Supporting Information

for

Fluorine-containing substituents: metabolism of the α,α-difluoroethyl thioether motif

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Further details of equipment specifications and compound characterisation
1. General procedures and methods

All the thioethers, sulfones and sulfoxides used were synthesised in the laboratory of Prof. David O’Hagan, either as published before (Tomita et al., 2018) or as detailed in the following sections. The rest of the chemicals, media and materials were bought from Sigma-Aldrich or Alfa Aesar. Fungus Cunninghamella elegans was originally donated by Dr. Cormac Murphy (University College Dublin, Dublin, Ireland), and stored as an agar gel at 4 °C, from which our current agar gels were prepared.

All the glassware, materials and media used for microbiological purposes were sterilised by autoclaving prior to their use. The aseptic conditions were maintained during the preparation, growth and incubation of the fungal cultures.

All the glassware used for chemical synthesis was oven-dried, cooled and used under nitrogen atmosphere, unless otherwise stated.

The progress of reactions was followed by thin-layer chromatography (TLC), using aluminium plates coated with silica gel (60F245 Merck). The TLC plates were examined under UV light at 254 and 266 nm, before being visualised with alkaline potassium permanganate.

Crude extracts were analysed by $^1$H and $^{19}$F NMR. Proton and proton decoupled ($^{19}$F($^1$H), $^{13}$C($^1$H)) nuclear magnetic resonance spectra were recorded on Bruker Avance III 500 or Bruker Avance III 500 HD spectrometers (500 MHz $^1$H, 476 MHz $^{19}$F, 126 MHz $^{13}$C). Bidimensional correlation spectra were also analysed for the correct assignment of signals. Chemical shifts (δ) are expressed in ppm, and quoted relative to the residual solvent signal. Proton coupling constants (J) are given in Hz, and quoted to the nearest 0.1 Hz. Identical coupling constants are averaged.

The fluorometabolites (sulfoxides and sulfones) were isolated using a Shimadzu Prominence (SIL-20A HT autosampler, CL-20AT ternary pump, DGU-20A3R solvent degasser, SPD 20A UV detector and CVM-20A controller module), equipped with a Phenomenex semi-preparative Luna C$_{18}$ column. The AcCN and water eluents used for HPLC were filtered and supplemented with 0.05% TFA.

High-resolution mass spectrometry was acquired using electrospray ionisation (ESI), on a ThermoFisher Excalibur Orbitrap Spectrometer, operating in positive and negative mode, from solutions of the analyte in methanol or acetonitrile. Mass analysis was done at the University of St Andrews Mass Spectrometry facility by Mrs. Caroline Horsburgh. Mass units are reported in Daltons (Da).

X-ray crystal structures were obtained on a Rigaku XtaLAB P200 diffractometer, using multi-layer mirror monochromed Mo-Kα radiation, by Prof. Alexandra Slawin (University of St Andrews). The data was analysed using CrystalMaker.
2. Experimental general procedures for metabolism studies

2.1 Preparation of cultures and inoculation of xenobiotics

Sterile Saboraud Dextrose Medium (SBD, 50 mL) was inoculated with a piece of fungal agar plate (1 cm x 1 cm) at room temperature. The cultures were left to grow for 72 h at 28 °C and rotary agitation (180 rpm). After 72 h, the corresponding thioethers were added dissolved in DMF solution (5–10 mg in 50 µL) to the grown cultures, and left to incubate for further 72 h at 28 °C and 180 rpm.

2.2 Extraction and purification of the metabolites

After incubation, the fungal biomass was separated from the liquid culture, and the supernatant was extracted with ethyl ether (3 × 50 mL) and DCM (3 × 50 mL). The combined organic phases were dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The extracts were analysed by ¹H and ¹⁹F NMR before further purification.

Purification of the metabolites was carried out by reversed-phase HPLC, using an eluent system of 60:40 AcCN/water (both supplemented with 0.05% TFA), at a flow rate of 1 mL/min. Structural analysis of the resulting metabolites and remaining starting materials was carried out by full NMR characterisation (¹H, ¹⁹F, ¹³C, COSY, HSQC and HMBC) and accurate mass spectrometry. X-ray structures were obtained when possible.

