New superacid synthesized (fluorinated) tertiary benzenesulfonamides acting as selective hCA IX inhibitors: toward a new mode of carbonic anhydrases inhibition by sulfonamides.

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A. GENERAL METHOD

The authors draw the reader’s attention to the dangerous features of superacidic chemistry. Handling of hydrogen fluoride and antimony pentafluoride must be done by experienced chemists with all the necessary safety arrangements in place.

Reactions performed in superacid were carried out in a sealed Teflon® flask with a magnetic stirrer. No further precautions have to be taken to prevent mixture from moisture (test reaction worked out in anhydrous conditions leads to the same results as expected).

Yields refer to isolated pure products.

\(^1\)H, \(^{13}\)C and \(^{19}\)F NMR were recorded on a 400 MHz Bruker Advance DPX spectrometer using CDCl\(_3\) as solvent. COSY \(^1\)H-\(^1\)H and \(^1\)H-\(^{13}\)C experiments were used to confirm the NMR peaks assignments.

Melting points were determined in a capillary tube with a device Buchi melting point B-545 and were uncorrected.

Mass Spectra (MS) were performed with a Liquid Chromatography–Coupled Tandem Mass Spectrometry (electronic impact).

All separations were done under flash-chromatography conditions on silica gel (15-40 \(\mu\)m).

High Resolution Mass Spectrometry (HRMS) spectra were performed at the Institut Lavoisier de Versailles of the University of Versailles St Quentin, France.

B. N-ALLYL-N-ARYLBENZENESULFONAMIDE

Procedure A: optimized procedure for \(N\)-sulfonylation of anilines

Into a round bottom flask cooled to 0°C, aniline derivative (1 eq), sulfonyl chloride (1.2 eq) and dichloromethane were introduced. Mixture was stirred under nitrogen’s atmosphere. Pyridine (3 eq) was slowly added. The mixture was magnetically stirred at room temperature for 48 hours. The reaction mixture was then neutralized with water-sodium carbonate solution (100 mL), extracted with dichloromethane (x 3). The combined organic layers were washed with hydrochloric acid 2M (x 4), dried over magnesium sulphate, filtered and concentrated in vacuo.

Procedure B: optimized procedure for \(N\)-allylation of \(N\)-arylbенzenesulfonamides

Into a round bottom flask at room temperature, \(N\)-arylbенzenesulfonamide derivative (1 eq), acetonitrile (60 mL) and potassium carbonate (10 eq) were introduced. Allyl bromide (3 eq) was slowly added. The mixture was magnetically stirred under nitrogen atmosphere at 80°C for 16 hours. The reaction mixture was then concentrated in vacuo, washed with water (x 1), dried over magnesium sulphate, filtered and concentrated in vacuo.

Products were isolated by column chromatography over silica gel.
Formation of compound 3a:

N-allyl-4-methyl-N-(4-nitrophenyl)benzenesulfonamide

This compound was obtained from 4-methyl-N-(4-nitrophenyl)benzenesulfonamide (1.38 g, 10.0 mmol) following the general procedure B. The reaction crude (without further purification) gave compound 3a (1.73 g, 98%).

Aspect: Yellow oil.

$^1$H NMR (CDCl$_3$, 400 MHz, ppm) δ: 8.15 (d, 2 H, J = 9.1 Hz, H$_3$), 7.47 (d, 2 H, J = 8.3 Hz, H$_2$), 7.29 - 7.25 (m, 4 H, H$_2$ and H$_3$), 5.70 (ddt, 1 H, J$_{trans}$ = 17.1 Hz, J$_{cis}$ = 10.3 Hz, J = 6.2 Hz, H$_{2''}$), 5.15 - 5.09 (dm, 1 H, J$_{trans}$ = 17.2 Hz, H$_{3''}$), 5.12 - 5.08 (dm, 1 H, J$_{cis}$ = 10.1 Hz, H$_{3''}$), 4.27 - 4.24 (dm, 2 H, J = 6.2 Hz, H$_{1''}$), 2.43 (s, 3 H, H$_{5'}$).

$^{13}$C NMR (CDCl$_3$, 100 MHz, ppm) δ: 148.0 (C$_1$ or C$_4$ or C$_1'$ or C$_4'$), 145.0 (C$_1$ or C$_4$ or C$_1'$ or C$_4'$), 144.3 (C$_1$ or C$_4$ or C$_1'$ or C$_4'$), 134.5 (C$_1'$ or C$_4'$), 131.8 (CH, C$_{2''}$), 129.7 (CH, C$_2$ or C$_3$), 128.1 (CH, C$_2$ or C$_3$), 127.4 (CH, C$_2$), 124.1 (CH, C$_3$), 119.7 (CH$_2$, C$_{3''}$), 52.7 (CH$_2$, C$_{1''}$), 21.5 (CH$_3$, C$_5$).

HRMS (MALDI/TOF, ES$^+$, CH$_3$CN): m/z calc for C$_{16}$H$_{16}$N$_2$O$_4$S [M+H]$^+$: 333.0909, m/z found: 333.0905.

Formation of compound 3b:

N-allyl-N-(4-methylphenyl)-4-nitrobenzenesulfonamide

This compound was obtained from 4-methylaniline (536 mg, 5.0 mmol) following the general procedure A then B. The reaction crude was filtered over silica gel with the eluent ethyl acetate, thereby obtaining compound 3b (1.61 g, 97% for two steps).

Aspect: Orange powder.

