Supporting Information

The Discovery of Setileuton, a Potent and Selective 5-Lipoxygenase Inhibitor

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Contents of Supporting Information:

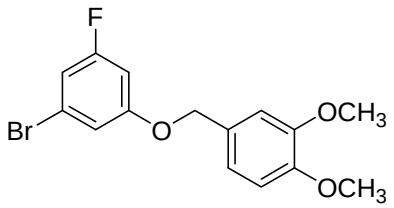
I. General Experimental Details

All non-aqueous reactions were carried out under an inert gas atmosphere (nitrogen or argon) at room temperature, unless otherwise noted. Commercial reagents and anhydrous solvents were used without further purification. Analytical thin layer chromatography (tlc) and flash chromatography were performed on Merck silica gel 60 (230-400 mesh). NMR spectra were recorded as solutions in acetone-d_6 or chloroform-*d* unless stated otherwise, at the field strength indicated and using solvent as internal standard. Conventional abbreviations used for signal shape are s: singlet, d: doublet, dd: doublet of doublets, t: triplet, m: multiplet, br: broad. The LC/MS studies were performed on PE Sciex API 2000 instruments, using positive and negative ionization. All compounds exhibited purity greater than 95% and purity was determined by analytical LC/MS/UV. HPLC conditions: Waters Sunfire C18, 3.5 μm, 20 x 2.1 mm column; flow gradient 1.25 mL/min; 50 °C; gradient 5-98% Eluent B over 1.1 min. Eluent A = 99.9% H_2O : 0.1% formic acid; Eluent B = 99.95% CH_3CN : 0.05% formic acid.

II: Detailed Experimental Procedures and Characterization Data

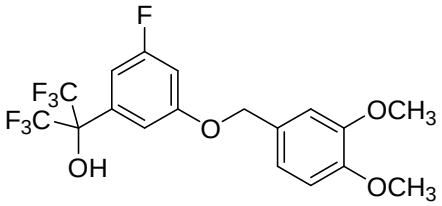
Scheme 1

4-[(3-bromo-5-fluorophenoxy)methyl]-1,2-dimethoxybenzene (17):



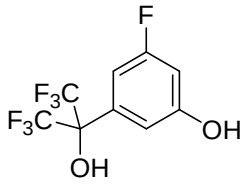
Sodium hydride 60% in mineral oil (1.66 g; 41.5 mmol) was added to a solution of 3,4-dimethoxybenzyl alcohol (5.38 g; 32 mmol) in 80 mL of DMF at 0° C. The solution was brought to rt for 30 min then back to 0° C. 1-Bromo-3,5-difluorobenzene (6.00 g; 31.0 mmol) in 10 mL of DMF was added and the temperature brought to rt. After stirring overnight, the solution was quenched in NH_4OAc and diluted with Et_2O. The organic phase was separated and the aqueous phase washed with more Et_2O. The ethereal phases were combined, dried and the solvent removed. The residue was triturated in hexane to give 8.8 g (83%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.1-6.95 (m, 5H), 6.85 (m, 1H), 5.1 (s, 2H), 3.82 (s, 6H).

2-{3-[(3,4-dimethoxybenzyl)oxy]-5-fluorophenyl}-1,1,1,3,3,3-hexafluoropropan-2-ol (18):



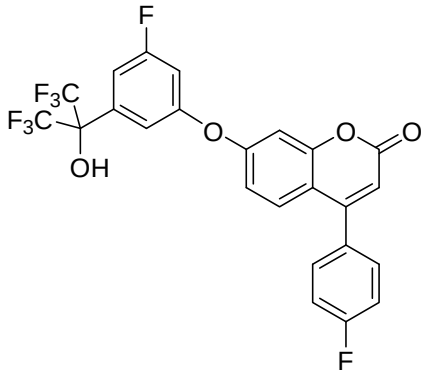
n-BuLi (15.0 mL; 24.0 mM) was added to a mixture of **17** (7.8 g; 22.8 mmol) in 125 mL of Et_2O and 70 mL of THF at -78 °C. After 30 min, hexafluoroacetone was bubbled in the reaction mixture. The solution was brought to rt and quenched with 10% NH_4Cl and diluted with EtOAc. The organic phase was separated, dried and the solvent removed. The residue was chromatographed on silica gel (hexanes: EtOAc; 8:2) to give 5.7 g (59%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.68 (s, 1H); 7.26 (s, 1H); 7.12 (m, 2H), 7.07 (m, 2H); 6.67 (m, 2H).

3-fluoro-5-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenol (19):



A mixture of compound **18** (0.79 g; 1.8 mmol), ammonium formate (0.58 g; 9.2 mmol) and Pd/C 10% (1.35 g) in MeOH (12 mL) was heated to reflux under nitrogen for 40 min. The reaction mixture was cooled to rt, filtered on celite and the solvent removed. The residue was chromatographed on silica gel (hexanes:EtOAc; 8:2) to give 0.40 g (80%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.18 (s, 1H), 7.0 (d, 1H), 6.78 (d, 1H).

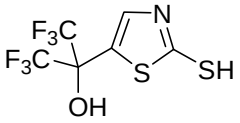
7-[3-fluoro-5-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenoxy]-4-(4-fluorophenyl)-2*H*-chromen-2-one (3):



A degassed mixture of compound **19** (0.217 g; 0.78 mmol), compound **20** (0.125 g; 0.39 mmol), 2,2,6,6-tetramethylheptane-3,5-dione (0.007 g; 0.04 mmol), Cs_2CO_3 (0.381 g; 1.17 mmol) and CuCl (0.039 g; 0.20 mmol) in NMP (1 mL) was heated at 120^o C for 16 h. The mixture was cooled to rt, diluted with EtOAc and quenched with NH_4Cl. The organic phase was separated and washed with water, dried and the solvent removed. The residue was chromatographed on silica gel (toluene:EtOAc; 99:1 to 97:3) to give 0.070 g (35%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.89 (br s, 1H), 7.67 (m, 2H), 7.59 (d, J=8 Hz, 2H), 7.4 (m, 4H), 7.2 (m, 1H), 7.05-7.10 (m, 2H). MS (CI, -ve ion) *m/z* 515.0, $(M-H)^-$.

Scheme 2

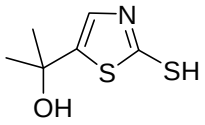
1,1,1,3,3,3-hexafluoro-2-(2-sulfanyl-1,3-thiazol-5-yl)propan-2-ol (21a):



To a solution of diisopropylamine (3.0 mL, 21.3 mmol) in 50 mL of THF at -78°C was added *n*-BuLi (2.6 M in hexanes, 8.19 mL, 21.3 mmol). After 5 min, a solution of 2-mercaptothiazole (1.0 g, 8.53 mmol) in 10 ml of THF was added dropwise. After 5 min, an excess of hexafluoroacetone was bubbled through the reaction mixture for 5 minutes. The mixture was stirred for 15 minutes at -78°C before being partitioned between water and diethyl ether. The organic layer was dried over anhydrous $MgSO_4$, the solvent was evaporated and the residue chromatographed on silica gel (hexanes: EtOAc; 9:1 to 7:3) to give the title compound as a cream colored solid (1.86 g, 77%): ^{1}H NMR (400 MHz, acetone-d_6) δ 7.55 (s); MS (CI, -ve ion) *m/z* 282.0, $(M-H)^-$.

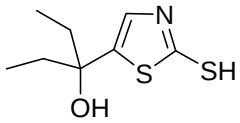
The same method was followed, using the appropriate ketone as starting material, to prepare the following tertiary alcohols.

2-(2-sulfanyl-1,3-thiazol-5-yl)propan-2-ol (21b):



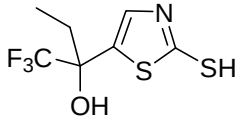
White solid; ^{1}H NMR (400 MHz, acetone-d_6) δ 11.6 (br s, 1H), 7.05 (s, 1H), 4.61 (br s, 1H), 1.55 (s, 6H).

3-(2-sulfanyl-1,3-thiazol-5-yl)pentan-3-ol (21c):



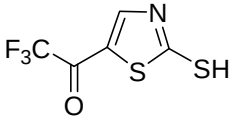
^{1}H NMR (400 MHz, acetone-d_6) δ 11.65 (br s, 1H), 7.01 (s, 1H), 4.26 (s, 1H), 1.76 (q, 4H), 0.90 (t, 6H).

