Supplementary Material for Organic & Biomolecular Chemistry

A new synthesis of difluoromethanesulfonamides – a novel pharmacophore for carbonic anhydrase inhibition.

Nicholas A. Boyle,[a†] W. Richard Chegwidden,[b§] and G. Michael Blackburn[a]*

[a] Krebs Institute, Chemistry Department, Sheffield University, Sheffield S3 7HF, UK. Fax: +44 1142 229346; Tel: +44 114 222 9462; E-mail: g.m.blackburn@shef.ac.uk. [b] Sheffield Hallam University, S1 1WB

Supplementary Data

Melting points were measured on a Kofler hot stage micro-melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker AC-250 spectrometer at 250 MHz. Chemical shifts are accurate to ±0.005 ppm at 250 MHz, and are reported using the δ scale in parts per million (increasing frequency corresponding to increasing ppm) with respect to tetramethylsilane (TMS). Coupling constants (*J*) are accurate to ±0.5 Hz. Carbon NMR spectra were recorded on a Bruker AC-250 at 62.9 MHz or a Bruker AM-400 at 100 MHz. Chemical shift values are quoted in ppm and δ_C values are accurate to ±0.05 ppm, coupling constants (*J*) are accurate to ±0.5 Hz. Fluorine NMR spectra were recorded on a Bruker AC-250 at 235 MHz. Chemical shifts are quoted downfield from $CFCl_3$ as the external reference. Chemical shift values are quoted in ppm and δ_F values are accurate to ±0.005 ppm, coupling constants (*J*) are accurate to ±0.5 Hz.

Low and high resolution electron impact (EI), chemical ionisation (CI), fast atom bombardment (FAB) and electrospray (ES) mass spectra were recorded on either a Kratos MS25 or a Fisons Prospec 3000 mass spectrometer. Fast atom bombardment (FAB) spectra were recorded on a Kratos MS80RF mass spectrometer or a Fisons Prospec 3000 mass spectrometer in conjunction with a DS55 data station. High resolution mass spectroscopy (HRMS) values are accurate to within 5 ppm.

pK_a Values were determined at ionic strength 0.1 and 25.0 °C by titration and, occasionally, by UV analysis, and are accurate to ± 0.05. With the titration method, pK_a values were determined directly by analysis of the pH-titration profile obtained using a PHM 82 standard pH meter. With the UV method, pK_a values were determined directly

by analysis of the pH-UV absorbency profile. With both methods, a combined calomel/glass electrode (GK2041B) was used for the pH measurements.

Carbonic anhydrase II was prepared from human erythrocytes by standard procedures and its purity established by SDS-PAGE e;lectrop0horesis.

(Benzenemethylthio)difluoroethanoyl chloride 5. 2-Chloro-2,2-difluoroethanoic acid (85.6 g, 656 mmol) was added dropwise over 1 h to a slurry of NaH (60 % dispersion in mineral oil) (57.7 g, 1.44 mol) in anhydrous dioxane (1 dm^3), under argon at -10°C. Benzenemethylmercaptan (85.6 g, 689 mmol) was added dropwise over 1 h and the mixture heated at reflux 4 h. After cooling to rt, the dioxane was removed *in vacuo* and DCM (500 ml) added to the brown residue. PCl_5 (143 g, 689 mmol) was added portionwise to the rapidly stirred mixture under argon at -15°C. After stirring 1 h at rt, the mixture was filtered under argon and the filtrate concentrated *in vacuo*. The resulting oil was distilled and the *title compound* **5** obtained as a clear, colourless oil (107 g, 70 %); bp 77.0°C/0.04 mm Hg; δ_H (250 MHz; $CDCl_3$) 7.36 (5H, m, 5 x Ar**H**), 4.12 (2H, SC**H**$_2$); δ_C (400 MHz; $CDCl_3$) 164.1 (t, J = 40 Hz, C2), 134.2 (C4), 129.1 (C5 and 9) 128.9 (C6 and 8), 128.1 (C7), 120.8 (t, J = 293 Hz, C1), 33.9 (C3); δ_F (250 MHz; $CDCl_3$) - 81.50; MS (EI^+) *m*/*z* 236 (M^+, 29 %); HRMS calcd for $C_9H_7ClF_2OS$: 235.9874. Found: 235.9876; IR (KBr, cm^{-1}) 1790, 1496, 1455, 1266, 1162, 1020.

