; LRMS (ESI) *m/z* calculated for [$C_{16}H_{15}O_3^-$] 255.10, found: 255.23.

Amide coupling – general procedure A

1.1 eq 3-(4-(benzyloxy)phenyl)propionic acid, 1.1 eq PyBOP, 0.5-1.1 eq HOBt·H_2O and 1.0 eq corresponding amine were dissolved in dry THF. Additionally, 1.5-3.0 eq DIPEA were added and the mixture stirred either for 16 h at room temperature or 1 h at 60 °C under microwave irradiation. After removal of the solvent the residue was dissolved in ethyl acetate and was washed three times with water and one time with brine. The organic phase was dried over $MgSO_4$ and filtered. The solvent was removed under reduce pressure and the obtained oil was purified via column chromatography. A solid was obtained.

Amide coupling – general procedure B

1.0 eq 3-(4-(benzyloxy)phenyl)propionic acid, 1.0-1.3 eq amine derivative, 1.2 eq EDC·HCl and a catalytic amount of 4-DMAP were dissolved in dry DCM. The mixture was heated to 60 °C for 60 min under microwave irradiation. The solvent was evaporated under reduced pressure and the residue was purified via column chromatography. The obtained solid was purified further with preparative HPLC to gain purities over 95%.

3-(4-(Benzyloxy)phenyl)-*N*-((1-phenethylpyrrolidin-3-yl)methyl)propanamide (**4a**)
procedure A; reagents: 3-(4-(benzyloxy)phenyl)propionic acid (83 mg, 0.33 mmol), PyBOP (186 mg, 0.36 mmol), HOBt·H_2O (55 mg, 0.36 mmol, 1.1 eq), [1-(2-phenylethyl)pyrrolidin-3-yl]methanamine (70 mg, 0.33 mmol), 10 mL THF and DIPEA (0.1 mL, 0.99 mmol, 3 eq); eluent of column chromatography hexane/acetone 2:1-1:1, yield: 0.08 g (0.18 mmol, 54%); $C_{29}H_{34}N_2O_2$, MW: 442.59 g/mol; ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.27 (s, 1H), 7.46 - 7.11 (m, 12H), 6.94 - 6.89 (m, 2H), 5.07 (s, 2H), 3.18 - 3.13 (m, 2H), 2.86 - 2.77 (m, 7H), 2.75 - 2.53 (m, 3H), 2.38 (t, J=7.5 Hz, 3H), 1.97 - 1.82 (m, 1H), 1.54 - 1.43 (m, 1H) ppm; ^{13}C-NMR (100 MHz, acetone-d_6) δ = 172.5, 172.4, 165.5, 158.1, 140.3, 138.5, 134.6, 130.2, 129.6, 129.3, 129.2, 128.5, 128.3, 127.0, 115.5, 70.3, 60.2, 57.2, 53.8, 38.6, 36.5, 32.6, 31.5, 28.2, 24.3 ppm; purity (HPLC-MS): 95%, t_R = 10.64 min; HRMS (MALDI) *m/z* calculated for [$C_{29}H_{34}N_2O_2$+H^+]: 443.26930, found: 443.26878.

3-(4-(Benzyloxy)phenyl)-*N*-((1-phenethylpiperidin-3-yl)methyl)propanamide (**4b**)
procedure B; reagents: 3-(4-(benzyloxy)phenyl)propionic acid (75 mg, 0.30 mmol), (1- phenethylpiperidin-3-yl)methanamine (70 mg, 0.32 mmol, 1.1 eq), EDC·HCl (67 mg, 0.35 mmol), cat. amount DMAP, 4 mL DCM; eluent of column chromatography: DCM/methanol$_{ammonia}$ 19:1-9:1, further purification occurred by preparative HPLC; yield: 100 mg (0.23 mmol, 75%); $C_{30}H_{36}N_2O_2$, MW: 456.62 g/mol; ^{1}H-NMR (500 MHz, acetone-d_6) δ = 8.39 (s, 1H), 7.48 - 7.10 (m, 12H), 6.94 - 6.88 (m, 2H), 6.29 (s, 1H), 5.06 (s, 2H), 3.22 - 3.18 (m, 2H), 3.03 - 2.81 (m, 10H), 2.78 - 2.72 (m, 1H), 2.48 - 2.38 (m, 3H), 1.99 - 1.91 (m, 1H), 1.61 - 1.55 (m, 1H) ppm, ^{13}C-NMR (125 MHz, acetone-d_6) δ = 172.5, 172.5, 166.3, 158.1, 140.0, 138.6, 134.6, 130.2, 129.5, 129.3, 129.2, 128.5, 128.3, 127.1. 115.5, 70.3, 57.6, 57.4, 53.8, 43.0, 42.9, 38.6 (d), 38.1, 34.1, 31.5, 28.4 ppm; purity (HPLC-MS): 95%, t_R = 10.74 min; HRMS (MALDI) *m/z* calculated for [$C_{30}H_{36}N_2O_2$+H^+]: 457.28495, found: 457.28445.