1,1,1-trifluoro-2-(2-sulfanyl-1,3-thiazol-5-yl)butan-2-ol (21e):



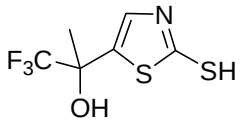
^{1}H NMR (400 MHz, acetone-d_6) δ 7.32 (s), 2.09 (q), 1.01 (t).

2,2,2-trifluoro-1-(2-sulfanyl-1,3-thiazol-5-yl)ethanone (22):



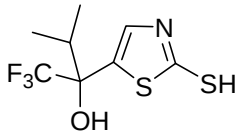
To a solution of diisopropylamine (3.11 mL, 22.2 mmol) in THF (25 mL) at 0 °C, was added *n*-BuLi (1.6 M in hexane, 13.3 mL, 21.3 mmol). The mixture was stirred at the same temperature for 10 min and cooled to −78 °C. A solution of commercially available 2-mercaptothiazole (1.00 g, 8.53 mmol) in THF (5 mL) was added and the resulting mixture was stirred for 30 min at −78 °C. Ethyl trifluoroacetate (1.12 mL, 9.38 mmol) was then added dropwise (10 min) and the reaction was maintained at the same temperature for 45 min. The mixture was then slowly allowed (1 h) to warm to −20 °C, quenched with saturated NH_4Cl and extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated, and the residue was purified by flash chromatography (acetone/toluene 20:80 until starting thiol was completely eluted out, then 20:80 + 0.25% AcOH, then 20:80 + 0.35% AcOH) to give **22** as a yellow solid (1.24 g, 68%). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.82 (br s, 1H), 8.49 (s, 1H).

1,1,1-trifluoro-2-(2-sulfanyl-1,3-thiazol-5-yl)propan-2-ol (21d):



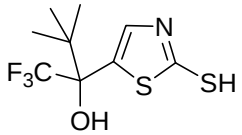
To a solution of **22** (500 mg, 2.34 mmol) in THF (5 mL) at 0 °C, was added methylmagnesium bromide (1.4 M in THF, 4.19 mL, 5.86 mmol) dropwise (5 min). After 25 min at the same temperature, the reaction was quenched with saturated NH_4Cl and extracted with EtOAc. The combined organic layers were washed with saturated NH_4Cl, dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (acetone/toluene 15:85) to give **21d** as an off-white solid (483 mg, 90%). ^{1}H NMR (500 MHz, acetone-d_6) δ 11.92 (br s, 1H), 7.36 (s, 1H), 6.22 (br s, 1H), 1.80 (s, 3H).

1,1,1-trifluoro-3-methyl-2-(2-sulfanyl-1,3-thiazol-5-yl)butan-2-ol (21f):



To a solution of **22** (350 mg, 1.64 mmol) in THF (3.5 mL) at 0 °C, was added isopropylmagnesium chloride (2.0 M in THF, 2.05 mL, 4.10 mmol) dropwise (5 min). After 1 h at the same temperature, the reaction was quenched with saturated NH_4Cl and extracted with EtOAc. The combined organic layers were washed with saturated NH_4Cl, dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (acetone/toluene 15:85) to give **21f** as a pale yellow gum (170 mg, 40%). ^{1}H NMR (400 MHz, acetone-d_6) δ 11.91 (br s, 1H), 7.27 (s, 1H), 5.91 (s, 1H), 2.38 (m, 1H), 1.11 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H).

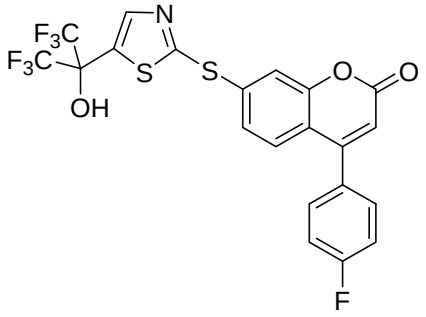
1,1,1-trifluoro-3,3-dimethyl-2-(2-sulfanyl-1,3-thiazol-5-yl)butan-2-ol (21g):



To a stirred solution of *t*-BuLi (1.7 M in pentane, 3.5 mL, 5.9 mmol) in diethylether at -78°C was added a solution of **22** (600 mg, 2.8 mmol) in diethyl ether (10 mL). The solution was warmed up to 0 °C and after 30 min, aqueous ammonium chloride was added. The mixture was extracted with ethyl acetate. The combined organic fractions were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified on silica gel (toluene/acetone; 9:1 to 8:2) to give the

title compound (200 mg, 26%). ^{1}H NMR (400 MHz, $CDC1_3$) δ 11.45 (s, 1H), 7.05 (s, 1H), 2.85 (s, 1H), 1.12 (s, 9H); MS (CI, -ve ion) *m/z* 269.9, $(M-H)^-$.

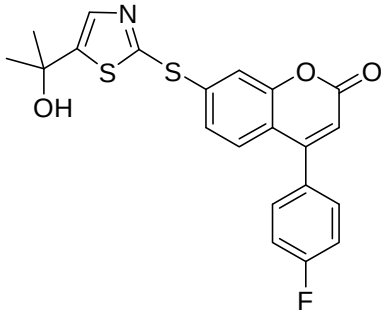
4-(4-fluorophenyl)-7-{[5-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-1,3-thiazol-2-yl]sulfanyl}-2*H*-chromen-2-one (4):



A solution of 7-bromo-4-(4-fluorophenyl)-2*H*-chromen-2-one (**20**) (2.38 g, 7.46 mmol), thiol **21a** (1.76 g, 6.21 mmol) and potassium carbonate (2.66 g, 19.25 mmol) in 50 mL of 1-methyl-2-pyrrolidinone (NMP) was heated at 120 °C overnight. The mixture was cooled to room temperature and partitioned between aqueous NH_4Cl and diethyl ether. The layers were separated and the aqueous phase was extracted with diethyl ether. The combined organic layers were washed with water, brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the residue chromatographed on silica gel (hexanes: EtOAc; 8:2 to 6:4) to give the title compound as a beige solid (1.63 g, 50%): ^{1}H NMR (400 MHz, acetone-d_6): δ 8.26 (s, 1H), 8.06 (s, 1H), 7.74 (d, 1H), 7.57-7.70 (m, 4H), 7.40 (t, 2H), 6.47 (s, 1H). MS (CI, -ve ion) *m/z* 520.1, $(M-H)^-$.

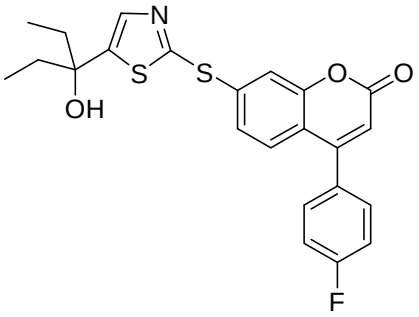
The same method was followed, using the appropriate 2-mercaptothiazole as starting material, to prepare the following compounds.

4-(4-fluorophenyl)-7-{[5-(2-hydroxypropan-2-yl)-1,3-thiazol-2-yl]sulfanyl}-2*H*-chromen-2-one (9):



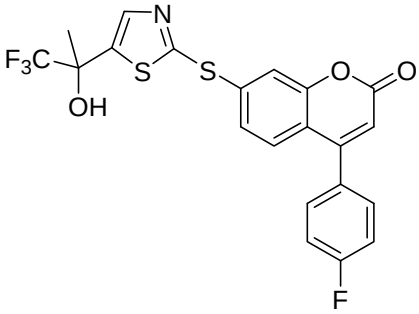
Beige solid; ^{1}H NMR (400 MHz, acetone-d_6): 7.74 (s, 1H), 7.63-7.67 (m, 2H), 7.50 (d, 1H), 7.43 (d, 1H), 7.34-7.40 (m, 3H), 6.37 (s, 1H), 4.86 (s, 1H), 1.64 (s, 6H); MS (CI, +ve ion) *m/z* 413.9, $(M+H)^+$.