Mp: 125.3 - 126.2 °C.
$^1$H NMR (CDCl$_3$, 400 MHz, ppm) $\delta$: 8.31 (d, 2 H, $J = 9.0$ Hz, $H_3$), 7.78 (d, 2 H, $J = 9.0$ Hz, $H_2$), 7.10 (d, 2 H, $J = 8.0$ Hz, $H_2$ or $H_3$), 6.90 (d, 2 H, $J = 8.3$ Hz, $H_2$ or $H_3$), 5.73 (ddt, 1 H, $J_{\text{trans}} = 17.1$ Hz, $J_{\text{cis}} = 10.2$ Hz, $J = 6.3$ Hz, $H_{2''}$), 5.13 - 5.07 (dm, 1 H, $J_{\text{trans}} = 17.1$ Hz, $H_{3''}$), 5.09 - 5.05 (dm, 1 H, $J_{\text{cis}} = 10.1$ Hz, $H_3$), 4.20 (ddd, 2 H, $J = 6.3$ Hz, $J = 1.2$ Hz, $J = 1.2$ Hz, $H_1''$), 2.32 (s, 3 H, $H_5$).

$^{13}$C NMR (CDCl$_3$, 100 MHz, ppm) $\delta$: 149.9 ($C_1$ or $C_4$ or $C_1'$ or $C_4'$), 144.2 ($C_1$ or $C_4$ or $C_1'$ or $C_4'$), 135.4 ($C_1$ or $C_4$ or $C_1'$ or $C_4'$), 132.1 ($CH$, $C_2''$), 129.8 ($CH$, $C_2$ or $C_3$ or $C_2'$), 128.7 ($CH$, $C_2$ or $C_3$ or $C_2'$), 123.9 ($CH$, $C_3''$), 119.3 ($CH$, $C_3'$), 53.9 ($CH_2$, $C_1''$), 21.0 ($CH_3$, $C_5$).

HRMS (MALDI/TOF, ES$^+$, CH$_3$CN): $m/z$ calc for C$_{16}$H$_{16}$N$_2$O$_4$S [M+H]$^+$: 333.0909, $m/z$ found: 333.0906.

**Compound 3c:**

![Chemical Structure](image)

**N-allyl-N-(4-bromophenyl)-4-methylbenzenesulfonamide**

This compound was obtained from N-(4-bromophenyl)-4-methylbenzenesulfonamide (879 mg, 2.7 mmol) following the general procedure B. The reaction crude was filtered over silica gel with the eluent ethyl acetate, thereby obtaining compound 3c (987 mg, 99%).

**Aspect:** White powder.

**Mp:** 64.8 - 65.5 °C.

$^1$H NMR (CDCl$_3$, 400 MHz, ppm) $\delta$: 7.50 (d, 2 H, $J = 8.3$ Hz, $H_2$), 7.42 (d, 2 H, $J = 8.8$ Hz, $H_3$), 7.28 (d, 2 H, $J = 8.0$ Hz, $H_3$), 6.95 (d, 2 H, $J = 8.8$ Hz, $H_2$), 5.72 (ddt, 1 H, $J_{\text{trans}} = 17.1$ Hz, $J_{\text{cis}} = 10.2$ Hz, $J = 6.3$ Hz, $H_{2''}$), 5.12 - 5.06 (dm, 1 H, $J_{\text{trans}} = 17.1$ Hz, $H_{3''}$), 5.09 - 5.04 (dm, 1 H, $J_{\text{cis}} = 10.1$ Hz, $H_3$), 4.17 (ddd, 2 H, $J = 6.3$ Hz, $J = 1.3$ Hz, $J = 1.3$ Hz, $H_{1''}$), 2.43 (s, 3 H, $H_5$).

$^{13}$C NMR (CDCl$_3$, 100 MHz, ppm) $\delta$: 143.6 ($C_1'$ or $C_4'$), 138.0 ($C_1$), 143.7 ($C_1'$ or $C_4'$), 132.2 ($CH$, $C_2''$), 131.8 ($CH$, $C_3$), 130.2 ($CH$, $C_2$), 129.4 ($CH$, $C_3$), 127.4 ($CH$, $C_2$), 121.4 ($C_4$), 119.0 ($CH_2$, $C_3''$), 53.1 ($CH_2$, $C_1''$), 21.3 ($CH_3$, $C_5$).

**Compound 3d:**

![](image)

*N*-allyl-*N*(4-chlorophenyl)-4-methylbenzenesulfonamide

This compound was obtained from *N*-(4-chlorophenyl)-4-methylbenzenesulfonamide (1.08 g, 3.8 mmol) following the general procedure B. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 90/10, thereby obtaining compound 3d (1.21 g, 98%).

**Aspect:** White powder.

**Mp:** 49.1 - 50.0 °C.

**$^1$H NMR (CDCl$_3$, 400 MHz, ppm)**: δ: 7.51 (d, 2 H, $J = 8.3$ Hz, $H_2'$), 7.30 - 7.26 (m, 4 H, $H_3$ and $H_3'$), 7.03 (d, 2 H, $J = 8.8$ Hz, $H_2$), 5.73 (ddt, 1 H, $J_{trans} = 17.1$ Hz, $J_{cis} = 10.2$ Hz, $J = 6.3$ Hz, $H_2''$), 5.13 - 5.05 (m, 2 H, $H_3''$), 4.21 - 4.18 (dm, 2 H, $J = 6.3$ Hz, $H_1''$), 2.43 (s, 3 H, $H_5'$).

**$^{13}$C NMR (CDCl$_3$, 100 MHz, ppm)**: δ: 143.5 ($C_1'$ or $C_4'$), 137.4 ($C_1$ or $C_4$ or $C_1$' or $C_4$'), 134.6 ($C_1$ or $C_4$ or $C_1$' or $C_4$'), 133.1 ($C_1$ or $C_4$ or $C_1$' or $C_4$'), 132.1 (CH, $C_2''$), 129.8 (CH, $C_2$), 129.3 (CH, $C_3$ or $C_3'$), 128.7 (CH, $C_3$ or $C_3'$), 127.3 (CH, $C_2$), 118.8 (CH$_2$, $C_2''$), 53.0 (CH$_2$, $C_1''$), 21.2 (CH$_3$, $C_5$).


**Compound 3e:**

![](image)

*N*-allyl-*N*(4-fluorophenyl)-4-methylbenzenesulfonamide

This compound was obtained from *N*-(4-fluorophenyl)-4-methylbenzenesulfonamide (1.06 g, 4.0 mmol) following the general procedure B. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 90/10, thereby obtaining compound 3e (1.20 g, 98%).