3. Thio- and oxyether metabolism

3.1 (1,1-Difluoroethyl)(4-methoxyphenyl)sulfane (4)

(1,1-Difluoroethyl)(4-methoxyphenyl)sulfane (4) was dissolved in DMF (50 µL) and added to a mature culture of C. elegans. The compound was left to incubate with the fungus for 72 h. at 28 °C and 180 rpm. After 72 h, the fungus was centrifuged down, and the supernatant was extracted with DCM (3 x 50 mL) and Et₂O (3 × 50 mL). The combined organic phases were dried over Na₂SO₄, filtered and evaporated under reduced pressure. Purification of the metabolite and residual starting material was achieved by reversed-phase HPLC, in a Phenomenex Luna, and eluting with a mixture of 60:40 AcCN:water, both supplemented with 0.05% of TFA, at a flow rate of 1 mL/min. This afforded 6 at a tᵣ = 24 min, 7 at a tᵣ = 16 min and 8 at a tᵣ = 21 min.

1-((1,1-Difluoroethyl)sulfinyl)-4-methoxybenzene (6)

¹H NMR (500 MHz, chloroform-d) δH = 7.64 (2H, d, J = 8.9 Hz, H-Ar), 7.06 (2H, d, J = 8.9 Hz, H-Ar), 3.88 (3H, s, OCH₃), 1.75 (3H, t, J = 18.4 Hz, CF₂CH₃); ¹⁹F{¹H} NMR (470 MHz, chloroform-d) δF = -94.2 (d, J = 225.8 Hz), -97.6 (d, J = 225.8 Hz); ¹³C NMR (126 MHz, chloroform-d) δC = 163.1 (s, C-4), 128.1 (t, J = 323.8 Hz, CF₂), 127.6 (s, C-2), 114.8 (s, C-3), 55.6 (s, OCH₃), 16.5 (t, J = 22.3 Hz, CF₂CH₃); HRMS (ESI⁺) m/z calc. for C₉H₁₁O₂F₂S [M+H]⁺ 221.0448, found 221.0440.
4-((1,1-Difluoroethyl)sulfinyl)phenol (7)

\(^1\text{H} \text{NMR} (500 \text{ MHz, chloroform-}d) \ \delta_H \ \text{7.60 (2H, d, } J = 8.9 \text{ Hz, H-Ar), 7.01 (2H, d, } J = 8.9 \text{ Hz, H-Ar), 1.75 (3H, t, } J = 18.4 \text{ Hz, CF}_2\text{CH}_3); \ ^{19}\text{F}^{(1}\text{H}) \ \text{NMR (470 MHz, chloroform-}d) \ \delta_F \ -94.1 \ \text{d, } J = 225.1 \text{ Hz, } -97.6 \ \text{d, } J = 225.1 \text{ Hz);} \ ^{13}\text{C} \ \text{NMR (126 MHz, chloroform-}d) \ \delta_C \ \text{159.7 (s, C-4), 127.9 (s, C-2), 125.4 (t, } J = 276.2 \text{ Hz, CF}_2, \text{ visible in HMBC), 116.4 (s, C-3), 16.5 (t, } J= 22.3 \text{ Hz, CF}_2\text{CH}_3); \ \text{HRMS (ESI') } m/z \ \text{calc. for C}_{8}H_{9}O_{2}F_{2}S \ [M+H]^+ 207.0291, \ \text{found 207.0286.}

4-((1,1-Difluoroethyl)sulfonyl)phenol (8)

\(^1\text{H} \text{NMR} (500 \text{ MHz, chloroform-}d) \ \delta_H \ \text{7.87 (2H, d, } J = 8.9 \text{ Hz, H-Ar), 7.01 (2H, d, } J = 8.9 \text{ Hz, H-Ar), 2.02 (3H, t, } J = 18.3 \text{ Hz, CF}_2\text{CH}_3); \ ^{19}\text{F}^{(1}\text{H}) \ \text{NMR (470 MHz, chloroform-}d) \ \delta_F \ -97.2 \ \text{s);} \ ^{13}\text{C} \ \text{NMR (126 MHz, chloroform-}d) \ \delta_C \ \text{161.8 (s, C-4), 133.5 (s, C-3), 123.6 (t, } J = 262.9 \text{ Hz, CF}_2, \text{ visible in HMBC), 116.3 (s, C-2), 16.6 (t, } J = 22.4 \text{ Hz, CF}_2\text{CH}_3); \ \text{HRMS (ESI') } m/z \ \text{calc. for C}_{8}H_{8}O_{3}F_{2}SNa \ [M+Na]^+ 245.0060, \ \text{found 245.0055; HRMS (ESI') } m/z \ \text{calc. for C}_{8}H_{7}O_{3}F_{2}S \ [M-H]^- 221.0084, \ \text{found 221.0087.}