4-(4-fluorophenyl)-7-{[5-(3-hydroxypentan-3-yl)-1,3-thiazol-2-yl]sulfanyl}-2*H*-chromen-2-one (10):



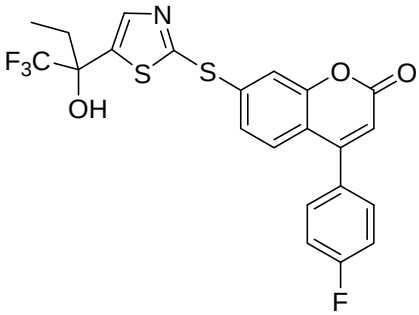
Beige solid; ^{1}H NMR (400 MHz, acetone-d_6): δ 7.70 (s, 1H), 7.64-7.67 (m, 2H), 7.50 (d, 1H), 7.35-7.42 (m, 4H), 6.38 (s, 1H), 4.45 (s, 1H), 1.88 (q, 4H), 0.88 (t, 6H). MS (CI, +ve ion) *m/z* 442.1, $(M+H)^+$.

4-(4-fluorophenyl)-7-{[5-(1,1,1-trifluoro-2-hydroxypropan-2-yl)-1,3-thiazol-2-yl]sulfanyl}-2*H*-chromen-2-one (11):



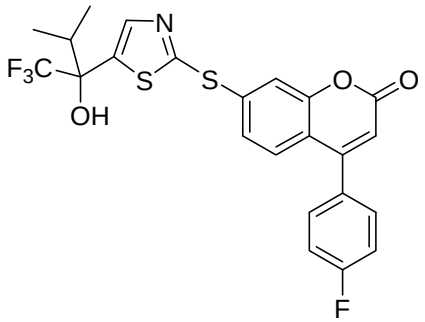
Off-white foam (55%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.93 (s, 1H), 7.69-7.66 (m, 2H), 7.60-7.58 (m, 1H), 7.56 (s, 1H), 7.48 (dd, J = 8.4, 1.8 Hz, 1H), 7.39 (m, 2H), 6.43 (s, 1H), 6.34 (s, 1H), 1.88 (s, 3H); MS (+ESI): MH^+ = 468.

4-(4-fluorophenyl)-7-{[5-(1,1,1-trifluoro-2-hydroxybutan-2-yl)-1,3-thiazol-2-yl]sulfanyl}-2*H*-chromen-2-one (12):



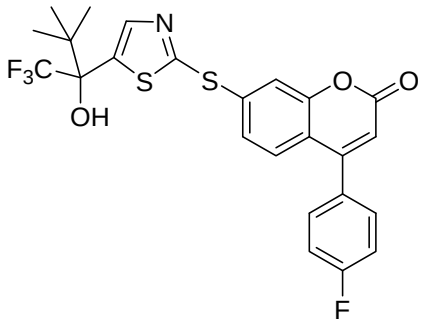
^{l}H NMR (400 MHz, CDC1$_3$): δ 7.76 (s, 1H), 7.56 (d, 1H), 7.44-7.48 (m, 3H), 7.36 (dd, 1H), 7.25 (m, 2H), 6.39 (s, 1H), 2.93 (br s, 1H), 2.12 (q, 2H), 0.97 (t, 3H). MS (CI, +ve ion) *m/z* 482.1.

4-(4-fluorophenyl)-7-{[5-(1,1,1-trifluoro-2-hydroxy-3-methylbutan-2-yl)-1,3-thiazol-2-yl]sulfanyl}-2*H*-chromen-2-one (13):



Beige solid (45%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.89 (s, 1H), 7.69-7.66 (m, 2H), 7.59-7.57 (m, 1H), 7.55 (s, 1H), 7.46 (dd, J = 8.4, 1.8 Hz, 1H), 7.39 (m, 2H), 6.42 (s, 1H), 6.06 (s, 1H), 2.49 (m, 1H), 1.08 (d, J = 6.8, 3H), 1.00 (d, J = 6.8, 3H); MS (+ESI): MH^+ = 496.

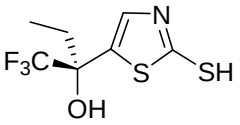
4-(4-fluorophenyl)-7-{[5-(1,1,1-trifluoro-2-hydroxy-3,3-dimethylbutan-2-yl)-1,3-thiazol-2-yl]sulfanyl}-2*H*-chromen-2-one (14):



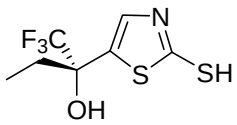
^{l}H NMR (400 MHz, CDC1$_3$): δ 7.82 (s, 1H), 7.55 (s, 1H), 7.43-7.48 (m, 3H), 7.33 (d, 1H), 7.22-7.28 (m, 2H), 6.39 (s, 1H), 2.82 (s, 1H), 1.12 (s, 9H). MS (CI, +ve ion) *m/z* 510.0, $(M+H)^+$.

Scheme 3

(2*S*)-1,1,1-trifluoro-2-(2-sulfanyl-1,3-thiazol-5-yl)butan-2-ol ((*S*)-21e) and (2*R*)-1,1,1-trifluoro-2-(2-sulfanyl-1,3-thiazol-5-yl)butan-2-ol ((*R*)-21e):



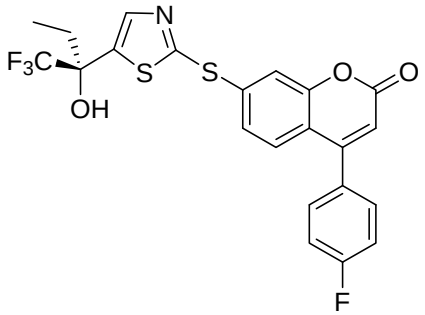
(*S*)-**21e**



(*R*)-**21e**

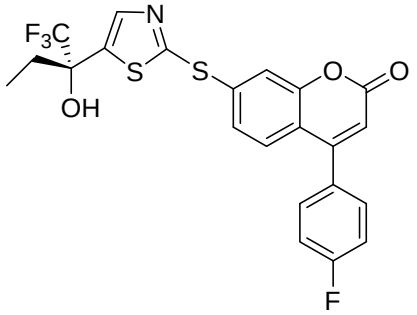
A solution of (±)-1,1,1-trifluoro-2-(2-sulfanyl-1,3-thiazol-5-yl)butan-2-ol (**21e**) (1.2 g) in EtOH / hexane (20 mL, 1:4) was injected onto a CHIRALPAK AD preparative (5 cm x 50 cm) HPLC column (eluting with hexane/EtOH, 4:1; at 75 mL/min with UV detection at 280 nm). The enantiomers were separated, with the fast-eluting enantiomer having a retention time of approximately 19 min (enantiomer # l, (*S*)-**21e**) and the slow-eluting enantiomer having a retention time of about 34 min (enantiomer # 2, (*R*)-**21e**). The eluents were concentrated to provide enantiomer #1 (0.468 g, 99% ee) and enantiomer #2 (0.426 g, 98% ee).

(+)-4-(4-fluorophenyl)-7-({5-[(2*S*)-1,1,1-trifluoro-2-hydroxybutan-2-yl]-1,3-thiazol-2-yl}sulfanyl)-2*H*-chromen-2-one ((*S*)-12):



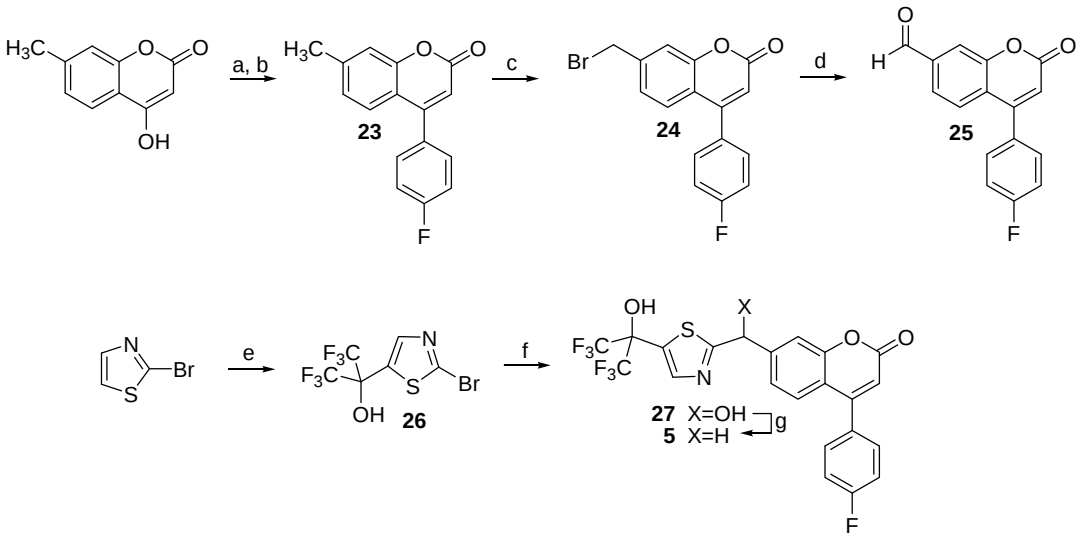
Employing the procedure used for the preparation of compound **4**, with thiol (*S*)-**21e**, the title compound was obtained.
$[\alpha]^{25}_{D}$ +16.2° (*c* = 0.26, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.56 (d, 1H), 7.44-7.48 (m, 3H), 7.36 (dd, 1H), 7.25 (m, 2H), 6.39 (s, 1H), 2.93 (br s, 1H), 2.12 (q, 2H), 0.97 (t, 3H).