3-(4-(Benzyloxy)phenyl)-*N*-((1-(thiophen-2-ylmethyl)piperidin-4-yl)methyl)propanamide (**4c**)
procedure B; reagents: 3-(4-(benzyloxy)phenyl)propionic acid (80 mg, 0.32 mmol), [1-(Thien-2-ylmethyl)piperid-4-yl]methylamine (70 mg, 0.32 mmol, 1.0 eq), EDC·HCl (73 mg, 0.38 mmol), cat. amount DMAP, 4 mL DCM; eluent of column chromatography: DCM/methanol$_{ammonia}$ 19:1-9:1; yield: 123 mg (0.27 mmol, 87%);$C_{27}H_{32}N_2O_2S$, MW: 448.62 g/mol; ^{1}H NMR (300 MHz, $CDCl_3$-d_1) δ = 7.50 - 7.28 (m, 5H),

7.20 (dd, J=5.1, 1.2 Hz, 1H), 7.17 - 7.05 (m, 2H), 6.95 - 6.83 (m, 4H), 5.36 (t, J=5.6 Hz, 1H), 5.02 (s, 2H), 3.69 (d, J=0.6 Hz, 2H), 3.08 (t, J=6.3 Hz, 2H), 2.93 - 2.86 (m, 4H), 2.43 (t, J=7.5 Hz, 2H), 1.94 (td, J=11.5, 2.4 Hz, 2H), 1.55 - 1.47 (m, 2H), 1.27 - 1.12 (m, 3H) ppm, ^{13}C NMR (75 MHz, $CDCl_3$-d_1) δ = 172.2, 157.5, 142.0, 137.2, 133.3, 129.5, 128.7, 128.1, 127.6, 126.5, 126.0, 125.0, 115.0, 70.2, 57.5, 53.1, 45.1, 39.0, 36.0, 31.1, 30.0 ppm; purity (HPLC-MS): 97%, t_R = 10.35 min; HRMS (MALDI) *m/z* calculated for [$C_{27}H_{32}N_2O_2S+H^+$]: 449.22573, found: 449.22532.

3-(4-(Benzyloxy)phenyl)-*N*-(2-(1-benzylpyrrolidin-3-yl)ethyl)propanamide (**4d**)
procedure B; reagents: 3-(4-(benzyloxy)phenyl)propionic acid (81 mg, 0.32 mmol), 2-(1-benzylpyrrolidin-3-yl)ethanamine (88 mg, 0.32 mmol, 1.0 eq), EDC·HCl (73 mg, 0.38 mmol), cat. amount DMAP, 4 mL DCM; eluent of column chromatography: DCM/methanol$_{ammonia}$ 19:1-9:1; yield: 39 mg (0.09 mmol, 28%); $C_{29}H_{34}N_2O_2$, MW: 442.59 g/mol; ^{1}H-NMR (250 MHz, $CDCl_3$-d_1) δ = 8.39 (s, 1H), 7.41 - 7.30 (m, 10H), 7.16 - 7.08 (m, 2H), 6.92 - 6.85 (m, 2H), 5.03 (d, J = 5.1 Hz, 2H), 4.05 (s, 1H), 3.73 (t, J = 14.8 Hz, 1H), 3.36 - 3.06 (m, 4H), 2.95 - 2.65 (m, 5H), 2.44 - 1.88 (m, 3H), 1.67- 1.46 (m, 2H), 1.17 (t, J = 7.3 Hz, 1H) ppm, ^{13}C-NMR (75 MHz, $CDCl_3$-d_1) δ =176.0, 172.5, 168.4, 157.4 (d), 137.2 (d), 133.4, 133.0, 129.9, 129.6 (d), 128.9, 128.8, 128.5, 128.1, 127.6, 115.1 (d), 70.2, 59.4, 58.4, 55.0, 52.8, 44.2, 38.8, 38.4, 38.1, 35.5, 35.2, 34.0, 30.9, 30.1 (d) ppm, purity (HPLC-MS): 98%, t_R = 10.54 min; HRMS (MALDI) *m/z* calculated for [$C_{29}H_{34}N_2O_2+H^+$]: 443.26930, found: 443.26991.

3-(4-(Benzyloxy)phenyl)-*N*-((1-(3-bromophenyl)pyrrolidin-3-yl)methyl)propanamide (**4e**)
procedure B; reagents: 3-(4-(benzyloxy)phenyl)propionic acid (81 mg, 0.32 mmol) [1-(3-bromophenyl)pyrrolidin-3-yl]methanamine (80 mg, 0.32 mmol, 1.0 eq), EDC·HCl (73 mg, 0.38 mmol), cat. amount DMAP, 4 mL DCM; eluent of column chromatography: hexane/acetone 2:3; yield: 55 mg (0.11 mmol, 35%); $C_{27}H_{29}BrN_2O_2$; MW: 493.44 g/mol; ^{1}H-NMR (400 MHz, $CDCl_3$-d_1) δ = 7.45 - 7.29 (m, 5H), 7.12 (d, 2H), 7.04 (t, J = 8.0 Hz, 1H), 6.97 - 6.85 (m, 2H), 6.77 (dd, J = 7.7, 1.9 Hz, 1H), 6.63 (t, J = 2.1 Hz, 1H), 6.41 (dd, J = 8.3, 2.4 Hz, 1H), 5.51 (t, J = 6.1 Hz, 1H), 5.00 (s, 2H), 3.35 - 3.17 (m, 5H), 2.94 - 2.85 (m, 3H), 2.48 - 2.39 (m, 3H), 2.05 - 1.96 (m, 1H), 1.66 - 1.57 (m, 1H) ppm, ^{13}C-NMR (100 MHz, $CDCl_3$-d_1) δ = 172.5, 157.5, 148.9, 137.2, 133.1, 130.5, 129.5, 128.7, 128.1, 127.6, 123.5, 118.6, 115.1, 114.5, 110.4, 70.1, 51.2, 47.1, 42.2, 38.8, 31.0, 29.0 ppm; purity (HPLC-MS): 97%, t_R = 17.73 min; HRMS (MALDI) *m/z* calculated for [$C_{27}H_{29}BrN_2O_2+H^+$]: 493.14852, found: 493.14714.