Reproducibility of results

| Structure | Incubation Number | Ratio by NMR | Ratio by HPLC |
|-----------|------------------|--------------|--------------|
| ![Structure 1](image1.png) | 1 | - | - |
| ![Structure 1](image2.png) | 2 | - | - |
| ![Structure 1](image3.png) | 3 | - | - |
| ![Structure 2](image4.png) | 1 | 1 | 1 |
| ![Structure 2](image5.png) | 2 | 1 | 1 |
| ![Structure 2](image6.png) | 3 | 1 | 1 |
| ![Structure 3](image7.png) | 1 | 0.6 | 0.6 |
| ![Structure 3](image8.png) | 2 | 0.8 | 0.7 |
| ![Structure 3](image9.png) | 3 | 0.8 | 0.6 |
| ![Structure 4](image10.png) | 1 | 0.1 | 0.1 |
| ![Structure 4](image11.png) | 2 | 0.1 | 0.1 |
| ![Structure 4](image12.png) | 3 | 0.15 | 0.1 |
(1,1-Difluoroethyl)napthalene-2-yl)sulfane (5) was dissolved in DMF (50 μL) and added to a mature culture of *C. elegans*. The compound was left to incubate with the fungus for 72 h. at 28 °C and 180 rpm. After 72 h, the fungus was centrifuged down, and the supernatant was extracted with DCM (3 × 50 mL) and Et₂O (3 × 50 mL). The combined organic phases were dried over Na₂SO₄, filtered and evaporated under reduced pressure. Purification of the metabolite and residual starting material was achieved by reversed-phase HPLC, in a Phenomenex Luna, and eluting with a mixture of 60:40 AcCN:water, both supplemented with 0.05% of TFA, at a flow rate of 1 mL/min. This afforded 11 at a \( t_R = 37 \) min, 12 at a \( t_R = 13 \) min and 13 at a \( t_R = 17 \) min.

2-((1,1-Difluoroethyl)sulfinyl)napthalene (11)

\[ ^1H \text{ NMR (500 MHz, chloroform-d): } \delta = 8.28 (s, 1H), 8.01 (d, \text{J} = 8.7 \text{ Hz}, 1H), 7.99 - 7.92 (m, 2H), 7.69 (dtd, \text{J} = 8.7, 2.6, 1.3 \text{ Hz}, 1H), 7.67 - 7.60 (m, 2H), 1.77 (t, \text{J} = 18.5 \text{ Hz}, 3H); \]^19F \text{ NMR (471 MHz, chloroform-d): } \delta = -92.9 (d, \text{J} = 227.0 \text{ Hz}), -95.6 (d, \text{J} = 227.0 \text{ Hz}); \text{ HRMS (ESI)} \text{ m/z calc. for C}_{12}H_{11}OF_2S [M+H]^+ 241.0420, found 241.0491. Data is consistent with the full characterisation carried out for 11 from chemical reaction.

6-((1,1-Difluoroethyl)sulfinyl)napthalene-1,4-diol (12)

\[ ^1H \text{ NMR (500 MHz, chloroform-d): } \delta = 7.81 (1H, d, \text{J} = 7.9 \text{ Hz}, H-\text{Ar}), 7.61 (1H, d, \text{J} = 7.9 \text{ Hz}, H-\text{Ar}), 7.44 (1H, s, H-\text{Ar}), 6.50 (1H, d, \text{J} = 11.0 \text{ Hz}, H-\text{Ar}), 6.12 (1H, d, \text{J} = 11.0 \text{ Hz}, H-\text{Ar}), 1.80 (3H, t, \text{J} = 18.4 \text{ Hz}, \text{CF}_2\text{CH}_3); \]^19F \text{ NMR (471 MHz, chloroform-d): } \delta = -93.2(d, \text{J} = 226.9 \text{ Hz}), -96.7 (d, \text{J} = 226.9 \text{ Hz}); \text{ HRMS (ESI)} \text{ m/z calc. for C}_{12}H_{10}O_2F_2S [M-H]^+ 271.0246, found 271.0245.

