Supplementary Information for

Synthesis of novel selenides bearing benzenesulfonamide derivatives as carbonic anhydrase I, II, IV, VII and IX inhibitors

Andrea Angeli,[a] Damiano Tanini,[b] Antonella Capperucci,[b] Claudiu T. Supuran[a]

[a] *Department of University of Florence, NEUROFARBA Dept., Sezione di Scienze Farmaceutiche, Via Ugo Schiff 6, 50019 Sesto Fiorentino (Florence), Italy*

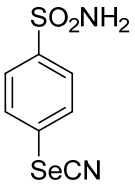
[b] *Department of University of Florence, Department of Chemistry "Ugo Schiff", Via della Lastruccia 13, I-50019 Sesto Fiorentino, Italy.*

Contents

1. General

All reactions were carried out in an oven-dried glassware under inert atmosphere (N_2). Ethanol was dried using a solvent purification system (Pure-Solv™). All commercial materials were used as received without further purification. Flash column chromatography purifications were performed with Silica gel 60 (230-400 mesh). Thin layer chromatography was performed with TLC plates Silica gel 60 F_{254}. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 with Varian Gemini 200, Mercury 400, and Bruker 400 Ultrashield spectrometers operating at 200 and 400 MHz (for ^{1}H), 50 and 100 MHz (for ^{13}C), and 76 MHz (for ^{77}Se). NMR signals were referenced to nondeuterated residual solvent signals (7.26 and 2.50 ppm for ^{1}H, 77.0 and 40.5 ppm for ^{13}C). $(PhSe)_2$ was used as an external reference for ^{77}Se NMR (δ = 461 ppm). ^{1}H NMR data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, ap d = apparent doublet, m = multiplet, dd = doublet of doublet, bs = broad singlet, bd = broad doublet, ecc.), coupling constant (*J*), and assignment.

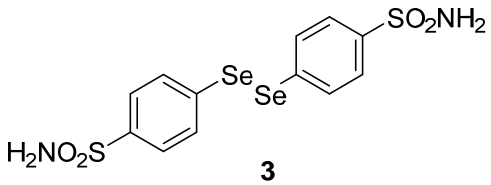
2. Preparation 4-selenocyanatobenzenesulfonamide 2



2

A suspension of 4-Aminonenzenesulfonamide **1** (1,72 g, 10 mmol) in H_2O (6 mL) with HCl (11 mL, 32 %) was cooled down to -5°C. Then, an aqueous solution of $NaNO_2$ (1.2 eq) was added dropwise and the mixture was kept stirring at the same temperature until a persistent pale yellow solution was formed (5–10 min). The resulting diazonium salt, kept at -5°C, was added KSeCN (1.2 eq). The reaction solution was stirred for 2 hours at the same temperature. The product was filtered off, washed with H_2O, dried under vacuo, and purified by flash column chromatography eluting with 1:1 mixture of hexane/ethyl acetate. (1.79 g, 83%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.95 (2H, d, *J*=8.6 Hz), 7.89 (2H, d, *J*=8.6 Hz), 7.52 (2H, bs, N**H$_2$**, exchange with D_2O). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 145.5, 134.1, 129.8, 127.9, 105.9. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 340.0. **MS** (ESI negative) *m/z* (%):261 $[M-H]^-$; ($[M-H]^-$ 260.93 required).

3. Preparation 4,4'-diselanediyldibenzenesulfonamide 3



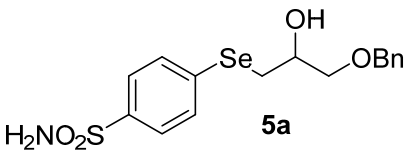
3

$NaBH_4$ (3 mmol) was added in small portions with caution to a solution of 4-selenocyanatobenzenesulfonamide **2** (3 mmol) in absolute ethanol (40 mL). The mixture was stirred at room temperature for 2 h. The solvents were removed under vacuum by rotary evaporation and the residue was treated with water. The mixture was extracted with ethyl acetate, dried with anhydrous Na_2SO_4, and purified by crystallization from EtOH. (529 mg, 75%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.87 (2H, d, *J*=8.3 Hz), 7.79 (2H, d, *J*=8.3 Hz), 7.43 (2H, bs, N**H$_2$**, exchange with D_2O). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 144.3, 135.2, 131.5, 127.5. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 446.7. **MS** (ESI negative) *m/z* (%):471 $[M-H]^-$; ($[M-H]^-$ 470.86 required).