(-)-4-(4-fluorophenyl)-7-({5-[(2*R*)-1,1,1-trifluoro-2-hydroxybutan-2-yl]-1,3-thiazol-2-yl}sulfanyl)-2*H*-chromen-2-one ((*R*)-12):



Employing the procedure used for the preparation of compound **4**, with thiol (*R*)-**21e**, the title compound was obtained.
$[\alpha]^{25}_{D}$ -18° (*c* = 0.25, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.56 (d, 1H), 7.44-7.48 (m, 3H), 7.36 (dd, 1H), 7.25 (m, 2H), 6.39 (s, 1H), 2.93 (br s, 1H), 2.12 (q, 2H), 0.97 (t, 3H). HRMS ($C_{22}H_{15}F_4NO_3S_2$ + H); calc. mass: 482.0508; found: 482.0510.

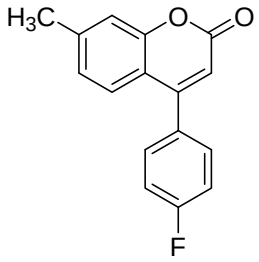
Scheme A



(a) Tf_2O, Et_3N, CH_2Cl_2; (b) 4-fluorophenylboronic acid, $Pd(OAc)_2$, tricyclohexylphosphine, KF, THF; (c) NBS, $(BzO)_2$, CCl_4; (d) 4-methylmorpholine-*N*-oxide, dioxane, 100 °C, 94%; (e) LDA, CF_3COCF_3, THF, -78 °C, 82%; (f) NaH, *n*-BuLi, **25**, THF, -78 °C, 52%; (g) I_2, H_3PO_2, AcOH, 90 °C, 47%.

The preparation of analogue **5** began with the conversion of 4-hydroxy-7-methyl-2*H*-chromen-2-one into the corresponding triflate, followed by Suzuki coupling with 4-fluorophenylboronic acid to afford coumarin **23**. Treatment with *N*-bromosuccinimide followed by oxidation with 4-methylmorpholine-*N*-oxide produced aldehyde **25**. In parallel, deprotonation of 2-bromothiazole with LDA and addition of hexafluoroacetone gave tertiary alcohol **26**. This intermediate, after bromine-lithium exchange, and treatment with aldehyde **25**, led to secondary alcohol **27,** that was reduced to 5-LO inhibitor **5** with iodine and hypophosphorous acid.

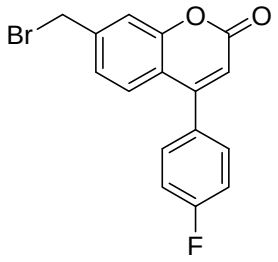
4-(4-fluorophenyl)-7-methyl-2*H*-chromen-2-one (23):



To a -30° C solution of 4-hydroxy-7-methyl-2*H*-chromen-2-one (50 g, 284 mmol) and triethylamine (48.8 g, 482 mmol) in 1.2 L of CH_2Cl_2, was slowly added a solution of triflic anhydride (128.1 g, 454 mmol) in 120 mL of CH_2Cl_2 (internal temp. < -30 ^{0}C). After 30 min of stirring, the solution was brought to 0° C and quenched with NH_4Cl. After extraction with CH_2Cl_2 the organic phase was washed three times with H_2O, dried over $MgSO_4$ and the solvent removed. The solid obtained was stirred in hexane-ether (9/1). After filtration, the triflate intermediate was dried. ^{1}H NMR (400 MHz, acetone-d_6): δ 7.68 (1H, d), 7.37 (2H, m), 6.61 (1H, s) and 2.52 (3H, s).

A mixture of the triflate (40 g, 130 mmol), 4-fluorophenylboronic acid (21.8 g, 156 mmol), $Pd(OAc)_2$ (0.87 g, 3.9 mmol), tricyclohexylphosphine (1.31 g, 4.7 mmol) and potassium fluoride (24.9 g, 428 mmol) in 500 mL of THF was stirred at rt overnight. The mixture was filtered over celite and the solvent removed. The crude product was then purified over a small pad of silica gel using CH_2Cl_2. The solvent was removed and the resulting solid stirred with CH_2Cl_2-hexane (1/9). After filtration, the product was dried to yield the title compound. ^{1}H NMR (400 MHz, acetone-d_6): δ 7.62 (m, 2H), 7.39 (m, 3H), 7.25 (s, 1H), 7.15 (d, 1H), 6.29 (s, 1H) and 2.45 (s, 3H).

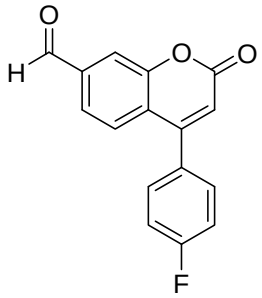
7-(bromomethyl)-4-(4-fluorophenyl)-2*H*-chromen-2-one (24):



A mixture of compound **23** (24.0 g, 94.3 mmol), *N*-bromosuccinimide (18.5 g, 103.8 mmol) and benzoyl peroxide (1.14 g, 4.72 mmol) in 470 mL of CCl_4 was brought to reflux. The solution was left overnight at reflux and then filtered hot. Once cooled to rt the solvent was removed, the compound was dissolved in CH_2Cl_2 and a purification was done with a small pad of silica gel using hexane-EtOAc (8/2) to (1/1). The solvent was removed and the solid triturated with hexane-EtOAc and filtered to give the title compound. The remaining solvent was removed to give additional compound contaminated with some starting material and dibromo compound. ^{1}H NMR (400 MHz,

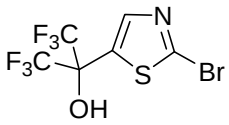
acetone-d_6): δ 7.67 (m, 2H), 7.55 (s, 1H), 7.50 (d, 1H), 7.40 (m, 3H), 6.40 (s, 1H) and 4.75 (s, 2H).

4-(4-fluorophenyl)-2-oxo-2*H*-chromene-7-carbaldehyde (25):



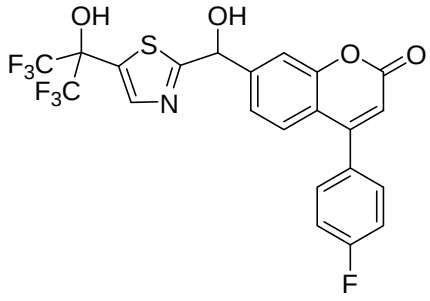
Compound **24** (11.42 g, 34.3 mmol) and 4-methylmorpholine-*N*-oxide (13.9 g, 102.8 mmol) in 110 mL of dioxane were heated to reflux for 6 h. The solution was cooled to rt and the solvent removed. The crude compound was diluted in EtOAc and washed with aqueous NH_4Cl, water, brine and dried over $MgSO_4$. The solvent was removed to yield 8.6 g (94%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6): δ 10.20 (s, 1H), 7.95 (s, 1H), 7.85 (m, 1H), 7.68 (m, 3H), 7.38 (m, 2H) and 6.51 (s, 1H).