General (1,1-difluoroethyl)sulfinyl)naphthalene (13)

\[ ^19F \text{ NMR (471 MHz, chloroform-d): } \delta = -96.8 (s); \text{ m/z calc. for C}_{12}H_{10}O_2F_2S [M]^+ 288, found 288. \]
Reproducibility of results

| Structure | Incubation Number | Ratio by NMR | Ratio by HPLC |
|-----------|-------------------|--------------|---------------|
| ![structure1](image1) | 1 | - | - |
| ![structure2](image2) | 2 | - | - |
| ![structure3](image3) | 3 | - | - |
| ![structure4](image4) | 1 | 1 | 1 |
| ![structure5](image5) | 2 | 1 | 1 |
| ![structure6](image6) | 3 | 1 | 1 |
| ![structure7](image7) | 1 | 0.35 | 0.30 |
| ![structure8](image8) | 2 | 0.40 | 0.30 |
| ![structure9](image9) | 3 | 0.35 | 0.35 |
| ![structure10](image10) | 1 | 0.25 | 0.05 |
| ![structure11](image11) | 2 | 0.26 | 0.05 |
| ![structure12](image12) | 3 | 0.29 | 0.06 |
3.3 1-(1,1-Difluoroethoxy)-4-methoxybenzene (14)

1-(1,1-Difluoroethoxy)-4-methoxybenzene (14) was dissolved in DMF (50 μL) and added to a mature culture of *C. elegans*. The compound was left to incubate with the fungus for 72 h. at 28 °C and 180 rpm. After 72 h, the fungus was centrifuged down, and the supernatant was extracted with EtOAc (3 × 50 mL). The combined organic phases were dried over Na₂SO₄, filtered and evaporated under reduced pressure. Purification of the metabolite and residual starting material was achieved by reversed-phase HPLC, in a Phenomenex Luna, and eluting with a mixture of 60:40 AcCN/water, both supplemented with 0.05% of TFA, at a flow rate of 1 mL/min. This afforded 15 at a *t*<sub>R</sub> = 25 min.

4-Acetoxyphenol (15)

\[ \text{1H NMR (500 MHz, chloroform-}d\text{)} \delta_{H} 6.95 (2H, d, J = 8.9 \text{ Hz, } H-\text{Ar}), 6.82 (2H, d, J = 8.9 \text{ Hz, } H-\text{Ar}), 2.28 (3H, t, J = 18.4 \text{ Hz, COCH}_3); \text{13C NMR (126 MHz, chloroform-}d\text{)} \delta_{C} 160.8 \text{ (s, CO, visible in HMBC), 122.7 (s, C-Ar), 116.1 (s, C-Ar), 21.1 (s, COCH}_3). \] 

Data is in good agreement with the literature values (Coombes et al., 2008).

4-(1,1-Difluoro)ethoxy phenol (16)

\[ \text{1H NMR (500 MHz, chloroform-}d\text{)} \delta_{H} 6.75 (2H, d, J = 2.7 \text{ Hz, } H-\text{Ar}), 6.64 (2H, d, J = 2.7 Hz, H-\text{Ar}), 1.87 (3H, t, J = 13.3 \text{ Hz, CF}_2CH_3); \text{19F{1H} NMR (470 MHz, chloroform-}d\text{)} \delta_{F} -64.6 \text{ (s); 13C NMR (126 MHz, chloroform-}d\text{)} \delta_{C} 114.4 \text{ (s, C-Ar), 110.1 (s, C-Ar), 22.5 (t, J = 32.0 Hz, CF}_2CH_3). \] 

Low amounts did not allow a full characterisation.
4. Synthesis of racemic materials for enantiomeric excess analysis and further characterisation of metabolites