4. General Procedure for the preparation of β-hydroxy selenide 5a-g from diselenide 3

$NaBH_4$ (23 mg, 0.60 mmol, 3.0 eq.) was portionwise added to a solution of 4,4'-diselanediyldibenzenesulfonamide **3** (94 mg, 0.20 mmol, 1.0 eq.) in EtOH (2 mL) at 0°C under inert atmosphere (N_2). After 30 min, the epoxide **4** (0.36 mmol, 1.8 eq.) was slowly added and the reaction mixture was stirred at room temperature for 2 h, until complete consumption of the starting material was observed by TLC. The reaction was quenched by addition of saturated aq. NH_4Cl (2 mL) and diluted with EtOAc (5 mL), The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield β-hydroxyselenides (**5**) bearing benzenesulfonamide moiety.

4-((3-(Benzyloxy)-2-hydroxypropyl)selanyl)benzenesulfonamide 5a

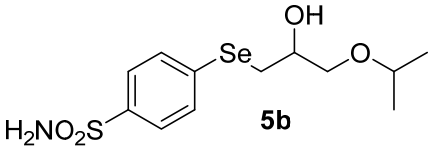


5a

Following the general procedure, 4,4'-diselanediyldibenzenesulfonamide **3** (94 mg, 0.20 mmol) and 2-((benzyloxy)methyl)oxirane **4a** (59 mg, 0. 36 mmol) gave after flash chromatography (petroleum ether/EtOAc 1:1) **5a** (128 mg, 89%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.63 (1H, bs, OH), 3.12 (1H, dd, *J* = 6.8, 12.8 Hz, C**H$_a$**H$_b$Se), 3.19 (1H, dd, *J* = 5.6, 12.8 Hz, CH$_a$**H$_b$**Se), 3.54 (1H, dd, *J* =

5.9, 9.5 Hz, C**H**$_a$H$_b$O), 3.59 (1H, dd, *J* = 4.2, 9.5 Hz, CH$_a$**H**$_b$O), 3.96-4.04 (1H, m, C**H**OH), 4.53 (2H, ap s, CH$_2$Ph), 4.82 (2H, bs, NH$_2$), 7.29-7.40 (5H, m), 7.59 (2H, d, *J* = 8.5 Hz), 7.76 (2H, d, *J* = 8.5 Hz). **^{13}C NMR** (100 MHz, CDCl$_3$) δ (ppm): 31.1 (CH$_2$Se), 69.5, 72.8, 73.5, 126.9, 127.8, 128.0, 128.5, 131.3, 137.5, 137.7, 139.9. **MS** (ESI positive) *m/z* (%): 401 [M+H]$^+$, (100). Elemental analysis: $C_{16}H_{19}NO_4SSe$ Calcd. C 48.00%, H 4.78%, N 3.50%. Found: C 48.11%, H 4.74%, N 3.46%.

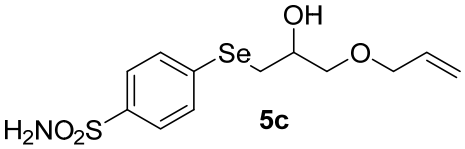
4-((2-Hydroxy-3-isopropoxypropyl)selanyl)benzenesulfonamide 5b



5b

Following the general procedure, 4,4'-diselanediyldibenzenesulfonamide **3** (94 mg, 0.20 mmol) and 2-(isopropoxymethyl)oxirane **4b** (42 mg, 0. 36 mmol) gave after flash chromatography (petroleum ether/EtOAc 1:1) **5b** (121 mg, 96%). **^{1}H NMR** (400 MHz, CDCl$_3$) δ (ppm): 1.15 (6H, d, *J* = 6.1 Hz), 2.78 (1H, bs, OH), 3.11 (1H, dd, *J* = 6.7, 12.7 Hz, C**H**$_a$H$_b$Se), 3.16 (1H, dd, *J* = 5.8, 12.7 Hz, CH$_a$**H**$_b$Se), 3.45 (1H, dd, *J* = 6.0, 9.4 Hz, C**H**$_a$H$_b$O), 3.53 (1H, dd, *J* = 4.1, 9.4 Hz, CH$_a$**H**$_b$O), 3.59 (1H, ept, *J* = 6.1 Hz, C**H**(CH$_3$)$_2$), 3.89-3.98 (1H, m, C**H**OH), 5.09 (2H, bs, NH$_2$), 7.58 (2H, d, *J* = 8.5 Hz), 7.75 (2H, d, *J* = 8.5 Hz). **^{13}C NMR** (100 MHz, CDCl$_3$) δ (ppm): 21.9, 22.0, 30.9 (CH$_2$Se), 69.6, 70.7, 72.4, 126.8, 131.2, 137.9, 139.9. **MS** (ESI positive) *m/z* (%): 375.8 [M+Na]$^+$, (100) ([M+Na]$^+$ 375.31 required). Elemental analysis: $C_{12}H_{19}NO_4SSe$ Calcd. C 40.91%, H 5.44%, N 3.98%. Found: C 40.82%, H 5.49%, N 3.94%.