2-(2-bromo-1,3-thiazol-5-yl)-1,1,1,3,3,3-hexafluoropropan-2-ol (26):



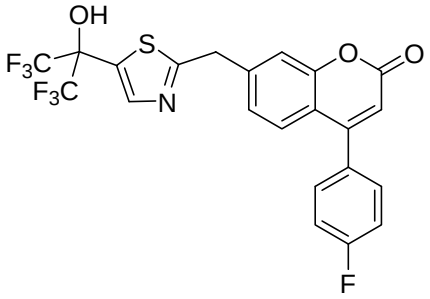
To a -78^o C solution of LDA prepared from diisopropylamine (1.9 mL, 13.3 mmol) and *n*-BuLi 1.6 M (8.00 mL, 12.8 mmol) was added 2-bromo-1,3-thiazole (2.00 g, 12.1 mmol) in 2 mL of THF. After 15 min hexafluoroacetone was bubbled for a few minutes. The mixture was brought to rt, quenched with NH_4Cl and diluted with EtOAc. The organic phase was separated, dried and the solvent removed. The residue was triturated in hexane and the solid filtered and dried to give 3.3 g (82%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.45 (br s, 1H), 7.93 (s, 1H).

4-(4-fluorophenyl)-7-{[5-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-1,3-thiazol-2-yl](hydroxy)methyl}-2*H*-chromen-2-one (27):



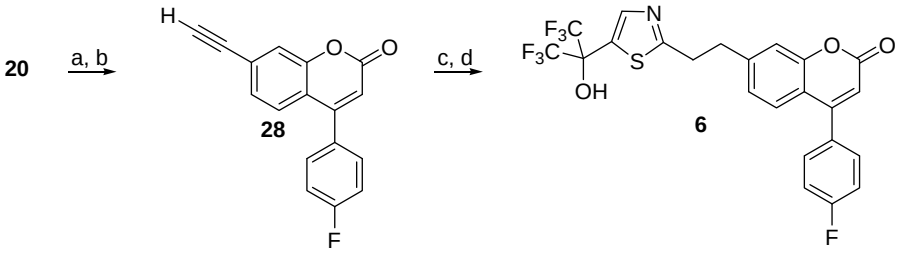
To a -78 ^{o}C solution of compound **26** (0.792 g; 2.4 mmol) in 10 mL of THF was added NaH 60% (0.115 g; 2.8 mmol) followed by *n*-BuLi 1.6 M (1.6 mL; 2.6 mmol). After 20 min this solution was added to a -78^{o} C solution of aldehyde **25** (0.64 g; 2.38 mmol). After 20 min, the reaction mixture was quenched with NH_4Cl and diluted with EtOAc. The organic phase was separated, dried and the solvent removed. The residue was chromatographed on silica gel (hexane:EtOAc; 6:4) to give 0.64 g (52%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.12 (br s, 1H), 7.97 (s, 1H), 7.65 (m, 3H), 7.5 (m, 2H), 7.38 (m, 2H), 6.39 (s, 1H), 6.28 (s, 1H).

4-(4-fluorophenyl)-7-{[5-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-1,3-thiazol-2-yl]methyl}-2*H*-chromen-2-one (5):



A mixture of compound **27** (0.120 g; 0.23 mmol), H_3PO_2 (0.32 mL) and iodine (0.175 g; 0.69 mmol) in acetic acid (2 mL) was heated to 90 ^{o}C for 24 h. The reaction mixture was cooled to rt, diluted with water and dichloromethane, and neutralized with solid Na_2CO_3. The organic phase was separated, dried and the solvent removed. The residue was chromatographed on silica gel (hexane:EtOAc; 6:4) to give 0.055 g (47%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.07 (s, 1H); 7.98 (s, 1H), 7.67 (m, 2H), 7.5 (m, 2H), 7.37 (m, 2H), 6.36 (s, 1H), 4.57 (s, 2H). MS (CI, -ve ion) *m/z* 502.0, $(M-H)^{-}$.

Scheme B

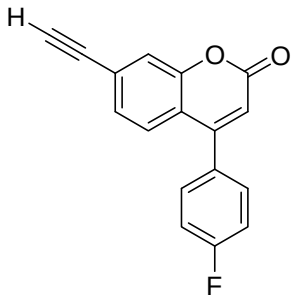


(a) Ethynyltrimethylsilane, $PdCl_2(PhCN)_2$, PPh_3, $Cu(OAc)_2$, diisopropylamine, 70 ^{o}C, 54%; (b) $Na_2B_4O_7$-10H_2O, MeOH, 99%; (c) **26**, $PdCl_2(PhCN)_2$, PPh_3, $Cu(OAc)_2$, diisopropylamine, 90 ^{o}C, 38%; (d) H_2, Pd-C 10%, EtOAc, 61%.

The synthesis of analog **6**, bearing an ethane linker, involved the palladium-catalyzed coupling of aryl bromide **20** with ethynyltrimethylsilane followed by desilylation to

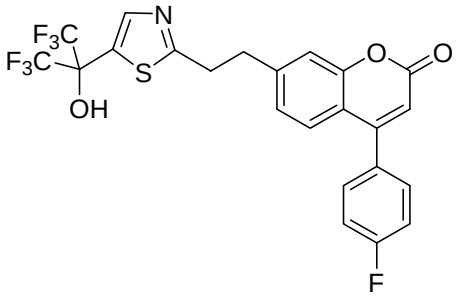
produce terminal alkyne **28**. This intermediate was reacted with 2-bromothiazole **26**, and then hydrogenated to yield the desired compound **6**.

7-ethynyl-4-(4-fluorophenyl)-2*H*-chromen-2-one (28):



A degassed mixture of compound **20**, ethynyltrimethylsilane (0.192 g; 1.95 mmol), $PdCl_2.(PhCN)_2$ (0.033 g; 0.08 mmol), triphenylphosphine (0.045 g; 0.17 mmol) and copper (II) acetate (0.014 g; 0.08 mmol) was heated to 70^o C in diisopropylamine (8 mL) for 10 h. The mixture was cooled to rt and diluted with EtOAc and water. The organic phase was separated, dried and the solvent removed. The residue was chromatographed on silica gel (hexane:EtOAc; 9:1) to give 0.311 g (54%) of the protected acetylene. This compound was taken in MeOH (8 mL) and a solution of Borax 0.1 M (1.8 mL) was added at 0 ^{o}C. The solution was brought to room temperature and let go overnight. The solution was diluted with saturated NH_4Cl and extracted with Et_2O. The ethereal phase was dried and the solvent removed to give 0.24 g (99%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.67 (m, 2H), 7.5 (m, 2H), 7.39 (m, 3H), 6.41 (s, 1H), 3.99 (s, 1H).

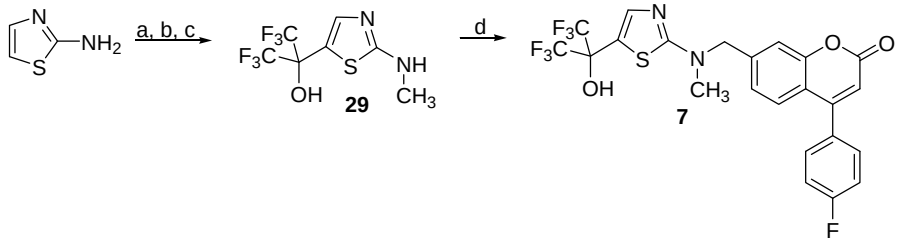
4-(4-fluorophenyl)-7-{2-[5-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-1,3-thiazol-2-yl]ethyl}-2*H*-chromen-2-one (6):



A degassed mixture of compound **28** (0.240 g; 0.91 mmol), compound **26** (0.306 g; 0.93 mmol), triphenylphosphine (0.035 g; 0.14 mmol), copper (II) acetate (0.007 g; 0.04 mmol) and $PdCl_2.(PhCN)_2$ (0.017g; 0.04 mmol) was heated to 90 ^{o}C in diisopropylamine for 10 h. The mixture was cooled to rt and diluted with a solution of NH_4OAc and EtOAc. The organic phase was separated, dried and the solvent removed. The residue was chromatographed on silica gel (hexane:EtOAc; 7:3) to give 0.176 g (38%) of the coupled product. This compound was hydrogenated over Pd/C 10% (20 mg) in EtOAc

overnight. The mixture was filtered over celite and the solvent removed. The residue was chromatographed on silica gel (hexane:EtOAc; 65:35) to give 0.061 g (61%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.95 (s, 1H), 7.65 (m, 2H), 7.35-7.45 (m, 4H), 7.29 (d, *J*=6.8 Hz, 1H), 6.32 (s, 1H), 3.47 (t, *J*=8.1 Hz, 2H), 3.31 (t, *J* =7.8 Hz, 2H). MS (CI, -ve ion) *m/z* 515.9, $(M-H)^-$.