3-(4-(Benzyloxy)phenyl)-*N*-((1-(3-phenylpropyl)piperidin-4-yl)methyl)propanamide (**4f**)
procedure A; reagents: 3-(4-(benzyloxy)phenyl)propionic acid (81 mg, 0.32 mmol), PyBOP (180 mg, 0.35 mmol), HOBt·H_2O (24 mg, 0.16 mmol, 0.5 eq), (1-(3-phenylpropyl)piperidin-4-yl)methanamine (73 mg, 0.32 mmol), 10 mL THF and DIPEA (0.1 mL, 0.79 mmol, 2.5 eq), the mixture reacted under microwave irradiation; eluent of column chromatography: DCM/methanol$_{ammonia}$ 9:1; yield: 0.11 g (0.24 mmol, 76%); $C_{31}H_{38}N_2O_2$, MW: 470.65 g/mol; ^{1}H-NMR (300 MHz, $CDCl_3$-d_1) δ = 7.39 - 6.96 (m, 12H), 6.85 - 6.76 (m, 2H), 5.45 - 5.40 (m, 1H), 4.95 (s, 2H), 3.03 - 2.98 (m, 2H), 2.94 - 2.73 (m, 4H), 2.57 - 2.51 (m, 3H), 2.39 - 2.31 (m, 4H), 1.90 - 1.71 (m, 4H), 1.51 - 1.09 (m, 4H) ppm.; ^{13}C-NMR (100 MHz, $CDCl_3$-d_1) δ = 172.4, 157.4, 141.9, 137.2, 133.2, 129.5, 128.7, 128.5 (d), 128.1, 127.6, 126.0, 115.0, 70.1, 58.3, 53.5, 44.9, 38.9, 35.7, 33.8, 31.1, 29.5, 28.4 ppm; purity (HPLC-MS): 97%, t_R = 10.77 min; ; HRMS (MALDI) *m/z* calculated for [$C_{31}H_{38}N_2O_2+H^+$]: 471.30068, found: 471.30140.

3-(4-(Benzyloxy)phenyl)-*N*-(4-(2-morpholinoethyl)phenyl)propanamide (**4g**)
procedure A; reagents: 3-(4-(benzyloxy)phenyl)propionic acid (0.18 g, 0.71 mmol), PyBOP (0.41 g, 0.78 mmol), HOBt·H_2O (0.06 g, 0.36 mmol, 0.5 eq), 4-(2-morpholinoethyl)aniline (0.15 g, 0.71 mmol), 10 mL THF and DIPEA (0.2 mL, 1.07 mmol, 1.5 eq), modified procedure: after column chromatography: hexane/acetone 2:1 further purification occurred by preparative HPLC, yield: 0.23 g (0.51 mmol, 71%); $C_{28}H_{32}N_2O_3$, MW: 444.57 g/mol; ^{1}H-NMR (400 MHz, $CDCl_3$-d_1) δ = 7.41 - 7.21 (m, 7H), 7.17 - 6.93 (m, 5H), 6.83 (d, J=8.5 Hz, 2H), 4.97 (s, 2H), 3.72 (t, J=4.7 Hz, 4H), 2.91 (t, J=7.4 Hz, 2H), 2.78 - 2.72 (m, 2H), 2.67 - 2.38 (m, 8H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$-d_1) δ = 170.5, 166.3, 157.6, 137.2, 136.3, 135.2, 133.1, 129.5, 129.3, 128.7, 128.1, 127.6, 120.3, 115.2, 70.2, 66.2, 60.2, 53.1,39.8, 31.8, 30.9 ppm; purity (HPLC-MS): 99%, t_R = 10.52 min; HRMS (MALDI) *m/z* calculated for [$C_{28}H_{32}N_2O_3$+H^+]: 445.24857, found: 445.24868.