**Aspect:** White solid.

**Mp:** 62.4 - 63.3 °C.
\[^1\text{H} \text{NMR}\ (\text{CDCl}_3, 400 \text{ MHz}, \text{ppm}) \delta:\ 7.51 \ (d, 2 \text{ H}, J = 8.3 \text{ Hz}, \text{H}_2'), 7.28 \ (d, 2 \text{ H}, J = 8.0 \text{ Hz}, \text{H}_3'), 7.06 - 6.95 \ (m, 4 \text{ H}, \text{H}_2 \text{ and } \text{H}_3), 5.74 \ (ddt, 1 \text{ H}, J_{\text{trans}} = 17.1 \text{ Hz}, J_{\text{cis}} = 10.1 \text{ Hz}, J = 6.3 \text{ Hz}, \text{H}_2''), 5.12 - 5.06 \ (dm, 1 \text{ H}, J_{\text{trans}} = 17.1 \text{ Hz}, \text{H}_3''), 5.08 - 5.04 \ (dm, 1 \text{ H}, J_{\text{cis}} = 10.1 \text{ Hz}, \text{H}_3''), 4.18 \ (ddd, 2 \text{ H}, J = 6.3 \text{ Hz}, J = 1.3 \text{ Hz}, J = 1.3 \text{ Hz}, \text{H}_1''), 2.43 \ (s, 3 \text{ H}, \text{H}_5').
\]

\[^13\text{C} \text{ NMR}\ (\text{CDCl}_3, 100 \text{ MHz}, \text{ppm}) \delta:\ 161.8 \ (d, J = 248.0 \text{ Hz}, \text{C}_4), 143.8 \ (\text{C}_1' \text{ or } \text{C}_4'), 135.1 \ (\text{C}_1' \text{ or } \text{C}_4'), 134.9 \ (d, J = 3.1 \text{ Hz}, \text{C}_1), 132.6 \ (\text{CH}, \text{C}_2''), 130.7 \ (d, \text{CH}, J = 8.7 \text{ Hz}, \text{C}_2), 129.5 \ (\text{CH}, \text{C}_3''), 127.7 \ (\text{CH}, \text{C}_2), 119.0 \ (\text{CH}_2, \text{C}_3''), 115.7 \ (d, \text{CH}, J = 22.6 \text{ Hz}, \text{C}_3), 53.7 \ (\text{CH}_2, \text{C}_1''), 21.5 \ (\text{CH}_3, \text{C}_5').
\]

\[^19\text{F} \{^1\text{H}\} \text{ NMR}\ (\text{CDCl}_3, 376 \text{ MHz}, \text{ppm}) \delta:\ -113.28 \ (\text{F}_4).
\]

\[^\text{HRMS}\ (\text{MALDI/TOF, ES}^+)\text{ C}_16\text{H}_{16}\text{FNO}_2\text{S }[M+H]^+: 306.0964, m/z \text{ found: 306.0961.}
\]

Compound 3f:

This compound was obtained from 4-methyl-N-(4-methylphenyl)benzenesulfonamide (1.05 g, 4.0 mmol) following the general procedure B. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 92/8, thereby obtaining compound 3f (1.18 mg, 98%).

Aspect: Pale yellow oil.

\[^1\text{H} \text{NMR}\ (\text{CDCl}_3, 400 \text{ MHz}, \text{ppm}) \delta:\ 7.48 \ (d, 2 \text{ H}, J = 8.3 \text{ Hz}, \text{H}_2'), 7.23 \ (d, 2 \text{ H}, J = 7.9 \text{ Hz}, \text{H}_3'), 7.06 \ (d, 2 \text{ H}, J = 8.0 \text{ Hz}, \text{H}_2 \text{ or } \text{H}_3), 6.91 \ (d, 2 \text{ H}, J = 8.3 \text{ Hz}, \text{H}_2 \text{ or } \text{H}_3), 5.72 \ (ddt, 1 \text{ H}, J_{\text{trans}} = 17.1 \text{ Hz}, J_{\text{cis}} = 10.2 \text{ Hz}, J = 6.2 \text{ Hz}, \text{H}_2''), 5.09 - 5.03 \ (dm, 1 \text{ H}, J_{\text{trans}} = 17.1 \text{ Hz}, \text{H}_3''), 5.03 - 4.99 \ (dm, 1 \text{ H}, J_{\text{cis}} = 10.2 \text{ Hz}, \text{H}_3''), 4.15 \ (ddd, 2 \text{ H}, J = 6.2 \text{ Hz}, J = 1.3 \text{ Hz}, J = 1.3 \text{ Hz}, \text{H}_1''), 2.39 \ (s, 3 \text{ H}, \text{H}_5), 2.29 \ (s, 3 \text{ H}, \text{H}_5).
\]

\[^13\text{C} \text{ NMR}\ (\text{CDCl}_3, 100 \text{ MHz}, \text{ppm}) \delta:\ 143.2 \ (\text{C}_1 \text{ or } \text{C}_4), 137.4 \ (\text{C}_1 \text{ or } \text{C}_4 \text{ or } \text{C}_1' \text{ or } \text{C}_4'), 135.2 \ (\text{C}_1 \text{ or } \text{C}_4 \text{ or } \text{C}_1' \text{ or } \text{C}_4'), 134.2 \ (\text{C}_1 \text{ or } \text{C}_4 \text{ or } \text{C}_1' \text{ or } \text{C}_4'), 132.7 \ (\text{CH}, \text{C}_2''), 129.3 \ (\text{CH}, \text{C}_2 \text{ or } \text{C}_3 \text{ or } \text{C}_3'), 129.2 \ (\text{CH}, \text{C}_2 \text{ or } \text{C}_3 \text{ or } \text{C}_3'), 128.4 \ (\text{CH}, \text{C}_2 \text{ or } \text{C}_3 \text{ or } \text{C}_3), 127.4 \ (\text{CH}, \text{C}_2), 118.4 \ (\text{CH}_2, \text{C}_3''), 53.3 \ (\text{CH}_2, \text{C}_1''), 21.3 \ (\text{CH}_3, \text{C}_5 \text{ or } \text{C}_5'), 20.8 \ (\text{CH}_3, \text{C}_5 \text{ or } \text{C}_5').
\]

**Compound 3g:**

![Chemical Structure](image)

*N*-**allyl-**N-(4-trifluoromethylphenyl)-4-methylbenzenesulfonamide

This compound was obtained from 4-trifluoromethylaniline (645 mg, 4.0 mmol) following the general procedure A then B. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 92/8, thereby obtaining compound 3g (1.46 g, 99 % for two steps).