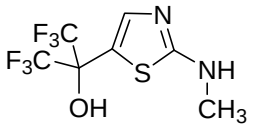
Scheme C



(a) $HC(OEt)_3$, cat. TFA, Δ; (b) $NaBH_4$, EtOH; (c) LDA, CF_3COCF_3, THF, -78 °C, 59%; (d) DIPEA, **24**, toluene, Δ, 48%.

2-(methylamino)thiazole **29** was prepared in three steps. First, 2-aminothiazole was formylated with triethyl orthoformate, second, the resulting amide was reduced with sodium borohydride and third, the resulting secondary amine was deprotonated with LDA and treated with hexafluoroacetone. Subsequently, amine **29** was alkylated with bromide **24** to produce inhibitor **7**.

1,1,1,3,3,3-hexafluoro-2-[2-(methylamino)-1,3-thiazol-5-yl]propan-2-ol (29):



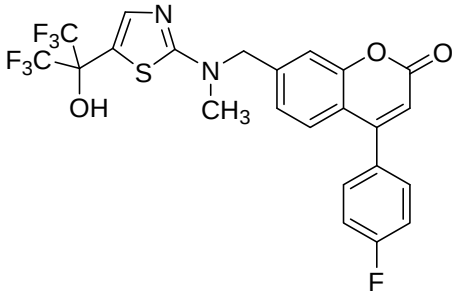
A mixture of 2-amino-1,3-thiazole (6.00 g, 60.0 mmol), triethylorthoformate (50 mL) and 5 drops of trifluoroacetic acid was heated to reflux for 10 h. The solution was brought to rt and the solvent removed.

The residue was taken in 100 mL of EtOH at 0 °C and $NaBH_4$ (2.6 g, 70 mmol) was added portionwise. The mixture was brought to 50 °C for 30 min and cooled to rt. The solvent was removed and the residue taken in EtOAc and quenched with aqueous NH_4OAc. The organic phase was separated, dried and the solvent removed. The solid was heated in EtOAc and filtered to give 1.8 g (26%) of *N*-methyl-2-amino-1,3-thiazole. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.04 (s, 1H), 6.52 (s, 1H), 2.93 (s, 3H).

To a -78 °C solution of LDA (33.0 mmol) prepared from *n*-BuLi and diisopropylamine in THF was added the previous methylamine (1.45 g; 12.7 mmol) in 15 mL of THF. After 30 min., hexafluoroacetone was bubbled in the solution. It was then brought to rt and quenched with a solution of NH_4OAc and extracted with EtOAc. The organic phase was dried and the solvent removed. Trituration in Et_2O-Hexane (7:3) and filtration gave 2.08

g (59%) of the title compound as a white solid. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.63 (br s, 1H), 7.31 (s, 1H), 6.99 (br s, 1H), 2.98 (s, 3H).

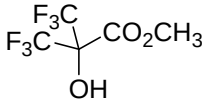
4-(4-fluorophenyl)-7-({[5-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-1,3-thiazol-2-yl](methyl)amino}methyl)-2*H*-chromen-2-one (7):



A mixture of compound **24** (0.050 g; 0.15 mmol), the amine **29** (0.046 g; 0.16 mmol), and diisopropylethylamine (0.028 mL; 0.16 mmol) in toluene was heated to reflux for 6 h. The solution was cooled to rt, quenched with a solution of NH_4OAc and diluted with EtOAc. The organic phase was separated, dried and the solvent removed. The residue was chromatographed on silica gel (toluene:EtOAc; 6:4) to give 0.038 g (48%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.72 (s, 1H), 7.67 (m, 2H), 7.47 (m, 2H), 7.38 (m, 2H), 7.31 (d, *J*= 6.7 Hz, 1H), 6.36 (s, 1H), 5.16 (s, 2H), 2.95 (s, 3H). MS (CI, -ve ion) *m/z* 531.3, $(M-H)^-$.

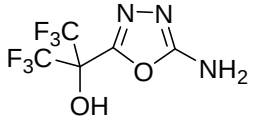
Scheme 4

methyl 3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl)propanoate (30):



A solution of 3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl)propanoic acid (3.6 g, 17.0 mmol) in diethylether at 0 ^{o}C was treated with an ethereal solution of diazomethane until a yellow color persisted. The solution was concentrated to give 3.8 g (100%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.48 (s, 1H), 4.09 (s, 3H).

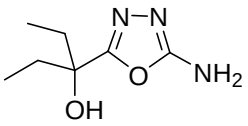
2-(5-amino-1,3,4-oxadiazol-2-yl)-1,1,1,3,3,3-hexafluoropropan-2-ol (31a):



A mixture of ester **30** (1.9 g, 8.4 mol) and hydrazine hydrate (0.8 mL) was heated to 120 ^{o}C for 3 h. The mixture was cooled to rt and excess reagent removed under vacuum. The

hydrazide thus obtained was stirred in water (40 mL) and $KHCO_3$ (907 mg, 9.0 mmol) was added followed by BrCN (915 mg, 8.6 mmol). After 90 min of stirring, the white solid was filtered, washed with water and dried to give 405 mg (20%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6): δ. 8.14 (s, 1H), 6.92 (s, 2H). MS (CI, +ve ion) *m/z* 251.9, $(M+H)^+$.

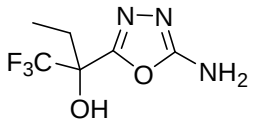
3-(5-amino-1,3,4-oxadiazol-2-yl)pentan-3-ol (31b):



Hydrazine hydrate (8.1 mL) in 9 mL of ethanol was added dropwise to diethyl oxalate (30.0 g, 202 mmol) in 60 mL of ethanol at -20 °C. After 15 min, the precipitate was filtered and washed with ethanol. To the liquid phase was added 15 mL of water and BrCN (16.5 g; 155 mmol) was added portionwise (formation of a precipitate). After 45 min of stirring, the mixture was filtered, washed with Et_2O and dried to give 9.6 g of ethyl 5-amino-1,3,4-oxadiazol-2-carboxylate. From the mother liquor, 4.0 g could also be recovered.

To a solution of ethylmagnesium bromide (200 mmol) in 300 mL of THF at 0 °C was added a suspension of ethyl 5-amino-1,3,4-oxadiazol-2-carboxylate (8.00 g, 50.9 mmol) in THF. The reaction mixture was brought to rt and, 30 min later, quenched with aqueous NH_4Cl. After extraction with EtOAc and drying over $MgSO_4$, the solvent was removed. The crude product thus obtained was triturated, filtered and dried to yield 5.0 g (57%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6): δ. 6.20 (br s, 2H), 4.25 (s, 1H), 1.85 (m, 4H), 0.88 (t, 6H).

2-(5-amino-1,3,4-oxadiazol-2-yl)-1,1,1-trifluorobutan-2-ol (31c):

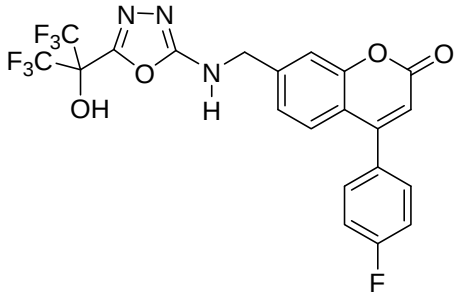


To a -78 °C solution of ethyl trifluoropyruvate (129.0 g 758 mmol) in ether was added dropwise, within 90 min, a solution of EtMgBr 3.0 M in ether (252 mL). The reaction mixture was brought over 1 h to -10 °C and poured in 2L of saturated NH_4Cl. The layers were separated and the aqueous phase extracted with ether (3 X 500 mL). The organic phases were combined, dried over $MgSO_4$ and the solvent removed. Distillation at 50-65 °C (30 mmHg) gave ethyl 2-hydroxy-2-(trifluoromethyl)butanoate. ^{1}H NMR (400 MHz, acetone- d_6): δ 5.4 (s, 1H), 4.35 (q, 2H), 2.07 (m, 1H), 1.83 (m, 1H), 1.3 (t, 3H) and 0.93 (t, 3H).