4-((3-(Allyloxy)-2-hydroxypropyl)selanyl)benzenesulfonamide 5c

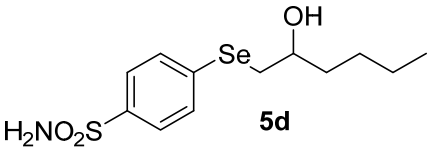


5c

Following the general procedure, 4,4'-diselanediyldibenzenesulfonamide **3** (71 mg, 0.15 mmol) and 2-((allyloxy)methyl)oxirane **4c** (31 mg, 0. 27 mmol) gave after flash chromatography (petroleum ether/EtOAc 7:3) **5c** (70 mg, 74%). **^{1}H NMR** (400 MHz, CDCl$_3$) δ (ppm) 2.71 (1H, bs, OH), 3.12 (1H, dd, *J* = 6.8, 12.8 Hz, C**H**$_a$H$_b$Se), 3.18 (1H, dd, *J* = 5.7, 12.8 Hz, CH$_a$**H**$_b$Se), 3.49 (1H, dd, *J* = 6.0, 9.5 Hz, C**H**$_a$H$_b$O), 3.55 (1H, dd, *J* = 4.0, 9.5 Hz, CH$_a$**H**$_b$O), 3.94-4.06 (3H, m, C**H$_2$**CH=CH$_2$ overlapped wih C**H**OH), 5.04 (2H, bs, NH$_2$), 5.19-5.32 (2H, m), 5.83-5.93 (1H, m), 7.59 (2H, d, *J* = 8.2 Hz), 7.77 (2H, d, *J* = 8.2 Hz). **^{13}C NMR** (100 MHz, CDCl$_3$) δ (ppm): 31.0 (CH$_2$Se), 69.5, 72.3, 72.7, 117.6, 126.9, 131.2, 134.1, 137.7, 140.0. **MS** (ESI positive) *m/z* (%): 373 [M+Na]$^+$, (100).

Elemental analysis: $C_{12}H_{17}NO_4SSe$ Calcd. C 41.15%, H 4.89%, N 4.00%. Found: C 41.07%, H 4.94%, N 4.05%.

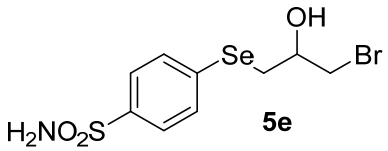
4-((2-Hydroxyhexyl)selanyl)benzenesulfonamide 5d



5d

Following the general procedure, 4,4'-diselanediyldibenzenesulfonamide **3** (94 mg, 0.20 mmol) and 2-butyloxirane **4d** (36 mg, 0. 36 mmol) gave after flash chromatography (petroleum ether/EtOAc 1:1) **5d** (82 mg, 68%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) 0.92 (3H, t, *J* = 7.1 Hz), 1.29-1.49 (4H, m), 1.54-1.63 (2H, m), 1.97. (1H, bs, OH), 3.03 (1H, dd, *J* = 8.2, 12.8 Hz, C**H$_a$**H$_b$Se), 3.23 (1H, dd, *J* = 3.7, 12.8 Hz, CH$_a$**H$_b$**Se), 3.76-3.82 (1H, m, C**H**OH), 5.07 (2H, bs, NH_2), 7.61 (2H, d, *J* = 8.5 Hz), 7.78 (2H, d, *J* = 8.5 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 14.0, 22.6, 27.9, 36.2, 36.6, 70.3, 126.9, 131.5, 137.6, 140.0. **MS** (ESI positive) *m/z* (%): 337 $[M+H]^+$, (100). Elemental analysis: $C_{12}H_{19}NO_3SSe$ Calcd. C 42.86%, H 5.69%, N 4.16%. Found: C 42.73%, H 5.76%, N 4.21%.