**Aspect:** Pale yellow oil.

**1H NMR** (CDCl₃, 400 MHz, ppm) δ: 7.55 (d, 2 H, J = 8.3 Hz, H₃), 7.48 (d, 2 H, J = 8.3 Hz, H₂), 7.27 (d, 2 H, J = 8.3 Hz, H₃'), 7.19 (d, 2 H, J = 8.2 Hz, H₂'), 5.72 (ddt, 1 H, J_{trans} = 16.9 Hz, J_{cis} = 10.3 Hz, J = 6.3 Hz, H₂''), 5.12 - 5.05 (dm, 1 H, J_{trans} = 16.9 Hz, H₃''), 5.10 - 5.05 (dm, 1 H, J_{cis} = 10.4 Hz, H₃''), 4.20 (ddd, 2 H, J = 6.2 Hz, J = 1.3 Hz, J = 1.3 Hz, H₁''), 2.43 (s, 3 H, H₅').

**13C NMR** (CDCl₃, 100 MHz, ppm) δ: 143.9 (C₁' or C₄'), 142.4 (C₁), 134.9 (C₁' or C₄), 132.2 (CH, C₂''), 129.9 (q, J = 32.9 Hz, C₄), 129.6 (CH, C₂'), 128.6 (CH, C₂), 127.6 (CH, C₂'), 125.9 (q, CH, J = 3.7 Hz, C₃), 123.8 (q, J = 272.8 Hz, C₅), 119.4 (CH₂, C₃''), 53.1 (CH₂, C₁''), 21.5 (CH₃, C₅).

**19F** {1H} NMR (CDCl₃, 376 MHz, ppm) δ: -62.52 (F₅).

**HRMS (MALDI/TOF, ES⁺, CH₃CN):** m/z calc for C₁₇H₁₈F₃NO₂S [M+H]⁺: 356.0932, m/z found: 356.0934.

**Compound 3h:**

![Chemical Structure](image)

*N*-**allyl-**N-(4-methoxyphenyl)-4-methylbenzenesulfonamide

This compound was obtained from N-(4-methoxyphenyl)-4-methylbenzenesulfonamide (1.11 g, 4.0 mmol) following the general procedure B. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 90/10, thereby obtaining compound 3h (769 mg, 61 %).

**Aspect:** Brown solid.

**Mp:** 53.7 - 54.9 °C.
**1H NMR (CDCl₃, 400 MHz, ppm)** δ: 7.49 (d, 2 H, J = 8.3 Hz, H₂'), 7.25 (d, 2 H, J = 8.1 Hz, H₃'), 6.92 (d, 2 H, J = 9.1 Hz, H₂), 6.79 (d, 2 H, J = 9.1 Hz, H₃), 5.73 (ddt, 1 H, J\text{trans} = 17.1 Hz, J\text{cis} = 10.2 Hz, J = 6.3 Hz, H₂''), 5.09 - 5.03 (dm, 1 H, J\text{trans} = 17.1 Hz, H₃''), 5.06 - 5.02 (dm, 1 H, J\text{cis} = 10.1 Hz, H₃''), 4.14 - 4.11 (dm, 2 H, J = 6.3 Hz, H₁''), 3.79 (s, 3 H, H₅), 2.43 (s, 3 H, H₅').

**13C NMR (CDCl₃, 100 MHz, ppm)** δ: 158.9 (C₄), 143.3 (C₁' or C₄'), 135.5 (C₁' or C₄'), 132.9 (CH, C₂''), 131.6 (C₁), 130.2 (CH, C₂), 129.4 (CH, C₃'), 127.3 (CH, C₂'), 118.7 (CH₂, C₃''), 114.0 (CH, C₃), 55.3 (CH₃, C₅), 53.8 (CH₂, C₁''), 21.5 (CH₃, C₅').


**Compound 3i:**

![Compound 3i](image)

**N-(4-acetylphenyl)-N-allyl-4-methylbenzenesulfonamide**

This compound was obtained from 4-acetylaniline (541 mg, 4.0 mmol) following the general procedure A then B. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 80/20, thereby obtaining compound 3i (1.23 mg, 93 % for two steps).

**Aspect:** Brown solid.

**Mp:** 60.9 - 61.7 °C.

**1H NMR (CDCl₃, 400 MHz, ppm)** δ: 7.88 (d, 2 H, J = 8.8 Hz, H₃), 7.47 (d, 2 H, J = 8.3 Hz, H₂), 7.26 (d, 2 H, J = 7.9 Hz, H₃), 7.17 (d, 2 H, J = 8.8 Hz, H₂), 5.71 (ddt, 1 H, J\text{trans} = 17.1 Hz, J\text{cis} = 10.2 Hz, J = 6.2 Hz, H₂''), 5.12 - 5.06 (dm, 1 H, J\text{trans} = 17.1 Hz, H₃''), 5.09 - 5.04 (dm, 1 H, J\text{cis} = 10.2 Hz, H₃''), 4.21 (dddt, 2 H, J = 1.4 Hz, J = 1.4 Hz, J = 1.4 Hz, H₁''), 2.59 (s, 3 H, H₅), 2.43 (s, 3 H, H₅').