The ethyl ester from the previous step (50.04 g, 250 mmol) and hydrazine hydrate (25.03 g, 50 mmol) were heated at 80 °C for 18 h. The excess hydrazine was removed under vacuum and the crude product was filtered through a pad of silica gel with EtOAc-hexane

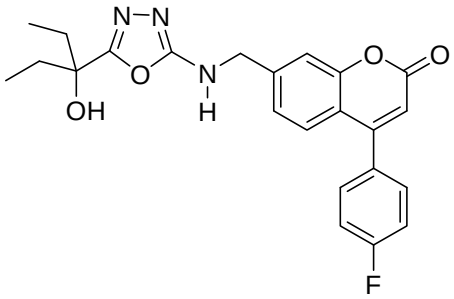
(ca. 3 L) to furnish 2-hydroxy-2-(trifluoromethyl)butanohydrazide. ^{1}H NMR (400 MHz, acetone-d_6): δ 9.7 (s, 1H), 6.10 (s, 1H), 2.25 (m, 1H), 1.85 (m, 1H) and 0.95 (t, 3H).
To the previous hydrazide (34.07 g, 183 mmol) of in 275 mL of water was added $KHCO_3$ (18.33 g, 183 mmol) followed by BrCN (19.39 g, 183 mmol) portionwise. After 3 h, the solid was filtered, washed with cold water and dried to afford the title compound. Additional compound could be recovered from the aqueous phase by extraction (ether-hexane, 1:1). ^{1}H NMR (400 MHz, acetone-d_6): δ 6.54 (s, 2H), 6.01 (s, 1H), 2.22 (m, 1H), 2.08 (m, 1H) and 0.99 (m, 3H).

4-(4-fluorophenyl)-7-({[5-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-1,3,4-oxadiazol-2-yl]amino}methyl)-2*H*-chromen-2-one (8):



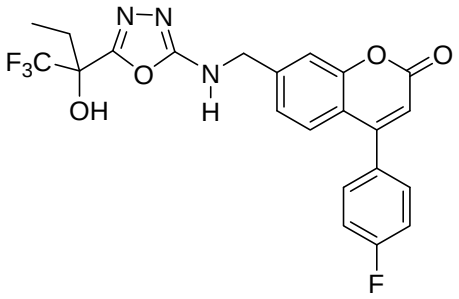
A mixture of aldehyde **25** (117 mg, 0.44 mmol) and amine **31a** (120 mg, 0.48 mmol) was heated to reflux in 10 mL of toluene with a Dean-Stark apparatus overnight. The solution was brought to 0 ^{o}C and diluted with EtOH (10 mL). $NaBH_4$ (17 mg, 0.44 mmol) was added portionwise and the mixture was stirred at rt. After 20 min, aqueous ammonium chloride was added. The mixture was extracted with ethyl acetate. The combined organic fractions were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified on silica gel (toluene: acetone; 7:3) to give the title compound (140 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.50 (m, 4H), 7.20-7.28 (m, 4H), 6.38 (s, 1H), 5.91 (s, 1H), 4.72 (s, 2H). MS (CI, +ve ion) *m/z* 504.1, $(M+H)^+$.

4-(4-fluorophenyl)-7-({[5-(3-hydroxypentan-3-yl)-1,3,4-oxadiazol-2-yl]amino}methyl)-2*H*-chromen-2-one (15):



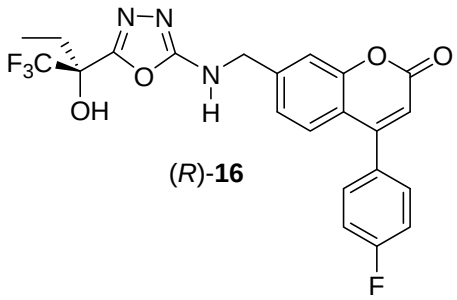
A mixture of aldehyde **25** (3.00 g; 11.1 mmol) and amine **31b** (2.48 g; 14.1 mmol) was heated to reflux in 45 mL of toluene with a Dean-Stark apparatus overnight. The solution was brought to 0 ^{o}C and diluted with EtOH (125 mL). $NaBH_4$ (0.419 g; 11.1 mmol) was added portionwise and the mixture brought to rt. After 15 min it was brought back to 0 ^{o}C, quenched with a solution of NH_4OAc and diluted with EtOAc. The organic phase was separated, dried and the solvent removed. The residue was chromatographed on silica gel (toluene:EtOAc; 1:9) to give 2.00 g (42%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.68 (m, 2H), 7.48 (m, 2H), 7.37 (m, 3H), 7.18 (t, J= 7.0 Hz, 1H), 6.32 (s, 1H), 4.65 (d, J= 7.0 Hz, 2H), 1.75 (m, 4H), 0.85 (t, J= 7.0 Hz, 6H). MS (CI, -ve ion) *m/z* 423.2, $(M-H)^-$.

4-(4-fluorophenyl)-7-({[5-(1,1,1-trifluoro-2-hydroxybutan-2-yl)-1,3,4-oxadiazol-2-yl]amino}methyl)-2*H*-chromen-2-one (16):

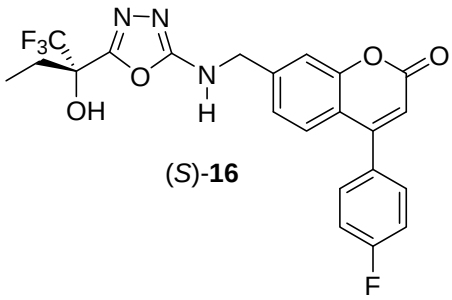


A mixture of oxadiazole **31c** (14.41 g, 68.2 mmol) and aldehyde **25** (14.1 g, 52.5 mmol) in toluene (160 mL) with 10% of pyridinium *p*-toluenesulfonate was refluxed overnight. The system was equipped with a Dean-Stark apparatus to collect water. The solvent was removed and the crude oil [^{1}H NMR (400 MHz, acetone-d_6): δ 9.33 (s, 1H, imine)] obtained was diluted in EtOH (ca. 75 mL) at 0^{o} C. To this solution was added $NaBH_4$ (1.9 g) portionwise and the reaction was quenched with a solution of NH_4Cl after 45 min. The mixture was saturated with NaCl and extracted with EtOAc (3 X 200 mL). The organic phases were combined and dried over $MgSO_4$. Purification over silica gel using toluene-EtOAc (55:45) gave 11.54 g (54%) of the title compound. ^{1}H NMR (400 MHz, acetone-d_6): δ 7.65 (m, 2H), 7.50 (m, 3H), 7.38 (m, 3H), 6.35 (s, 1H), 6.06 (s, 1H), 4.70 (m, 2H), 2.21 (m, 1H), 2.11 (m, 1H) and 0.98 (t, 3H).

Separation of 4-(4-fluorophenyl)-7-[({5-[(2*R*)-1,1,1-trifluoro-2-hydroxybutan-2-yl]-1,3,4-oxadiazol-2-yl}amino)methyl]-2*H*-chromen-2-one ((*R*)-16) and 4-(4-fluorophenyl)-7-[({5-[(2*S*)-1,1,1-trifluoro-2-hydroxybutan-2-yl]-1,3,4-oxadiazol-2-yl}amino)methyl]-2*H*-chromen-2-one ((*S*)-16):



(*R*)-**16**

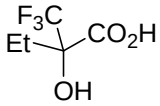


(*S*)-**16**

A solution of (±)-4-(4-fluorophenyl)-7-[({5-[1-hydroxy-1-(trifluoromethyl)propyl]-1,3,4-oxadiazol-2-yl}amino)methyl]-2H-chromen-2-one (**16**) (0.5-0.6 g) in EtOH-Hexane (30:70, ca. 40 mL) was injected on a CHIRALPAK AD preparative (5 cm x 50 cm) HPLC column (eluting with EtOH/Hexane, 30/70 with UV detection at 280 nm). The enantiomers were separated with the fast-eluting enantiomer having a retention time of ~ 34 min for the (-) and the slow-eluting enantiomer having a retention time of ~ 49 min for the (+)-enantiomer.