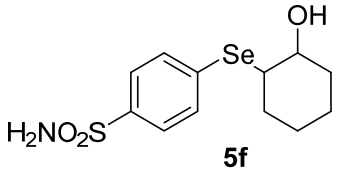
4-((3-Bromo-2-hydroxypropyl)selanyl)benzenesulfonamide 5e



5e

Following the general procedure, 4,4'-diselanediyldibenzenesulfonamide **3** (141 mg, 0.30 mmol) and 2-(bromomethyl)oxirane **4e** (74 mg, 0. 54 mmol) gave after flash chromatography (petroleum ether/EtOAc 3:2) **5e** (189 mg, 94%). **^{1}H NMR** (400 MHz, DMSO-*d$_6$*) δ (ppm) 3.15 (1H, dd, *J* = 7.1, 12.4 Hz, C**H$_a$**H$_b$Se), 3.24 (1H, dd, *J* = 5.1, 12.4 Hz, CH$_a$**H$_b$**Se), 3.54 (1H, dd, *J* = 5.6, 10.2 Hz, C**H$_a$**H$_b$O), 3.61 (1H, dd, *J* = 4.7, 10.2 Hz, CH$_a$**H$_b$**O), 3.85-3.92 (1H, m, C**H**OH), 5.68 (1H, d, *J* = 5.3 Hz, OH), 7.34 (2H, bs, NH_2), 7.65 (2H, d, *J* = 8.6 Hz), 7.69 (2H, d, *J* = 8.6 Hz). **^{13}C NMR** (100 MHz, DMSO-*d$_6$*) δ (ppm): 32.8, 39.8, 70.2, 127.1, 131.3, 137.1, 142.8. **MS** (ESI negative) *m/z* (%): 372 $[M-H]^-$, (100). Elemental analysis: $C_9H_{12}BrNO_3SSe$ Calcd. C 28.97%, H 3.24%, N 3.75%. Found: C 29.02%, H 3.19%, N 3.69%.

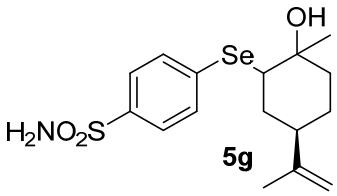
4-((2-Hydroxycyclohexyl)selanyl)benzenesulfonamide 5f



5f

Following the general procedure, 4,4'-diselanediyldibenzenesulfonamide **3** (71 mg, 0.15 mmol) and 7-oxabicyclo[4.1.0]heptane **4f** (26 mg, 0. 27 mmol) gave after flash chromatography (petroleum ether/EtOAc 1:1) **5f** (37 mg, 41%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) 1.25-1.37 (4H, m), 1.63-1.72 (1H, m), 1.74-1.83 (1H, m), 2.12-2.26 (2H, m), 2.62 (1H, bs, OH), 3.05-3.12 (1H, m, CHSe), 3.40-3.48 (1H, m, CHO), 4.91 (2H, bs, NH_2), 7.70 (2H, d, J = 8.4 Hz), 7.80 (2H, d, J = 8.4 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 24.4, 26.8, 33.5, 34.4, 53.9, 73.1, 126.6, 134.6, 138.2, 141.0. **MS** (ESI positive) *m/z* (%): 357 $[M+Na]^+$, (100). Elemental analysis: $C_{12}H_{17}NO_3SSe$ Calcd. C 43.12%, H 5.13%, N 4.19%. Found: C 43.05%, H 5.19%, N 4.23%.

4-(((5*R*)-2-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexyl)selanyl)benzenesulfonamide 5g