**13C NMR (CDCl₃, 100 MHz, ppm)** δ: 197.2 (C₅), 143.9 (C₁ or C₄ or C₁' or C₄'), 143.5 (C₁ or C₄ or C₁' or C₄'), 135.7 (C₁ or C₄ or C₁' or C₄'), 135.0 (C₁ or C₄ or C₁' or C₄'), 132.3 (CH, C₂''), 129.6 (CH, C₃'), 128.9 (CH, C₃), 128.1 (CH, C₂), 127.6 (CH, C₂), 119.3 (CH₂, C₃''), 52.9 (CH₂, C₁''), 26.6 (CH₃, C₆), 21.6 (CH₃, C₅).

C. HYDROFLUORINATION REACTION

Procedure C: optimized procedure in superacid media

To a mixture of hydrofluoric acid and antimony pentafluoride (8 mL, 3.8 mol% antimony pentafluoride) maintained at -65 °C, was added \( N \)-allyl-\( N \)-arylbenzenesulfonamide derivative. The mixture was magnetically stirred at the same temperature during 10 minutes. The reaction mixture was then neutralized with water-ice-sodium carbonate solution, extracted with dichloromethane (x 3). The combined organic phases were dried over magnesium sulphate, filtered and concentrated in vacuo. Products were isolated by column chromatography over silica gel.

Compound 4b:

\[
\begin{align*}
\text{F} & 2'' \quad 3'' \\
\text{N} & 1' \quad \text{2' or 3'} \\
\text{O} & 4' \quad \text{5} \\
\text{O} & 1 \quad \text{2} \quad \text{3}
\end{align*}
\]

\( N \)-(2-fluoropropyl)-\( N \)-(4-methylphenyl)-4-nitrobenzenesulfonamide

This compound was obtained from \( N \)-allyl-\( N \)-(4-methylphenyl)-4-nitrobenzenesulfonamide 3b (133 mg, 0.400 mmol) following the general procedure C. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 90/10, thereby obtaining compound 4b (47 mg, 33%).

Aspect: Yellow viscous oil.

\(^1\)H NMR (CDCl\(_3\), 400 MHz, ppm) \( \delta \): 8.23 (d, 2 H, \( J = 9.0 \) Hz, \( H_{3'} \)), 7.72 (d, 2 H, \( J = 9.0 \) Hz, \( H_{2'} \)), 7.07 (d, 2 H, \( J = 8.0 \) Hz, \( H_2 \) or \( H_3 \)), 6.87 (d, 2 H, \( J = 8.3 \) Hz, \( H_2 \) or \( H_3 \)), 4.73 - 4.53 (dm, 1 H, \( J = 48.8 \) Hz, \( H_2'' \)), 3.82 (td, 1 H, \( J = 14.4 \) Hz, \( J = 6.7 \) Hz, \( H_{1''} \)), 3.54 (ddd, 1 H, \( J = 25.4 \) Hz, \( J = 14.4 \) Hz, \( J = 3.8 \) Hz, \( H_{1''} \)), 2.28 (s, 3 H, \( H_5 \)), 1.27 (dd, 3 H, \( J = 23.6 \) Hz, \( J = 6.3 \) Hz, \( H_{3''} \)).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz, ppm) \( \delta \): 150.0 (\( C_1 \) or \( C_4 \)), 144.4 (\( C_1' \) or \( C_4' \)), 139.0 (\( C_1 \) or \( C_4 \)), 135.9 (\( C_1' \) or \( C_4' \)), 130.2 (CH, \( C_2 \) or \( C_3 \)), 128.9 (CH, \( C_2 \) or \( C_3 \) or \( C_2' \)), 128.8 (CH, \( C_2 \) or \( C_3 \) or \( C_2' \)), 124.0 (CH, \( C_3' \)), 88.1 (d, \( C_1 \), \( J = 171.4 \) Hz, \( C_{2''} \)), 56.3 (d, \( C_2' \)), 21.1 (d, \( C_3' \)), 18.3 (d, \( C_3' \), \( J = 21.8 \) Hz, \( C_{3''} \)).

\(^{19}\)F \( \{1\} \)H NMR (CDCl\(_3\), 376 MHz, ppm) \( \delta \): -178.28 (\( F_{2''} \)).

HRMS (MALDI/TOF, ES\(^+\), CH\(_3\)CN): m/z calc for \( C_{16}H_{17}FN_2O_2S \) [\( M+H \)^+]: 353.0971, m/z found: 353.0977.
**Compound 4c:**

N-(4-bromophenyl)-N-(2-fluoropropyl)-4-methylbenzenesulfonamide

This compound was obtained from N-allyl-N-(4-bromophenyl)-4-methylbenzenesulfonamide 3c (90 mg, 0.256 mmol) following the general procedure C. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 92/8, thereby obtaining compound 4c (10 mg, 11%).

**Aspect:** Colourless viscous oil.

**1H NMR (CDCl₃, 400 MHz, ppm) δ:** 7.46 (d, 2 H, J = 8.3 Hz, H₉), 7.43 (d, 2 H, J = 8.8 Hz, H₈), 7.26 (d, 2 H, J = 7.9 Hz, H₇), 6.94 (d, 2 H, J = 8.8 Hz, H₆), 4.84 - 4.63 (dm, 1 H, J = 48.7 Hz, H₂''), 3.71 (td, 1 H, J = 14.5 Hz, J = 6.9 Hz, H₁''), 3.63 (ddd, 1 H, J = 23.1 Hz, J = 14.4 Hz, J = 4.2 Hz, H₁''), 2.42 (s, 3 H, H₅').

**13C NMR (CDCl₃, 100 MHz, ppm) δ:** 143.9 (C₁' or C₄'), 139.0 (C₁'), 135.0 (C₁' or C₄'), 132.3 (CH, C₃), 130.5 (CH, C₂), 129.6 (CH, C₃), 127.6 (CH, C₂'), 122.0 (C₄), 88.8 (d, CH, J = 170.8 Hz, C₂''), 55.8 (d, CH₂, J = 24.3 Hz, C₁''), 21.5 (CH₃, C₅'), 18.4 (d, CH₃, J = 21.7 Hz, C₃').