2-(Benzenemethylthio)-2,2-difluoroethanoyl (*N*-methyl)anilide 6. *N*-Methyl-aniline (54.4 g, 508 mmol) was added dropwise to **5** (57 g, 240 mmol) in ether (1 dm^3) under argon at -78°C. After complete addition, the mixture was warmed to rt, filtered and the filter-cake washed with ether (5 x 50 ml). The combine filtrate and washings were vigorously washed with HCl (2 x 500 ml, aq, 1 M). The ether was dried with sodium sulfate and evaporated to give the *title compound* **6** as an orange oil which crystallised overnight (73.7 g, quant); recrystallised from ether mp 42.0°C; δ_H (250 MHz; $CDCl_3$) 7.18 (10H, m, 10 x Ar**H**), 3.82 (2H, s, SC**H**$_2$), 3.17 (3H, s, NC**H**$_3$); δ_C (250 MHz; $CDCl_3$) 161.1 (t, J = 30 Hz, C2), 141.6 (C4), 136.0 (C11), 129.3, 129.1, 128.7, 128.5, 127.8, 127.6 (C5-9, 12-16), 124.6 (t, J = 273 Hz, C1); δ_F (250 MHz; $CDCl_3$) -73.83; MS (EI^+) *m*/*z* 307 (M^+, 13 %); HRMS calcd for $C_{16}H_{16}F_2NOS$ (M^+ + H): 308.0921. Found: 308.0910; IR (KBr, cm^{-1}) 1673, 1594, 1495, 1162, 1074, 1027.

2-Chlorosulfonyl-2,2-difluoroethanoyl (*N*-methyl)anilide 7. Chlorine gas was bubbled into a vigorously stirred suspension of **6** (70.0 g, 227 mmol) in glacial ethanoic acid (150 ml) and water (300 ml) at 0°C for 1 h. The resulting yellow mixture was poured onto excess ice with vigorous stirring and extracted with DCM (4 x 50 ml). The combined organic extracts were washed with cold brine then water, dried over sodium sulfate, and evaporated. The resulting mixture of product and benzyl chloride was triturated with hexanes (100 ml) and solid material collected by filtration and washed with hexanes (3 x 30 ml) to give the *title compound* **7** as a white crystalline solid (54.3 g, 84 %); mp 91.2°C; δ_H (250 MHz; $CDCl_3$) 7.47 (3H, m, 3 x Ar**H**), 7.29 (2H, m, 2 x Ar**H**), 3.39 (3H, s, NC**H**$_3$); δ_C (400 MHz; $CDCl_3$) 155.8 (t, *J* = 22 Hz, C2), 139.6 (C4), 130.3 (C6 and 8), 130.0 (C7), 127.6 (C5 and 9), 117.3 (t, *J* = 321 Hz, C1), 40.5 (C3); δ_F (250 MHz; $CDCl_3$) -88.28; MS (EI^+) *m/z* 283 (M^+, 73 %); HRMS calcd for $C_9H_8ClF_2NO_3S$: 282.9881. Found: 282.9874; IR (KBr, cm^{-1}) 1679, 1592, 1493, 1405, 1378, 1185, 1156, 1062; calcd for $C_9H_8ClF_2NO_3S$: C, 38.11; H, 2.84; N, 4.94; Cl, 12.50. Found: C, 38.39; H, 2.81; N, 4.87; Cl, 12.34.