5g

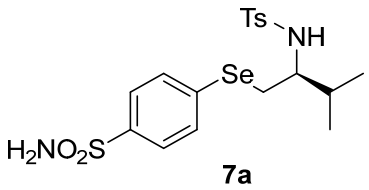
Following the general procedure, 4,4'-diselanediyldibenzenesulfonamide **3** (71 mg, 0.15 mmol) and (4*R*)-1-methyl-4-(prop-1-en-2-yl)-7-oxabicyclo[4.1.0]heptane **4g** (41 mg, 0. 27 mmol) gave after flash chromatography (petroleum ether/EtOAc 2:1) **5g** (51 mg, 56%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) 1.39 (3H, s), 1.63-1.66 (1H, m), 1.63-1.84 (1H, OH partially overlapped), 1.69 (3H, s), 1.72-1.90 (4H, m), 2.23-2.35 (2H, m), 3.54-3.59 (1H, m, CHSe), 4.72 (2H, ap d, J = 14.7 Hz), 5.14 (2H, bs, NH_2), 7.64 (2H, d, J = 8.3 Hz), 7.77 (2H, d, J = 8.3 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 21.3, 26.1, 29.6, 33.8, 35.4, 39.9, 54.1, 72.5, 109.5, 126.8, 132.8, 138.1, 140.2, 148.4. **MS** (ESI positive) *m/z* (%): 411 $[M+Na]^+$, (100). Elemental analysis: $C_{16}H_{23}NO_3SSe$ Calcd. C 49.48%, H 5.97%, N 3.61%. Found: C 49.36%, H 6.04%, N 3.65%.

5. General Procedure for the preparation of *N*- protected β-amino selenide 7a-c

$NaBH_4$ (23 mg, 0.60 mmol, 3.0 eq.) was portionwise added to a solution of 4,4'-diselanediyldibenzenesulfonamide **3** (94 mg, 0.20 mmol, 1.0 eq.) in EtOH (2 mL) at 0°C under inert atmosphere (N_2). After 30 min, a solution of aziridine **6** (0.36 mmol, 1.8 eq.) in THF (1 mL) was slowly added and the reaction mixture was stirred at room temperature for 12 h. The reaction was quenched by addition of saturated aq. NH_4Cl (2 mL) and diluted with EtOAc (5 mL), The layers

were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield *N*-protected β-aminoselenides **7-ac** bearing benzenesulfonamide moiety.

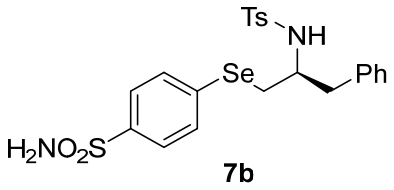
(*S*)-4-Methyl-*N*-(3-methyl-1-((4-sulfamoylphenyl)selanyl)butan-2-yl)benzenesulfonamide 7a



7a

Following the general procedure, 4,4'-diselanediyldibenzenesulfonamide **3** (71 mg, 0.15 mmol) and (*S*)-2-isopropyl-1-tosylaziridine **6a** (65 mg, 0. 27 mmol) gave after flash chromatography (petroleum ether/EtOAc 3:2) **7a** (94 mg, 73%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm) 0.71 (6H, d, J = 6.8 Hz), 1.81-1.93 (1H, m), 2.36 (3H, s), 2.81 (1H, dd, J = 6.1, 12.2 Hz, $CH_a\mathbf{H_b}Se$), 3.03 (1H, dd, J = 6.9, 12.2 Hz, $CH_a\mathbf{H_b}Se$), 3.07-3.15 (1H, m, C**H**NH), 7.31 (2H, d, J = 8.0 Hz), 7.36 (2H, bs, NH_2), 7.39 (2H, d, J = 8.4 Hz), 7.61 (2H, d, J = 8.0 Hz), 7.64 (2H, d, J = 8.4 Hz), 7.71 (1H, bd, J = 7.8 Hz, NH). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 12.8, 19.6, 21.9, 31.3, 31.6, 58.9, 127.1, 127.5, 130.3, 131.5, 136.4, 139.6, 143.0, 143.3. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 268.8. **MS** (ESI positive) *m/z* (%): 476 $[M+H]^+$, (100). Elemental analysis: $C_{18}H_{24}N_2O_4S_2Se$ Calcd. C 45.47%, H 5.09%, N 5.89%. Found: C 45.34%, H 5.15%, N 5.93%.