***N*-(4-(2-(Azepan-1-yl)ethoxy)phenyl)-3-(4-(benzyloxy)phenyl)propanamide** (**4h**)
procedure B; reagents: 3-(4-(benzyloxy)phenyl)propionic acid (80 mg, 0.31 mmol), 4-(2-(azepan-1-yl)ethoxy)aniline (100 mg, 0.40 mmol, 1.3 eq), EDC·HCl (72 mg, 0.38 mmol), cat. amount DMAP, 4 mL DCM; eluent of column chromatography: DCM/methanol$_{ammonia}$ 100:0-9:1; yield: 88 mg (0.19 mmol, 60%); $C_{30}H_{36}N_2O_3$, MW: 472.62 g/mol;^{1}H-NMR (300 MHz, $CDCl_3$-d_1) δ = 8.54 (s, 1H), 7.68 (s, 1H), 7.43 - 7.31 (m, 6H), 7.17 - 7.11 (m, 2H), 6.92 - 6.87 (m, 2H), 6.80 - 6.73 (m, 2H), 5.03 (s, 2H), 4.23 (t, J=5.1 Hz, 2H), 3.29 (t, J=5.0 Hz, 2H), 3.20 - 3.14 (m, 4H), 2.98 (t, J=7.5 Hz, 2H), 2.60 (t, J=7.6 Hz, 2H), 1.89 - 1.79 (m, 4H), 1.70 - 1.64 (m, 4H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$-d_1) δ = 170.7, 168.4, 157.5, 154.5, 137.2, 133.3, 132.2, 129.5, 128.7, 128.1, 127.6, 121.9, 115.1, 114.9, 70.2, 64.2, 55.5, 54.8, 39.6, 30.9, 27.1, 24.3 ppm; purity (HPLC-MS): 98%, t_R = 10.91 min; HRMS (MALDI) *m/z* calculated for [$C_{30}H_{36}N_2O_3$+H^+]: 473.27987, found: 473.27987.

3-(4-(Benzyloxy)phenyl)-*N*-(4-(*N*-(2,2,2-trifluoroethyl)sulfamoyl)phenyl)propanamide (**4i**)
procedure B; reagents: 3-(4-(benzyloxy)phenyl)propionic acid (100 mg, 0.39 mmol), 4-amino-*N*-(2,2,2-trifluoroethyl)benzenesulfonamide (136 mg, 0.51 mmol, 1.3 eq), EDC·HCl (90 mg, 0.47 mmol), cat. amount DMAP, 4 mL DCM; eluent of column chromatography: hexane/acetone 2:1-1:1; yield: 39 mg (0.08 mmol, 20%); $C_{24}H_{23}F_3N_2O_4S$, MW: 492.51 g/mol; ^{1}H-NMR (400 MHz, acetone-d_6) δ = 9.48 (s, 1H), 7.88 - 7.70 (m, 4H), 7.50 - 7.23 (m, 6H), 7.21 - 7.16 (m, 2H), 6.95 - 6.90 (m, 2H), 5.08 (s, 2H), 3.80 - 3.69 (m, 2H), 3.07 - 2.83 (m, 2H), 2.73 - 2.68 (m, 2H) ppm; ^{13}C-NMR (100 MHz, acetone-d_6) δ = 171.9, 158.3, 144.3, 138.6, 135.6, 134.3, 130.2, 129.3, 128.8, 128.4, 126.7, 123.9, 119.7, 115.6, 70.4, 45.5, 45.1, 44.8, 44.4 (q), 39.7 ppm; purity (HPLC-MS): 98%, t_R = 15.77 min; HRMS (MALDI) *m/z* calculated for [$C_{24}H_{23}F_3N_2O_4S$+H^+]: 493.14034, found: 493.13901.

Diethyl (4-(3-(4-(benzyloxy)phenyl)propanamido)phenyl)phosphonate (**4j**)
procedure A; reagents: 3-(4-(benzyloxy)phenyl)propionic acid (53 mg, 0.21 mmol), PyBOP (119 mg, 0.23 mmol), HOBt·H_2O (35 mg, 0.23 mmol, 1.1 eq), diethyl (4-aminophenyl)phosphonate (50 mg, 0.21 mmol), 10 mL THF and DIPEA (0.1 mL, 0.62 mmol, 3 eq); eluent of column chromatography: hexane/acetone 2:1-1:1, yield: 0.02 g (0.05 mmol, 23%); $C_{26}H_{30}NO_5P$, MW: 467.49 g/mol; ^{1}H-NMR (400 MHz, $CDCl_3$-d_1) δ = 8.07 (s, 1H), 7.67 - 7.50 (m, 4H), 7.36 - 7.20 (m, 5H), 7.08 - 7.01 (m, 2H), 6.84 - 6.78 (m, 2H), 4.95 (s, 2H), 4.07 - 3.88 (m, 4H), 2.90 (t, J=7.6 Hz, 2H), 2.58 (t, J=7.6 Hz, 2H), 1.21 (t, J=7.1 Hz, 6H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$-d_1) δ = 171.3, 157.5, 142.3, 142.2, 137.2, 133.1, 132.1, 129.5, 128.7, 127.6, 123.7, 121.7, 119.4, 119.2, 115.2, 115.0, 62.3, 39.7, 30.7, 16.5, 16.4 ppm; purity

(HPLC-MS): 97%, t_R = 15.50 min; HRMS (MALDI) *m/z* calculated for [$C_{26}H_{30}NO_5P+H^+$]: 468.19344, found: 468.19267.