4.1 1-((1,1-Difluoroethyl)sulfonyl)-4-methoxybenzene (9)

(1,1-Difluoroethyl)(4-methoxyphenyl)sulfane (4, 5 mg, 0.025 mmol) was added to a round bottom flask containing a stirring bar, and dissolved in CH₂Cl₂ (1.5 mL). mCPBA was added to the solution (21 mg, 0.122 mmol), and the mixture was stirred at r.t. overnight. The reaction was quenched by addition of a saturated solution of NaHCO₃. The aqueous phase was extracted with CH₂Cl₂ (3 × 3 mL). The combined organic phases were combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure, yielding to 9 with 100% conversion. Further purification was carried out by column chromatography, starting with 100% petroleum ether, followed by 15% EtOAc in petroleum ether, affording 8 in quantitative yield.

1H NMR (500 MHz, chloroform-d): δH 7.84 (d, J = 8.6 Hz, 2H), 7.02 (d, J = 8.6 Hz, 2H), 3.91 (s, 3H), 2.02 (t, J = 18.3 Hz, 3H)

19F NMR (471 MHz, chloroform-d): δF -97.3 (s)

13C NMR (126 MHz, chloroform-d): δC 165.2, 133.1, 122.9, 114.7, 55.8, 16.6 (t, J = 22.2 Hz)

HMRS (ESI) C₉H₁₁F₂O₃S [M+H]+ calculated for 236.0310, found 236.0390; [M+Na]+ calculated for 259.0211, found 259.0216.

4.2 Racemic 1-((1,1-difluoroethyl)sulfinyl)-4-methoxybenzene (6)

(1,1-Difluoroethyl)(4-methoxyphenyl)sulfane (4, 5 mg, 0.025 mmol) was added to a round bottom flask with a stirring bar and dissolved in a mixture of DCM (3 mL) and methanol (0.3 mL). The solution was stirred at room temperature until homogenisation (5 min). AlCl₃ (1.8 mg, 0.012 mmol) was added, and the solution stirred for 5 min, prior to the addition of BAIB ((bisacetoxyiodo)benzene, 7.3 mg, 0.025 mmol). The reaction was left to stir overnight. After 16 h, the solvents were evaporated under reduced pressure. The remaining mixture showed the formation of 6 with 66% conversion from the starting material 4. Further purification was achieved by reversed-phase HPLC in a Phenomenex Luna SP column, with 60:40 AcCN/water (supplemented with 0.05% TFA) at a flow rate of 1 mL/min. The product 6 was isolated at tᵣ = 24 min, which was consistent with the metabolic experiment’s data.

1H NMR (500 MHz, chloroform-d): δH 7.65 (d, J = 8.9 Hz, 2H), 7.08 (d, J = 8.9 Hz, 2H), 3.89 (s, 1H), 1.81 (t, J = 18.4 Hz, 1H)

19F NMR (471 MHz, chloroform-d): δF -93.4 (d, J = 225.1 Hz), -97.1 (d, J = 225.1 Hz).
4.3 Racemic 2-((1,1-difluoroethyl)sulfinyl)naphthalene (11)