(*S*)-4-methyl-*N*-(1-phenyl-3-((4-sulfamoylphenyl)selanyl)propan-2-yl)benzenesulfonamide 7b

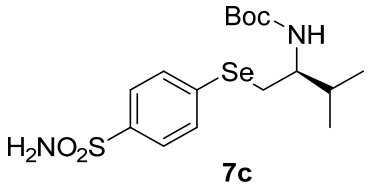


7b

Following the general procedure, 4,4'-diselanediyldibenzenesulfonamide **3** (36 mg, 0.1 mmol) and (*S*)-2-benzyl-1-tosylaziridine **6b** (52 mg, 0. 18 mmol) gave after flash chromatography (petroleum ether/EtOAc 3:2) **7b** (65 mg, 69%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm) 2.33 (3H, s), 2.66 (1H, dd, J = 6.9, 13.5 Hz, $C\mathbf{H_a}H_bPh$), 2.82 (1H, dd, J = 6.4, 13.5 Hz, $CH_a\mathbf{H_b}Ph$), 2.96 (1H, dd, J = 5.1, 11.6 Hz, $C\mathbf{H_a}H_bSe$), 3.01 (1H, dd, J = 4.9, 11.6 Hz, $CH_a\mathbf{H_b}Se$), 3.35-3.42 (1H, m, C**H**NH), 7.00-7.06 (2H, m), 7.15-7.22 (5H, m), 7.31 (2H, d, J = 8.4 Hz), 7.37 (2H, bs, NH_2), 7.44 (2H, d, J = 8.2 Hz), 7.60 (2H, d, J = 8.4 Hz), 7.97 (1H, bd, J = 5.8 Hz, NH). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 21.9, 32.1 (CH_2Se), 40.9 (CH_2Ph, partially overlapped with DMSO-d_6 signal), 55.9 (CHNH), 127.0, 127.1, 127.2, 129.1, 130.1, 130.3, 131.0, 136.4, 138.4, 138.8, 142.8, 143.3. **MS**

(ESI positive) *m/z* (%): 547 $[M+Na]^+$, (100). Elemental analysis: $C_{22}H_{24}N_2O_4S_2Se$ Calcd. C 50.47%, H 4.62%, N 5.35%. Found: C 50.35%, H 4.68%, N 5.38%.

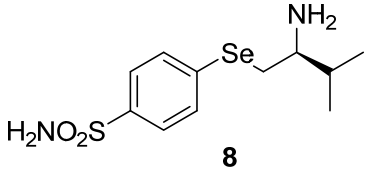
***tert*-Butyl (*S*)-(3-methyl-1-((4-sulfamoylphenyl)selanyl)butan-2-yl)carbamate 7c**



7c

Following the general procedure, 4,4'-diselanediyldibenzenesulfonamide **3** (94 mg, 0.20 mmol) and *tert*-butyl (*S*)-2-isopropylaziridine-1-carboxylate **6c** (67 mg, 0. 36 mmol) gave after flash chromatography (petroleum ether/EtOAc 3:1) **7c** (108 mg, 71%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) 0.91 (3H, d, *J* = 6.9 Hz), 0.94 (3H, d, *J* = 6.8 Hz), 1.42 (9H, s), 1.83-1.93 (1H, m), 3.13 (2H, ap d, *J* = 5.9 Hz, CH_2Se), 3.62-3.74 (1H, m, C**H**NH), 4.57 (1H, bd, *J* = 9.0 Hz, CHN**H**), 5.08 (2H, bs, NH_2), 7.58 (2H, d, *J* = 8.3 Hz), 7.76 (2H, d, *J* = 8.3 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 17.7, 19.6, 28.4, 31.6, 55.3, 79.5, 126.8, 131.5, 138.0, 140.0, 155.6. **^{77}Se NMR** (76 MHz, $CDCl_3$) δ (ppm): 265.6. **MS** (ESI positive) *m/z* (%): 422 $[M+H]^+$, (100). Elemental analysis: $C_{16}H_{26}N_2O_4SSe$ Calcd. C 45.60%, H 6.22%, N 6.65%. Found: C 45.71%, H 6.18%, N 6.60%.

6. Preparation of (*S*)-4-((2-Amino-3-methylbutyl)selanyl)benzenesulfonamide 8



8

Following a reported procedure,[1] a solution of acetyl chloride (107µL, 1.5 mmol, 15 eq.) in MeOH (1 mL) was slowly added to a solution of *tert*-butyl (*S*)-(3-methyl-1-((4-sulfamoylphenyl)selanyl)butan-2-yl)carbamate **7c** (42 mg, 0.1 mmol, 1.0 eq.) in MeOH (1 mL) at 0 °C under inert atmosphere (N_2). The reaction mixture was stirred at 0 °C for 6 h and the solvent was removed under vacuum to afford the crude product. Flash column chromatography (petroleum ether/ethyl acetate 1:2) gave (*S*)-4-((2-amino-3-methylbutyl)selanyl)benzenesulfonamide **8** (17 mg, 52%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm) 0.92 (6H, ap t, *J* = 6.3 Hz), 1.97-2.1 (1H, m), 3.01-3.13 (1H, m), 3.23 (1H, dd, *J* = 6.9, 13.1 Hz), 3.27-3.38 (1H, m), 7.38 (2H, bs), 7.67-7.74 (4H, m), (8.20 (2H, bs). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 18.2, 18.9, 27.6, 30.6, 56.5, 127.2, 132.0, 135.4, 143.4. **MS** (ESI positive) *m/z* (%): 320 $[M-H]^-$, (100). Elemental analysis: $C_{11}H_{18}N_2O_2SSe$ Calcd. C 41.12%, H 5.65%, N 8.72%. Found: C 41.22%, H 5.59%, N 8.67%.