3-(4-(Benzyloxy)phenyl)-*N*-(4-(trifluoromethyl)oxazol-2-yl)propanamide (**4k**)

Under argon atmosphere 3-(4-(benzyloxy)phenyl)propionic acid (120 mg, 0.47 mmol, 1.5 eq), Fluoro-*N*,*N*,*N*′,*N*′-bis(tetramethylen)formamidinium hexafluorophosphate (151 mg, 0.47 mmol, 1.5 eq) and DIPEA (0.3 mL, 1.42 mmol, 4.5 eq) were dissolved in 3 mL dry DCM in a microwave vial and the vial was sealed. The mixture was heated to 50 °C for 4 h. Additionally, 4-trifluoromethyl-oxazol-2-ylamine (50 mg, 0.32 mmol, 1.0 eq), dissolved in 3 mL dry DCM, was added and the sealed tube was heated to 50 °C for another 72 h. The reaction mixture was diluted with ethyl acetate and washed with water (3x). The precipitate, which was generated during the washing steps, was filtered. The organic phase was dried over $MgSO_4$, filtered and the solvent was evaporated. The residue was purified with column chromatography (eluent: hexane:acetone 4:1) and further with preparative HPLC. A white solid was obtained; yield: 18 mg (0.11 mmol, 35%); $C_{20}H_{17}F_3N_2O_3$, MW: 390,36 g/mol; ^{1}H-NMR (300 MHz, $CDCl_3$-d_1) δ = 9.06 (s, 1H), 7.66 (q, J=1.6 Hz, 1H), 7.40 - 7.21 (m, 5H), 7.10 - 7.04 (m, 2H), 6.85 - 6.79 (m, 2H), 4.96 (s, 2H), 3.20 - 2.70 (m, 4H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$-d_1) δ = 1576, 154.4, 137.2, 134.8, 129.6, 128.7, 128.1, 127.6, 123.5, 121.3, 119.2, 117.1, 115.1, 77.4, 77.2, 76.9, 70.2, 38.4, 29.9 ppm; purity (HPLC-MS): 97%, t_R = 15.76 min; HRMS (MALDI) *m/z* calculated for [$C_{20}H_{17}F_3N_2O_3+H^+$]: 391.12640, found: 391.12622.

sEH expression and purification

The sEH full length (sEH-FL; aa1-aa555) was expressed and purified as published previously by Lukin et. al.[1] In brief, the enzyme was expressed in *E. coli* BL21-(DE3) cells, grown in ZYP5052 autoinduction media with kanamycin as selection marker at 16 °C for 36 h. After expression the protein was isolated by nickel affinity chromatography followed by a size exclusion chromatography. Buffer for the size exclusion chromatography was 50 mM Tris, 500 mM NaCl, pH8 (HCl). Protein was supplemented with 25% (v/v) glycerol and aliquots of the protein were flash frozen in liquid nitrogen and stored at - 80 °C.

sEH-H activity assay with PHOME

The fluorescence-based activity assay was adapted from the protocols published by Lukin et.al.[1] and Hahn et. al.[2] The assay was performed in black flat bottom 96-well plates with a final assay volume of 100 µL. As substrate the non-fluorescent PHOME (3-phenyl-cyano-(6-methoxy-2-naphthalenyl)methyl ester-2-oxirane-acetic acid) was used. PHOME is hydrolyzed by the sEH to the fluorescent 6-methoxynaphtaldehyde, which is monitored at λ_{ex} = 330 nm and λ_{em} = 465 nm by a Tecan Infinite F200 Pro plate reader (Männedorf, Switzerland). A dilution series of the tested compounds in DMSO was generated. 1 µL of each concentration of the dilution series was pipetted in the wells and 89 µL of a mixture of recombinant human full length sEH (3 nM c_{final}) in 25 Mm Bis-Tris buffer (pH 7) with 0.1 mg/ml BSA and Triton-X 100 (0.01% (w/v) c_{final}) was added. The plates were incubated for 45 min at room temperature. The reaction was started by the addition of 10 µl substrate solution (25 Mm Bis-Tris (pH 7) with 0.1 mg/ml BSA, 50 µM c_{final}) and monitored for 45 mins (one point each minute). A blank control (1% pure DMSO, without protein) as well as a positive control (1% pure DMSO, with protein) was carried out as well. All measurements were performed in three independent experiments and in triplicates. 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. For further fitting GraphPad Prism 7 was used (fit: sigmoidal dose response curve fit, variable slope with 4 parameters).

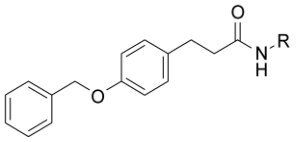
LTA4H expression and purification

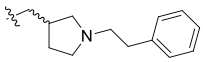
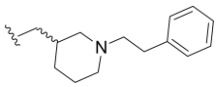
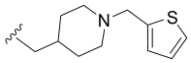
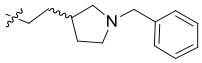
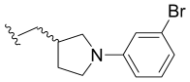
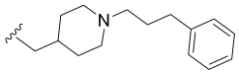
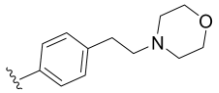
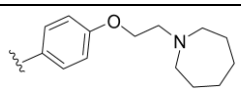
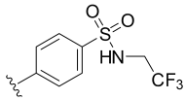
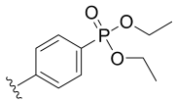
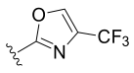
The protocol steps for the expression and isolation of recombinant human LTA4H was published by Moser et al.[3] In brief, a pET 24 (+) vector encoding the full length LTA4H with a hexahistidin tag (C-terminus) and a kanamycin resistance was used for the expression. The overexpression of the protein was performed in *E. coli* BL21(DE3) cells overnight at 21 °C. After harvesting and lysis of the cell, the protein was purified by metal ion affinity chromatography. Fractions with protein were further purified by a Superdex200 column. The running buffer contained 50 mM Tris, 50 mM NaCl (pH 8.0) and aliquots of the protein were flash frozen in liquid nitrogen and stored at - 80 °C.