(1,1-Difluoroethyl)(naphthalene-2-yl)sulfane (5, 5 mg, 0.022 mmol) was added to a round bottom flask with a stirring bar and dissolved in a mixture of DCM (3 mL) and methanol (0.3 mL). The solution was stirred at room temperature until homogenisation (5 min). AlCl₃ (1.5 mg, 0.011 mmol) was added, and the solution stirred for 5 min, prior to the addition of BAIB (11.1 mg, 0.022 mmol). The reaction was left to stir overnight. After 16 h, the solvents were evaporated under reduced pressure. Further purification was achieved by reversed-phase HPLC in a Phenomenex Luna SP column, with 60:40 AcCN/water (supplemented with 0.05% TFA) at a flow rate of 1 mL/min, which afforded 11 in 30% yield. The product 11 was isolated at tᵣ = 37 min, which was consistent with the metabolic experiments’ data. **¹H NMR** (500 MHz, chloroform-d): 6H 8.28 (s, 1H), 8.01 (d, 6.77 Hz, 1H), 7.99 – 7.89 (m, 2H), 7.69 (ddt, 6.77, 1.3 Hz, 1H), 7.67 – 7.60 (m, 2H), 1.77 (t, 6.77 Hz, 3H); **¹⁹F NMR** (471 MHz, chloroform-d): δF -92.9 (d, 6.77 Hz), -96.0 (d, 6.77 Hz); **¹³C NMR** (126 MHz, chloroform-d) δC 145.9 (C-Ar, visible in HMBC), 135.1 (s, C-Ar), 133.6 (t, 6.77 Hz, CF₃), 129.3 (s, C-Ar), 128.8 (s, C-Ar), 128.5 (s, C-Ar), 128.1 (s, C-Ar), 127.5 (s, C-Ar), 126.8 (s, C-Ar), 121.0 (s, C-Ar), 111.7 (C-Ar, visible in HMBC), 16.5 (t, 6.77 Hz, CF₃CH₃); **HRMS** (ESI⁺) m/z calc. for C₁₂H₁₁OF₂S [M+H]⁺ 241.0420, found 241.0491.
5. Enantiomeric excess analysis

5.1 1-((1,1-Difluoroethyl)sulfinyl)-4-methoxybenzene (6)

HPLC data for compound 6: Chiralcel ID (95:5 hexane:IPA, flow rate 1 mL/min, 254 nm, 30 °C), t_R(A): 15.6 min, t_R(B): 16.5 min; 20:80 ee.
5.2 2-\{(1,1-Difluoroethyl)sulfinyl\}naphthalene (11)

HPLC data for compound 11: Chiralcel IC (95:5 hexane:IPA, flow rate 1 mL/min, 254 nm, 30 °C), $t_R$ (A): 18.9 min, $t_R$ (B): 16.5 min; 23:77 ee.
6. Single crystal X-ray analysis
4-((1,1-Difluoroethyl)sulfinyl)phenol (7)

Code: agdh14
Structure Type: Crystal
Chemical Formula: C₈ H₈ F₂ O₂ S
Display Formula: C₈ H₈ F₂ O₂ S
Spacegroup: P 2₁ 2₁ 2₁
(Allows Chirality)

Crystal System: Orthorhombic

a: 8.5526 Å
b: 9.2693 Å
c: 10.9710 Å

Asymmetric Unit: 21 sites
Unit Cell: 84 sites per unit cell
Site Density: 0.0966 sites/Å³
Visible Atoms: 21
Cell Volume: 869.744 Å³
Density: 1.5748 g/cm³
7. NMR Spectra

1-((1,1-Difluoroethyl)sulfinyl)-4-methoxybenzene (6)

\[ \text{H-NMR} \]

\[ \text{F-NMR} \]
4-((1,1-Difluoroethyl)sulfinyl)phenol (7)

$^1$H NMR

$^{19}$F NMR
4-((1,1-Difluoroethyl)sulfonyl)phenol (8)

$\text{H NMR}$

$\text{F NMR}$
1-((1,1-Difluoroethyl)sulfonyl)-4-methoxybenzene (9)

$^1$H NMR

$^{19}$F NMR
2-((1,1-difluoroethyl)sulfinyl)naphthalene (11)

$^1$H NMR

$^{19}$F NMR
6-((1,1-Difluoroethyl)sulfinyl)naphthalene-1,4-diol (12)

\[ \text{\^19F NMR} \]

(1,1-difluoroethyl)sulfinyl)naphthalene (13)

\[ \text{\^19F NMR} \]
4-Acetoxyphenol (15)

\[^1\text{H NMR}\]

4-(1,1-Difluoro)ethoxy phenol (16)

\[^1\text{H NMR}\]
8. References

Coombes CL, Moody CJ (2008) First syntheses of 2,2-dimethyl-7-(2’-methylbut-3’-en-2’-yl)-2H-chromen-6-ol and 2-(3’-Methylbut-2’-enyl)-5-(2’-methylbut-3’-en-2’-yl)-1,4-benzoquinone, novel prenylated quinone derivatives from the New Zealand brown alga Perithalia capillaris. J Org Chem 73:6758–6762. https://doi.org/10.1021/jo801057x

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