2-Aminosulfonyl-2,2-difluoroethanoyl (*N*-methyl)anilide 8. Ammonia gas was bubbled into a solution of **7** (50.0 g, 176 mmol) in DCM (400 ml) for 2 h. The mixture was concentrated *in vacuo* to approximately 25 % of the original volume, the solid collected by filtration, and the filter-cake washed with DCM (4 x 30 ml). The white solid (53.0 g, 84 % - based on a purified sample) containing the *product* (as the ammonium salt) and ammonium chloride was used without further purification in the synthesis of **9**. A portion (1.0 g) of this white solid was suspended in water (20 ml) and the suspension adjusted to pH 4 with conc HCl. The mixture was extracted with EtOAc (3 x 30 ml) and the combined organic extracts washed with cold brine, water, dried over sodium sulfate, and evaporated. The resulting pale brown residue was triturated with hot $CHCl_3$ to give a yellow solid. Recrystallisation from EtOAc gave the *title compound* **8** as a colourless, crystalline solid (0.74 g); TLC (40 % EtOAc in petrol): R_f = 0.4; mp 176°C; δ_H (250 MHz; d_6-DMSO) 8.19 (2H, bs, N**H**$_2$), 7.42 (5H, m, 5 x Ar**H**), 3.72 (3H, s, NC**H**$_3$); δ_C (400 MHz; d_6-DMSO) 157.6 (t, *J* = 23 Hz, C2), 141.4 (C4), 129.3 (C6 and 8), 128.4 (C7), 127.3 (C5 and 9), 114.2 (t, *J* = 295 Hz, C1), 40.2 (C3); δ_F (250 MHz; d_6-DMSO) -99.17; MS (EI^+) *m/z* 264 (M^+, 77 %); HRMS calcd for $C_9H_{10}F_2N_2O_3S$: 264.0380. Found: 264.0370; IR (KBr, cm^{-1}) 3371, 3244, 1664, 1549,

1496, 1359, 1204, 1108; calcd for $C_9H_{10}F_2N_2O_3S$: C, 40.91; H, 3.81; N, 10.6; S, 12.13. Found: C, 40.61; H, 3.64; N, 10.49; S, 12.37.

Carboxydifluoromethanesulfonamide 9. Conc HCl (200 ml) and conc H_2SO_4 (25 ml) were added successively to **8** (52 g (crude), 146 mmol) and the suspension heated at gentle reflux overnight. The resulting solution was cooled to rt, concentrated *in vacuo* to 25 % of the original volume and extracted with ether (100 ml) for 24 h using a continuous liquid phase extractor. The extract was dried over sodium sulfate, filtered and concentrated *in vacuo* to give the *title compound* **9** as a clear oil which solidified upon prolonged exposure to high vacuum (25.5 g, quant); δ_H (250 MHz; d_6-DMSO) 9.55 (1H, bs, $CO_2\mathbf{H}$), 8.34 (2H, s, $N\mathbf{H}_2$); δ_C (250 MHz; d_6-DMSO) 161.1 (t, J = 28 Hz, C2), 113.0 (t, J = 289 Hz, C1); δ_F (250 MHz; d_6-DMSO) -108.79; MS (EI^+) m/z 176 (M^+ + H, 32 %); HRMS calcd for $C_2H_4F_2NO_4S$, (M^+ + H): 175.9829. Found: 175.9835; IR (KBr, cm^{-1}) 3800-3000, 1760, 1636, 1368, 1158.