LTA4H activity assay with L-arginine-7-amido-4-methylcoumarine

To determine the inhibitory activity of the synthesized compounds a fluorescence-based LTA4H activity assay was performed according the protocol published by Wittmann et.al.[4] with minor modifications. As substrate the non-fluorescent L-arginine-7-amino-4-methylcoumarine (Sigma Aldrich, US) was used which is cleaved by the LTA4H in L-arginine as well as the fluorescent amino-4-methylcoumarine. The assay was performed in 96-well black flat bottom polystyrene plates in a final volume of 100 µL. 1 µL of inhibitor solution in DMSO was pipetted in the wells and a mixture of LTA4H (200 nM c_{final}) in 50 mM Tris, 50 mM NaCl, pH 8.0 supplemented with 0.01% Triton-X 100 was added. After an incubation time of 30 min 10 µL substrate solution (in 50 mM Tris, 50 mM NaCl, pH 8.0; 180 µM c_{final}) was added and the change in the fluorescence intensity was measured by a Tecan Infinite F200 Pro Plate Reader (λ_{ex} = 360 nm, λ_{em} = 465 nm) for 30 minutes (one point each minute) at room temperature. As blank 1% pure DMSO without protein was used and as positive control 1% DMSO with protein solution. All measurements were performed in triplicates as well as in three independent experiments. The determination of IC_{50} values were described above in section sEH-H activity assay with PHOME**.**

Table S1. Inhibitory activity values of all compounds



Cpd Nr.	R	LTA4H (IC_{50} or % inhibition at different concentrations)[a]	sEH (IC_{50} or % inhibition at different concentrations)[a]
2	-	-	0.013 ± 0.0003 µM
3	-	0.27 ± 0.003 µM	-
4a		0.67 ± 0.04 µM	64 % @300µM, 25 % @30µM; 4 % @10µM
4b		0.69 ± 0.08 µM	15 ± 8 µM
4c		0.75 ± 0.09 µM	0.5 ± 0.2 µM
4d		3.2 ± 0.6 µM	28 ± 1 µM
4e		12 % @ 300µM; 5% @100µM; 4 % @10µM	10 ± 4 µM
4f		18.3 ± 0.8 µM	69 % @ 300 µM; 39 % @ 100 µM; 20 % @30µM; 7 % @10µM
4g		30% @100 µM; 16% @10µM	1.3 ± 0.1 µM
4h		30% @100 µM; 16% @10µM	16 ± 1.3 µM
4i		26 % @300µM; 10 % @100µM; 9% @10µM	9 % @100µM; 6 % @10µM
4j		4.2 ± 0.8 µM	4.7 ± 0.8 µM
4k		0.57 ± 0.08 µM	0.317 ± 0.008 µM

Training of the self-organizing map (SOM)

For the training of the SOM, sEH and LTA4H inhibitors from ChemblDB v. 24 were extracted using OSIRIS DataWarrior (Idorsia Pharmaceuticals). The data was filtered for compounds with an annotated IC_{50} value. 1794 sEH inhibitors and 748 LTA4H inhibitors remained. A 50x50 SOM was trained on FragFP representations of molecular structures and analyzed manually.

Machine learning optimization

Table S2: Optimal partitioning scheme for targets of interest and each of the five fingerprints used.

LTA4H	Train set size	Test set size
PLIF	90	10
AtomPair	75	25
Morgan	85	15
FeatMorgan	75	25
MACCS	85	15
sEH		
PLIF	90	10
AtomPair	85	15
Morgan	75	25
FeatMorgan	80	20
MACCS	80	20

Table S3: Parameters optimized by grid search.

Machine learning algorithm	Parameter	Tested values
XGBoost	Max depth	10-200 | 10
	Learning rate	0.01, 0.001
	Estimators	100-1,000 | 100
	Alpha	0.0, 0.005
Random Forest	Estimators	10-1,000 | 10
AdaBoost	Estimators	10-200 | 10

For each machine learning algorithm different parameters were optimized to achieve an optimal model. In a grid search, the following parameters were optimized with accuracy calculation (Table S3). For XGBoost classification (XGB) max depth (maximum tree depth for base learners), learning rate (boosting learning rate), number of estimators (number of boosted trees to fit) and alpha (L1 regularization term on weights) were optimized. For Random Forest classification (RF) the number of estimators (number of trees) was optimized. For AdaBoost classification (ADA) the number of estimators (maximum number of estimators at which boosting is terminated) was optimized. For Support Vector Classification (SVC) the settings were set default.

Table S4: Optimized settings for ligand-based approach.

Machine learning algorithm	Fingerprint	Target	Parameter	Optimized values
Random Forest	AtomPair	LTA4H	Estimators	30
Random Forest	AtomPair	sEH	Estimators	490

Table S5: Optimized settings for ligand-based approach.