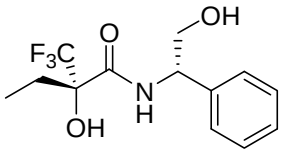
Scheme 5

2-hydroxy-2-(trifluoromethyl)butanoic acid (32):



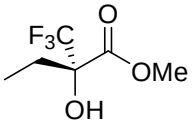
To a -78 ^{o}C solution of ethyl trifluoropyruvate (129.0 g 758 mmol) in ether was added dropwise, within 90 min, a solution of EtMgBr 3.0 M in ether (252 mL). The solution was brought over 1 h to -10 ^{o}C and poured over 2 L of saturated NH_4Cl. The layers were separated and the aqueous phase extracted with ether (3 X 500 mL). The organic phases were combined, dried over $MgSO_4$ and the solvent removed. Distillation at 50-65 ^{o}C (30 mm Hg) gave 115.4 g (76%) of the desired ethyl ester. ^{1}H NMR (400 MHz, acetone-d_6): δ 5.4 (s, 1H), 4.35 (q, 2H), 2.07 (m, 1H), 1.83 (m, 1H), 1.3 (t, 3H) and 0.93 (t, 3H).
To a stirred solution of ethyl 2-hydroxy-2-(trifluoromethyl)butanoate (116.9 g, 584 mmol) in THF (800 mL) and MeOH (100 mL) was added 2 M NaOH (730 mL, 1460 mmol). The solution was stirred at rt overnight after which concentrated HCl was added until pH=1. The mixture was extracted with ethyl acetate. The combined organic fractions were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was triturated in hexanes and filtered to give the title compound as a white solid (90.2 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.06-2.19 (m, 1H), 1.94-2.06 (m, 1H), 1.02 (t, 3H).

(2*S*)-2-hydroxy-*N*-[(1*S*)-2-hydroxy-1-phenylethyl]-2-(trifluoromethyl)butanamide ((*S, S*)-33):



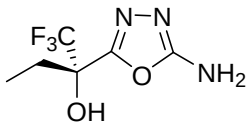
To a stirred solution of **32** (90.2 g, 524 mmol) in DMF (700 mL) were added (*S*)-2-phenylglycinol (75.5 g, 551 mmol), HATU (219.3 g, 576 mmol) and DIPEA (183 mL, 1048 mmol). The solution was stirred at rt for 3 h after which aqueous ammonium chloride was added. The mixture was extracted with ethyl ether. The combined organic fractions were washed with water, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified on silica gel (Hexanes/ EtOAc; 8:2 to 1:1) to give the title compound (least polar diastereomer, 62.7 g, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25-7.43 (m, 5H), 7.05 (s, 1H), 5.13 (m, 1H), 4.11 (s, 1H), 3.93 (s, 2H), 1.90-2.10 (m, 3H), 0.97 (t, 3H).

methyl (2*S*)-2-hydroxy-2-(trifluoromethyl)butanoate ((*S*)-34):



To a stirred solution of (*S*,*S*)-**33** (50.0 g, 172 mmol) in dioxane (150 mL) was added H_2SO_4 (12 M, 150 mL). The solution was stirred at 105 °C for 6 h, cooled down and EtOAc was added. The aqueous phase was poured dropwise into NaOH (7.5 M, 800 mL) at 0 °C. The organic phase was added and the mixture was extracted with EtOAc. The combined organic fractions were washed with water, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was triturated in hexanes, filtered dissolved in ethyl ether and treated at 0 °C with an ethereal solution of diazomethane until a yellow color persisted. The solution was concentrated to give the title compound as a brownish liquid (23.7 g, 74% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 3H), 3.79 (s, 1H), 2.00-2.12 (m, 1H), 1.89-1.99 (m, 1H), 0.93 (t, 3H).

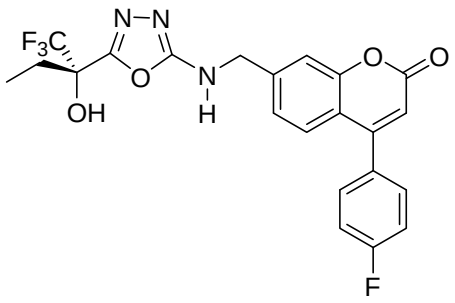
(2*S*)-2-(5-amino-1,3,4-oxadiazol-2-yl)-1,1,1-trifluorobutan-2-ol ((*S*)-35):



A mixture of (***S***)-**34** (51.0 g, 274 mmol) and hydrazine hydrate (27 mL, 548 mmol) were heated at 120 °C for 5 h. The excess hydrazine was removed under vacuum and the crude product was filtered through a pad of silica gel with EtOAc-hexane (95:5). To a solution of the hydrazide obtained (42.6 g, 229 mmol) in water (300 mL) was added $KHCO_3$ (24.1 g, 240 mmol) followed by BrCN (24.7 g, 233 mmol) portionwise. After 2 h, the solid was filtered, washed with cold water and dried to afford the title compound as a white

solid. (41.0 g, 71% over 2 steps). ^{1}H NMR (400 MHz, acetone-d_6): δ 6.54 (s, 2H), 6.01 (s, 1H), 2.18-2.12 (m, 1H), 2.00-2.12 (m, 1H), 0.99 (t, 3H).

4-(4-fluorophenyl)-7-[({5-[(2*S*)-1,1,1-trifluoro-2-hydroxybutan-2-yl]-1,3,4-oxadiazol-2-yl}amino)methyl]-2*H*-chromen-2-one ((*S*)-16, MK-0633, setileuton):



A mixture of oxadiazole **(*S*)-35** (41.9 g, 156 mmol) and aldehyde **25** (39.2 g, 186 mmol) in toluene (2 L) with 10% of pyridinium *p*-toluenesulfonate was refluxed overnight. The system was equipped with a Dean-Stark apparatus to collect water. The solvent was removed and the crude oil [^{1}H NMR (400 MHz, acetone-d_6): δ 9.33 (s, 1H, imine)] obtained was diluted in THF (600 mL) and EtOH (100 mL). To this solution was added at 0 ^{o}C $NaBH_4$ (7.2 g) portionwise. After 1 h of stirring, aqueous ammonium acetate was added. The mixture was extracted with ethyl acetate. The combined organic fractions were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified on silica gel (toluene/EtOAc; 1:1) to give the title compound (39.4 g, 54%). ^{1}H NMR (400 MHz, acetone-d_6): δ 7.65 (m, 2H), 7.50 (m, 3H), 7.38 (m, 3H), 6.35 (s, 1H), 6.06 (s, 1H), 4.70 (m, 2H), 2.21 (m, 1H), 2.11 (m, 1H), 0.98 (t, 3H); HRMS calcd for $C_{22}H_{17}F_4N_3O_4$ [MH+]: 464.1233; found: 464.1228.

III) H5-LO Assay Protocol

The activity of 5-lipoxygenase was measured using a spectrophotometric assay and recombinant human 5-lipoxygenase as a source of enzyme. Human 5-lipoxygenase was purified from Sf9 cells infected with the recombinant baculovirus rvH5LO (8-1) containing the coding sequence for human 5-lipoxygenase as described by Percival et al. (*Eur. J. Biochem.* **1992,** *210*, 109-117). The enzymatic activity was measured using a spectrophotometric assay from the optimal rate of conjugated diene formation (absorbance at 238 nm) using the procedure described in Riendeau et al. (*Biochem. Pharmacol.* **1989,** *38*, 2313-2321), with minor modifications. The incubation mixture contained 25 mM potassium phosphate, pH 7.5, 0.1 mM EDTA, 0.3 mM $CaCl_2$, 24 μg/mL phosphatidylcholine, 0.1 mM ATP, 0.5 mM DTT, 20 μM arachidonic acid (2 μL from a 100-fold solution in ethanol), inhibitor (2 μL aliquot from a 100-fold solution in DMSO) and an aliquot of purified 5-lipoxygenase. Reactions were initiated by the addition of purified 5-lipoxygenase (~10 nM) and the rate of conjugated diene production

was followed for 5 min at room temperature. The reaction was performed in a Costar UV plate (Cat. # 3635) and the absorbance changes at 238 nm were recorded with a Molecular Devices UV/VIS 96 well spectrophotometer (Spectra Max 190) using SOFTmax PRO software. Enzymatic activity was calculated from the optimal rate of the reaction by a linear fit of the increase in absorbance at 238 nm over 36 seconds (typically 0.1 Absorbance Unit/min). When the rate of diene formation is low (<0.01 Absorbance Unit/min) the linear fit is performed over 180 seconds. The results are expressed as percentage of inhibition of the reaction rate relative to controls (typically between 0.001-0.005 Absorbance Unit/min) containing the DMSO vehicle.