**19F (1H) NMR (CDCl₃, 376 MHz, ppm) δ:** -177.81 (F₂'').

**HRMS (MALDI/TOF, ES⁺, CH₃CN):** m/z calc for C₁₆H₁₇BrFNO₂S [M+H]⁺: 386.0226, m/z found: 386.0225.

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**Compound 4d:**

N-(4-chlorophenyl)-N-(2-fluoropropyl)-4-methylbenzenesulfonamide

This compound was obtained from N-allyl-N-(4-chlorophenyl)-4-methylbenzenesulfonamide 3d (103 mg, 0.320 mmol) following the general procedure C. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 92/8, thereby obtaining compound 4d (53 mg, 48%).

**Aspect:** Colourless viscous oil.
$^1$H NMR (CDCl$_3$, 400 MHz, ppm) δ: 7.46 (d, 2 H, J = 8.3 Hz, H$_2'$), 7.28 - 7.24 (m, 4 H, H$_3$ and H$_3'$), 7.01 (d, 2 H, J = 8.8 Hz, H$_2$), 4.84 - 4.63 (dm, 1 H, J = 48.6 Hz, H$_{2''}$), 3.72 (td, 1 H, J = 14.5 Hz, J = 6.9 Hz, H$_1''$), 3.63 (ddd, 1 H, J = 23.3 Hz, J = 14.4 Hz, J = 4.2 Hz, H$_1''$), 2.43 (s, 3 H, H$_5'$), 1.34 (dd, 3 H, J = 23.7 Hz, J = 6.3 Hz, H$_3''$).

$^{13}$C NMR (CDCl$_3$, 100 MHz, ppm) δ: 143.8 (C$_1'$ or C$_4'$), 138.4 (C$_1$ or C$_4$), 135.0 (C$_1'$ or C$_4'$), 133.9 (C$_1$ or C$_4$), 130.2 (CH, C$_2$), 129.5 (CH, C$_3$ or C$_3'$), 129.3 (CH, C$_3$ or C$_3'$), 127.6 (CH, C$_2$), 88.8 (d, CH, J = 170.9 Hz, C$_{2''}$), 55.9 (d, CH, J = 24.3 Hz, C$_{1''}$), 21.5 (CH, C$_{5'}$), 18.4 (d, CH, J = 21.9 Hz, C$_{3''}$).

$^{19}$F {1H} NMR (CDCl$_3$, 376 MHz, ppm) δ: -112.77 (F$_4$), -178.03 (F$_{2''}$).

HRMS (MALDI/TOF, ES$^+$, CH$_3$CN): m/z calc for C$_{16}$H$_{17}$ClFNO$_2$S [M+Na]$^+$: 364.0550, m/z found: 364.0553.

**Compound 4e:**

![Compound structure](image)

$\text{N-(4-fluorophenyl)-N-(2-fluoropropyl)-4-methylbenzenesulfonamide}$

This compound was obtained from $\text{N}$-allyl-$\text{N}$-(4-fluorophenyl)-4-methylbenzenesulfonamide 3e (191 mg, 0.625 mmol) following the general procedure C. The reaction crude was filtered over silica gel with the eluent ethyl acetate, thereby obtaining compound 4e (185 mg, 97 %).

**Aspect:** Brown solid.

**MP:** 88.9 - 90.3 °C.

$^1$H NMR (CDCl$_3$, 400 MHz, ppm) δ: 7.47 (d, 2 H, J = 8.3 Hz, H$_2'$), 7.26 (d, 2 H, J = 7.9 Hz, H$_3$), 6.96 - 7.06 (m, 4 H, H$_2$ and H$_3$), 4.84 - 4.63 (dm, 1 H, J = 48.6 Hz, H$_{2''}$), 3.72 (td, 1 H, J = 14.4 Hz, J = 7.0 Hz, H$_1''$), 3.63 (ddd, 1 H, J = 23.4 Hz, J = 14.4 Hz, J = 4.2 Hz, H$_1''$), 2.43 (s, 3 H, H$_5'$), 1.35 (dd, 3 H, J = 23.7 Hz, J = 6.3 Hz, H$_3''$).

$^{13}$C NMR (CDCl$_3$, 100 MHz, ppm) δ: 161.8 (d, J = 248.5 Hz, C$_4$), 143.7 (C$_1'$ or C$_4'$), 135.7 (d, J = 3.1 Hz, C$_1$), 135.0 (C$_1'$ or C$_4'$), 130.8 (d, CH, J = 8.8 Hz, C$_2$), 129.5 (CH, C$_3$), 127.6 (CH, C$_2$), 115.9 (d, CH, J = 22.7 Hz, C$_3$), 88.6 (d, CH, J = 170.8 Hz, C$_{2''}$), 56.0 (d, CH, J = 24.2 Hz, C$_{1''}$), 21.4 (CH$_3$, C$_{3''}$), 18.3 (d, CH$_3$, J = 21.8 Hz, C$_{3''}$).

$^{19}$F {1H} NMR (CDCl$_3$, 376 MHz, ppm) δ: -112.77 (F$_4$), -178.03 (F$_{2''}$).

HRMS (MALDI/TOF, ES$^+$, CH$_3$CN): m/z calc for C$_{18}$H$_{17}$F$_2$NO$_2$S [M+H]$^+$: 326.1026, m/z found: 326.1024.
**Compound 4f:**

![Chemical Structure](image)

**N-(2-fluoropropyl)-N-(4-methylphenyl)-4-methylbenzenesulfonamide**

This compound was obtained from *N*-allyl-4-methyl-*N*-(4-methylphenyl)benzenesulfonamide 3f (150 mg, 0.498 mmol) following the general procedure C. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 92/8, thereby obtaining compound 4f (52 mg, 33%).