Machine learning algorithm	Fingerprint	Target	Parameter	Optimized values
Random Forest	PLIF	LTA4H	Estimators	130
Random Forest	PLIF	sEH	Estimators	130

2D-fingerprints

The AtomPair fingerprint (RDKit) describes two atoms with atom descriptions and the distance between the two atoms. The atom description includes its chemical atom type, the number of non-hydrogen atoms and the number of bonding π electrons.[5] The Morgan fingerprint (RDKit) is an ECFP-like circular fingerprint (Extended-connectivity fingerprints). Structures are represented by assigning numbers to heavy atoms combining several connectivity features (element type, number of heavy atoms, number of hydrogens, charge etc.). Those substructure features are translated into a fingerprint scheme.[6] The FeatMorgan fingerprint (RDKit) is a FCFP-like (functional-class fingerprint) circular fingerprint based on the Morgan algorithm. The FCFP is an abstraction of the ECFP fingerprint, where atom identifiers are a set of pharmacophoric identifiers (hydrogen-bond acceptor and donor, negatively and positively ionizable, aromatic, halogen).[6] The MACCS fingerprint (Molecular ACCess System) (RDKit) is translated into SMARTS pattern, corresponding to 166 MACCS keys describing possible substructures. The SMARTS pattern are used to describe a molecular structure in a fingerprint scheme.[MDL Information Systems/Symyx, MACCS-II, Santa Clara, CA, 1984] All 2D-fingerprints were calculated in KNIME using the RDKit Fingerprint node (provided by RDKit).[*Open-Source Cheminformatics Software, "RDKit2018.09.1 documentation,"*]

Docking procedure

Docking was performed using the MOE software suite. For sEH, the PDB structure 4Y2T was used, for LTA4H the PDB structure 3CHO. After downloading, the structures were prepared for docking using MOE's QuickPrep routine (structure check, adjustment of the protonation state, refinement of the complex by energy minimization). In order to define the maximal volume of the binding site, all available X-ray structures were downloaded from the PDB and superposed using the Superposition routine in MOE. The binding site was defined by the volume occupied by all crystallized ligands, respectively. This space was filled with dummy atoms using the Site Finder function in the MOE GUI. A template-based docking procedure was conducted on the combinatorial library data set. In a template-based docking one part of the compounds to dock is fixed according to the position and orientation of the overlapping structural part found in the crystal structure. Template based docking saves computation time because the complexity of the optimization problem is dramatically reduced. Fragment **1** without the hydroxyl group was selected as the template substructure. The position and orientation of the amine building blocks were optimized during docking. An MOE batch file was generated for each of the receptors. Ligand structures were loaded as an MDB file. Triangle Matcher was selected as the secondary placement method with the scoring function London dG and a maximum generation 0f 30 poses. Rigid receptor was selected as the refinement method with the scoring function GBVI/WSA dG, a maximum of 5 poses was generated.