(2-Amino-1,3,4-thiadiazol-5-yl)-difluoromethanesulfonamide 3. $POCl_3$ (29.1 g, 190 mmol) was added dropwise to a mixture of **9** (16.6 g, 94.9 mmol), and thiosemicarbazide (8.64 g, 94.9 mmol) under argon at 0˚C. The stirred suspension was heated 3 h at 70˚C then cooled and poured into ice-water (50 ml). After adjusting the solution to pH 6, the resulting precipitate was collected by filtration. The filter-cake was dissolved in hot EtOAc (150 ml), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a white solid, recrystallised from MeOH/EtOAc (7.1 g, 33 %); TLC (100 % EtOAc): R_f = 0.6 (stains brown with ninhydrin); mp 238°C (decomposition); δ_H (250 MHz; d_6-DMSO) 8.44 (2H, d, J = 5 Hz, $SO_2N\mathbf{H}_2$), 7.92 (2H, s, C3-$N\mathbf{H}_2$); δ_C (400 MHz; d_6-DMSO) 172.0 (C3), 144.2 (t, J = 26 Hz, C2), 116.3 (t, J = 281 Hz, C1); δ_F (250 MHz; d_6-DMSO) -97.74; MS (EI^+) m/z 230 (M^+, 30 %); HRMS calcd for $C_3H_4F_2N_4O_2S_2$: 229.9744. Found: 229.9744; IR (KBr, cm^{-1}) 3432, 3364, 3260, 3099, 1622, 1509, 1367, 1181; calcd for $C_3H_4F_2N_4O_2S_2$: C, 15.65; H, 1.75; N, 24.34. Found: C, 15.56; H, 1.59; N, 24.04.

(2-Ethanoylamino-1,3,4-thiadiazol-5-yl)-difluoromethanesulfonamide 10. A suspension of **3** (0.5 g, 2.15 mmol) in ethanoic anhydride (10.0 ml, excess) was stirred at rt overnight. The solid was collected by filtration and washed extensively with

$CHCl_3$ to give the *title compound* **10** as a white solid (0.5 g, 86 %); TLC (100 % EtOAc): R_f = 0.7 (stains red with ninhydrin); recrystallised from ethanol mp 245-252°C (decomposition); δ_H (250 MHz; d_6-DMSO) 13.14 (1H, s, CON**H**), 8.55 (2H, d, *J* = 5 Hz, $SO_2N\mathbf{H}_2$), 2.26 (3H, s, $COC\mathbf{H}_3$); δ_C (400 MHz; d_6-DMSO) 169.5 (C3), 161.7 (C4), 151.0 (t, *J* = 26 Hz, C2), 116.7 (t, *J* = 282 Hz, C1), 22.4 (C5); δ_F (250 MHz; d_6-DMSO) -97.95; MS (EI^+) *m/z* 272 (M^+, 22 %); HRMS calcd for $C_5H_6F_2N_4O_3S_2$: 271.9849. Found: 271.9862; IR (KBr, cm^{-1}) 3380, 3272, 3158, 1699, 1565, 1542, 1473, 1379, 1318, 1276, 1243, 1192, 1155, 1108, 1093; calcd for $C_5H_6F_2N_4O_3S_2$: C, 22.06; H, 2.22; N, 20.59. Found: C, 21.92; H, 2.07; N, 20.32.

(2-Trifluoroethanoylamino-1,3,4-thiadiazol-5-yl)-difluoromethanesulfonamide 11. Trifluoroethanoic anhydride (3.0 ml, excess) was added to **3** (0.2 g, 0.87 mmol) at rt. The suspension was stirred overnight then solid collected by filtration and washed extensively with $CHCl_3$ to give the *title compound* **11** as a white solid (190 mg, 67 %); recrystallised from ethyl ethanoate mp 179°C; δ_H (250 MHz; d_6-DMSO) 8.6 (2H, d, *J* = 5 Hz, $SO_2N\mathbf{H}_2$); δ_C (400 MHz; d_6-DMSO) 167.8 (C3), 160.9 (q, *J* = 38 Hz, C4), 150.5 (t, *J* = 27 Hz, C2), 116.5 (q, *J* = 286 Hz, C5), 116.3 (t, *J* = 282 Hz, C1); δ_F (250 MHz; d_6-DMSO) -73.49, -99.52; MS (EI^+) *m/z* 326 (M^+, 8 %); HRMS calcd for $C_5H_3F_5N_4O_3S_2$: 325.9567. Found: 325.9557; IR (KBr, cm^{-1}) 3432, 3402, 3298, 1749, 1568, 1372, 1289, 1236, 1193, 1179, 1166, 1151, 1091.