**Aspect:** white solid.

**MP:** 62.9 - 63.7 °C.

**1H NMR** (CDCl$_3$, 400 MHz, ppm) $\delta$: 7.48 (d, 2 H, $J = 8.3$ Hz, H$_2'$), 7.24 (d, 2 H, $J = 8.0$ Hz, H$_3'$), 7.10 (d, 2 H, $J = 8.0$ Hz, H$_3$), 6.94 (d, 2 H, $J = 8.3$ Hz, H$_2$), 4.81 - 4.60 (dm, 1 H, $J = 48.5$ Hz, H$2''$), 3.75 (td, 1 H, $J = 14.1$ Hz, H$_1''$), 3.60 (ddd, 1 H, $J = 22.4$ Hz, $J = 14.2$ Hz, H$1''$), 2.42 (s, 3 H, H$_5'$), 2.33 (s, 3 H, H$_5$), 1.34 (dd, 3 H, $J = 23.7$ Hz, $J = 6.3$ Hz, H$_3''$).

**13C NMR** (CDCl$_3$, 100 MHz, ppm) $\delta$: 143.5 (C$_1'$ or C$_4'$), 138.1 (C$_1$ or C$_4$), 137.1 (C$_1$ or C$_4$), 135.4 (C$_1'$ or C$_4'$), 129.7 (CH, C$_3$), 129.4 (CH, C$_3$), 128.7 (CH, C$_2$), 127.7 (CH, C$_2$), 88.6 (d, CH, $J = 170.4$ Hz, C$_2''$), 55.9 (d, CH, $J = 25.1$ Hz, C$_1''$), 21.5 (CH$_3$, C$_5$ or C$_9$), 21.1 (CH$_3$, C$_5$ or C$_9$), 18.5 (d, CH$_3$, $J = 21.7$ Hz, C$_3''$).

**19F (1H) NMR** (CDCl$_3$, 376 MHz, ppm) $\delta$: -178.19 (F$_2$-).

**HRMS** (MALDI/TOF, ES$^+$, CH$_3$CN): m/z calc for C$_{17}$H$_{20}$FNO$_2$S [M+Na]$^+$: 344.1096, m/z found: 344.1095.

**Compound 4g:**

![Chemical Structure](image)

**N-(2-fluoropropyl)-4-methyl-N-(4-trifluoromethylphenyl)benzenesulfonamide**

This compound was obtained from *N*-allyl-4-methyl-*N*-(4-trifluoromethylphenyl)benzenesulfonamide 3g (91 mg, 0.256 mmol) following the general procedure C. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 92/8, thereby obtaining compound 4g (93 mg, 97%).

**Aspect:** White solid.

**MP:** 89.4 - 90.0 °C.
**1H NMR (CDCl₃, 400 MHz, ppm)**: δ: 7.57 (d, 2 H, J = 8.4 Hz, H₃), 7.46 (d, 2 H, J = 8.3 Hz, H₂), 7.27 (d, 2 H, J = 8.0 Hz, H₃'), 7.22 (d, 2 H, J = 8.3 Hz, H₂'), 4.87 - 4.66 (dm, 1 H, J = 48.6 Hz, H₂''), 3.80 - 3.64 (m, 2 H, H₁''), 2.43 (s, 3 H, H₅'), 1.35 (dd, 3 H, J = 23.6 Hz, J = 6.3 Hz, H₃'').

**13C NMR (CDCl₃, 100 MHz, ppm)**: δ: 144.1 (C₁ or C₁' or C₄'), 143.3 (C₁ or C₁' or C₄'), 134.9 (C₁' or C₄'), 129.9 (q, J = 32.9 Hz, C₄), 129.6 (CH, C₃'), 129.1 (CH, C₂), 127.5 (CH, C₂'), 126.2 (q, CH, J = 3.7 Hz, C₃), 123.7 (q, J = 272.2 Hz, C₅), 88.9 (d, CH₂, J = 171.0 Hz, C₂''), 55.7 (d, CH₂, J = 24.0 Hz, C₁''), 21.5 (CH₃, C₅'), 18.3 (d, CH₃, J = 21.9 Hz, C₃'').

**19F {1H} NMR (CDCl₃, 376 MHz, ppm)**: δ: -62.55 (F₅), -177.68 (F₂'').

**HRMS (MALDI/TOF, ES⁺, CH₃CN)**: m/z calc for C₁₇H₁₇F₄NO₂S [M+H]⁺: 376.0994, m/z found: 376.0997.

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**Compound 4h:**

![Compound 4h structure](image)

**N-(2-fluoropropyl)-N-(4-methoxyphenyl)-4-methylbenzenesulfonamide**

This compound was obtained from *N*-allyl-*N*-(4-methoxyphenyl)-4-methylbenzenesulfonamide 3h (124 mg, 0.391 mmol) following the general procedure C. The reaction crude was filtered over silica gel with the eluent ethyl acetate, thereby obtaining compound 4h (127 mg, 96%).

**Aspect:** Colourless viscous oil.

**1H NMR (CDCl₃, 400 MHz, ppm)**: δ: 7.47 (d, 2 H, J = 8.3 Hz, H₂), 7.23 (d, 2 H, J = 8.0 Hz, H₃), 6.95 (d, 2 H, J = 9.0 Hz, H₂), 6.79 (d, 2 H, J = 9.0 Hz, H₃), 4.79 - 4.59 (dm, 1 H, J = 48.6 Hz, H₂''), 3.76 (s, 3 H, H₂'), 3.73 (td, 1 H, J = 14.1 Hz, J = 7.1 Hz, H₁''), 3.57 (ddd, 1 H, J = 23.2 Hz, J = 14.2 Hz, J = 4.4 Hz, H₁''), 2.40 (s, 3 H, H₅'), 1.32 (dd, 3 H, J = 23.7 Hz, J = 6.3 Hz, H₃'').