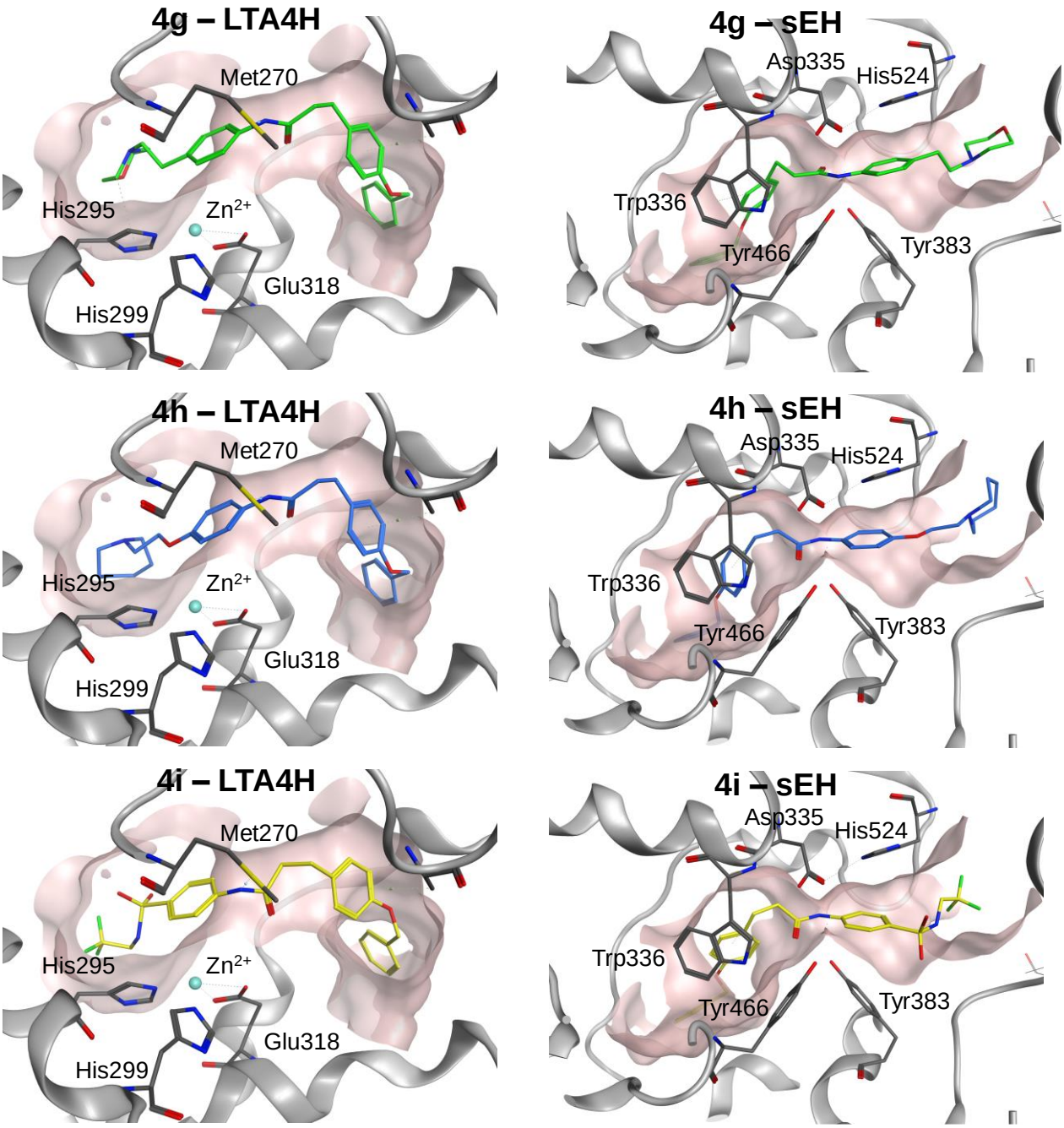


Figure S1: Docking modes of compounds **4g-4i** from the structure-based strategy in complex with LTA4H and sEH.

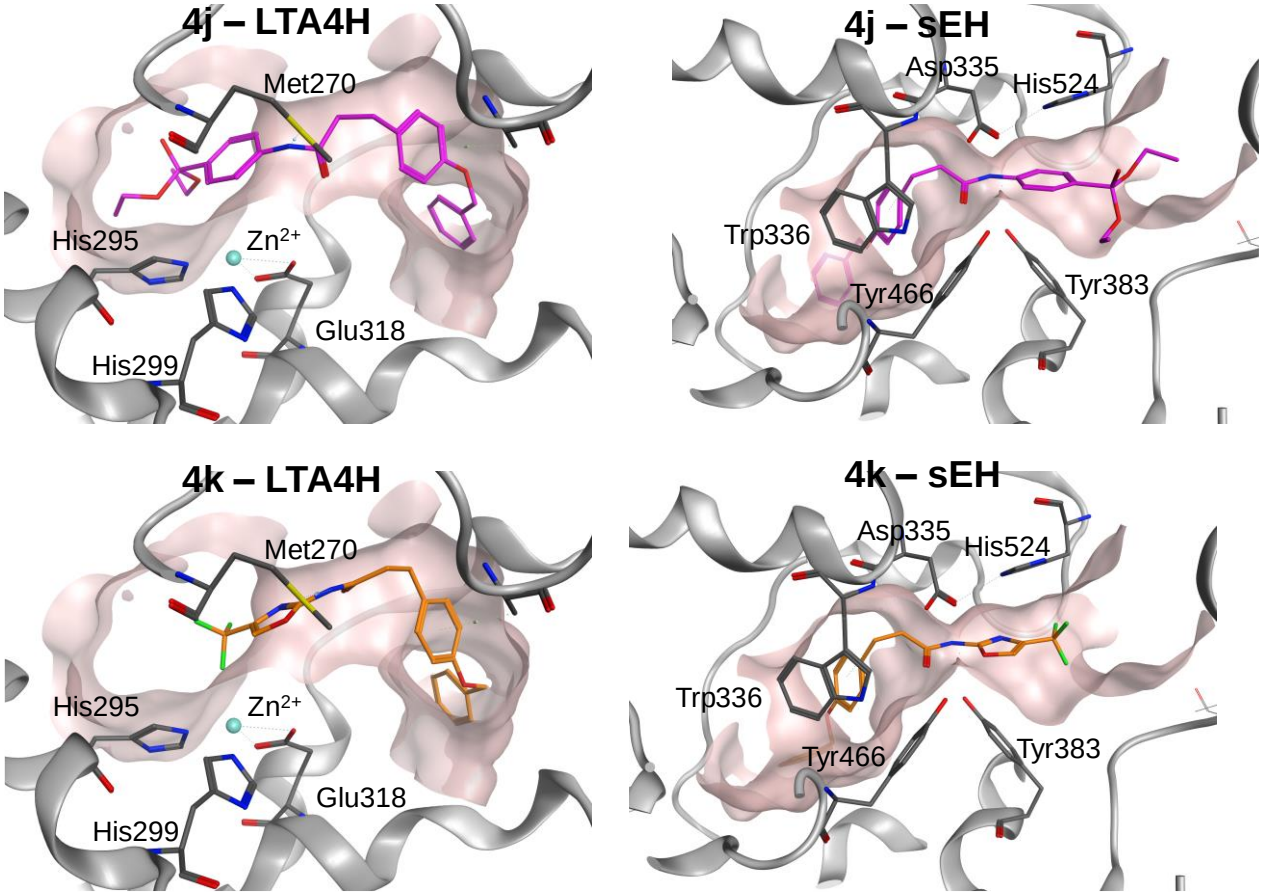


Figure S2: Docking modes of compounds **4j** and **4k** from the structure-based strategy in complex with LTA4H and sEH.

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