**13C NMR (CDCl₃, 100 MHz, ppm)**: δ: 159.0 (C₄), 143.4 (C₁' or C₄'), 135.3 (C₁' or C₄'), 132.1 (C₁'), 130.1 (CH, C₂), 129.3 (CH, C₃'), 127.6 (CH, C₂'), 114.2 (CH, C₃), 88.4 (d, CH, J = 170.5 Hz, C₂''), 56.1 (d, CH₂, J = 24.9 Hz, C₁''), 55.3 (CH₃, C₅'), 21.4 (CH₃, C₅'), 18.3 (d, CH₃, J = 21.9 Hz, C₃'').

**19F {1H} NMR (CDCl₃, 376 MHz, ppm)**: δ: -178.28 (F₂'').

**HRMS (MALDI/TOF, ES⁺, CH₃CN)**: m/z calc for C₁₇H₂₀FNO₂S [M+Na]⁺: 360.1046, m/z found: 360.1042.
**Compound 4i:**

\[
\begin{array}{c}
\text{N} \text{-(4-acetylphenyl)-N-(2-fluoropropyl)-4-methylbenzenesulfonamide} \\
\end{array}
\]

This compound was obtained from \(N\)-\(4\)-\(acetylphenyl\)-\(N\)-allyl-4-methylbenzenesulfonamide 3i (110 mg, 0.334 mmol) following the general procedure C. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 80/20, thereby obtaining compound 4i (104 mg, 89\%).

**Aspect:** White solid.

**MP:** 92.9 - 94.3 °C.

\(^{1}\)H NMR (CDCl\(_3\), 400 MHz, ppm) \(\delta\): 7.89 (d, 2 H, \(J = 8.7 \text{ Hz}\), \(H_3\)), 7.43 (d, 2 H, \(J = 8.3 \text{ Hz}\), \(H_2\)), 7.24 (d, 2 H, \(J = 8.0 \text{ Hz}\), \(H_3\)), 7.19 (d, 2 H, \(J = 9.0 \text{ Hz}\), \(H_2\)), 4.85 - 4.65 (dm, 1 H, \(J = 48.5 \text{ Hz}\), \(H_{2''}\)), 3.76 (td, 1 H, \(J = 14.6 \text{ Hz}, J = 6.7 \text{ Hz}\), \(H_{1''}\)), 3.69 (ddd, 1 H, \(J = 22.8 \text{ Hz}, J = 14.5 \text{ Hz}, J = 4.2 \text{ Hz}\), \(H_{1''}\)), 2.58 (s, 3 H, \(H_6\)), 2.41 (s, 3 H, \(H_{5'}\)), 1.34 (dd, 3 H, \(J = 23.7 \text{ Hz}, J = 6.3 \text{ Hz}\), \(H_{3''}\)).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz, ppm) \(\delta\): 197.1 (\(C_5\)), 144.3 (\(C_1 \text{ or } C_4 \text{ or } C_1' \text{ or } C_4'\)), 143.0 (\(C_1 \text{ or } C_4 \text{ or } C_1' \text{ or } C_4'\)), 136.1 (\(C_1 \text{ or } C_4 \text{ or } C_1' \text{ or } C_4'\)), 134.9 (\(C_1 \text{ or } C_4 \text{ or } C_1' \text{ or } C_4'\)), 129.6 (CH, \(C_3\)), 129.2 (CH, \(C_3\)), 128.6 (CH, \(C_2\)), 127.5 (CH, \(C_2\)), 88.9 (d, CH, \(J = 171.0 \text{ Hz}\), \(C_{2''}\)), 55.5 (d, CH, \(J = 24.3 \text{ Hz}\), \(C_{1''}\)), 26.6 (CH, \(C_6\)), 21.5 (CH, \(C_9\)), 18.4 (d, CH, \(J = 21.9 \text{ Hz}\), \(C_{3''}\)).

\(^{19}\)F {\(^1\)H} NMR (CDCl\(_3\), 376 MHz, ppm) \(\delta\): -177.52 (\(F_{2''}\)).

HRMS (MALDI/TOF, ES\(^+\), CH\(_3\)CN): m/z calc for \(C_{18}H_{20}FNO_3S\) [M+H]\(^+\): 350.1226, m/z found: 350.1230.
D. NMR SPECTRA:

Compound 3a: $^1$H NMR
Compound 3a: $^{13}$C NMR
Compound 3b: $^1\text{H NMR}$
Compound 3b: $^{13}$C NMR
Compound 3c: $^1$H NMR
Compound 3c: $^{13}$C NMR
Compound 3d: $^1$H NMR
Compound 3d: $^{13}$C NMR
Compound 3e: $^1$H NMR
Compound 3e: $^{13}$C NMR
Compound 3f: $^1$H NMR
Compound 3f: $^{13}$C NMR
Compound 3g: $^1$H NMR
Compound 3g: $^{13}$C NMR
Compound 3h: $^1$H NMR
Compound 3h: $^1$H NMR
Compound 3i: $^1$H NMR
Compound 3i: $^{13}$C NMR
Compound 4b: $^1$H NMR
Compound 4b: $^{13}$C NMR
Compound 4c: $^1$H NMR
Compound 4c: $^{13}$C NMR
Compound 4d: $^1$H NMR
Compound 4d: $^{13}$C NMR
Compound 4e: $^1$H NMR
Compound 4e: $^{13}$C NMR
Compound 4f: $^1$H NMR
Compound 4f: $^{13}$C NMR
Compound 4g: $^1$H NMR
Compound 4g: $^{13}$C NMR
Compound 4h: $^1$H NMR
Compound 4h: $^{13}$C NMR
Compound 4i: $^1$H NMR
Compound 4i: $^{13}$C NMR
A. CA INHIBITION ASSAY

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO\(_2\) hydration activity.\(^1\) Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na\(_2\)SO\(_4\) (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO\(_2\) hydration reaction for a period of 10-100 s. The CO\(_2\) concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5-10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min – 6 h at room temperature (15 min) or 4 °C (6h) prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3, as reported earlier,\(^2\) and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.\(^3,4\)

References