7. General procedure for the synthesis of β-hydroxyselenides 5 and β-aminoselenides 6 from selenocyanate 2

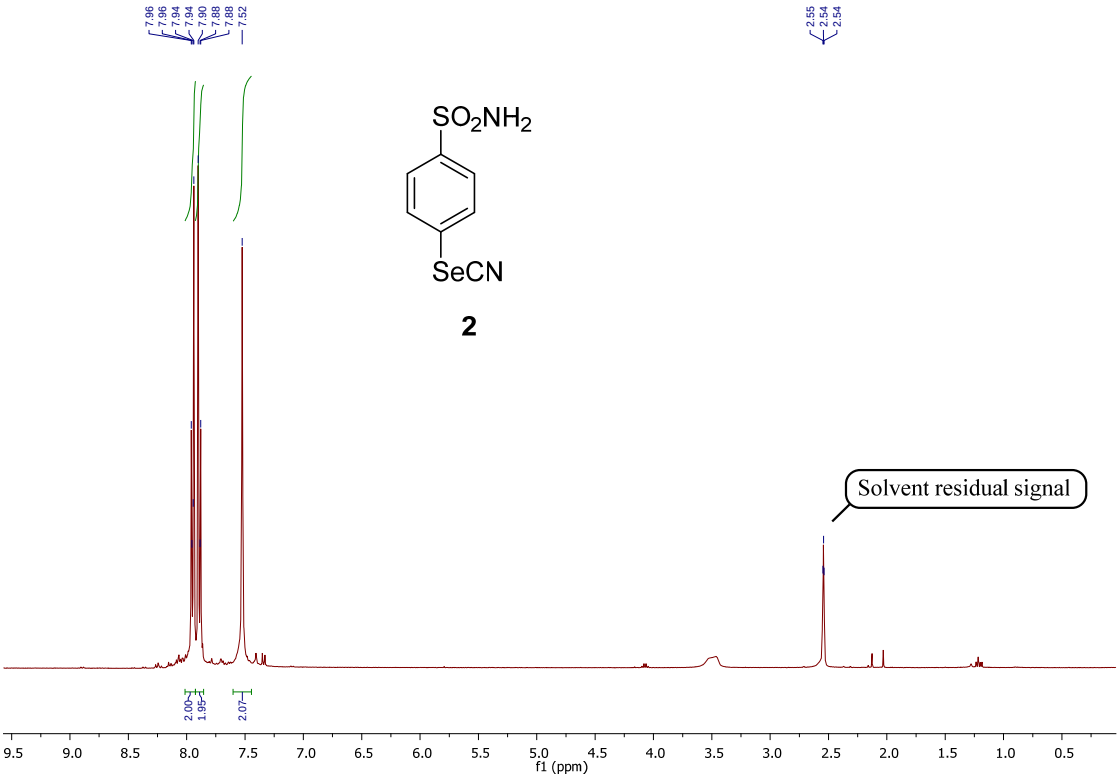
$NaBH_4$ (30 mg, 0.80 mmol, 4.0 eq.) was portionwise added to a solution of 4-selenocyanatobenzenesulfonamide **2** (52 mg, 0.20 mmol, 1.0 eq.) in EtOH (2 mL) at room temperature under inert atmosphere (N_2). After 1 h, the epoxide **4** or the aziridine **6** (0.18 mmol, 0.9 eq.) was slowly added and the reaction mixture was stirred at room temperature for 3 h, until complete consumption of the starting material was observed by TLC. The reaction was quenched by addition of saturated aq. NH_4Cl (2 mL) and diluted with EtOAc (5 mL), The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield selenides **5** and **6**, bearing benzenesulfonamide moiety.

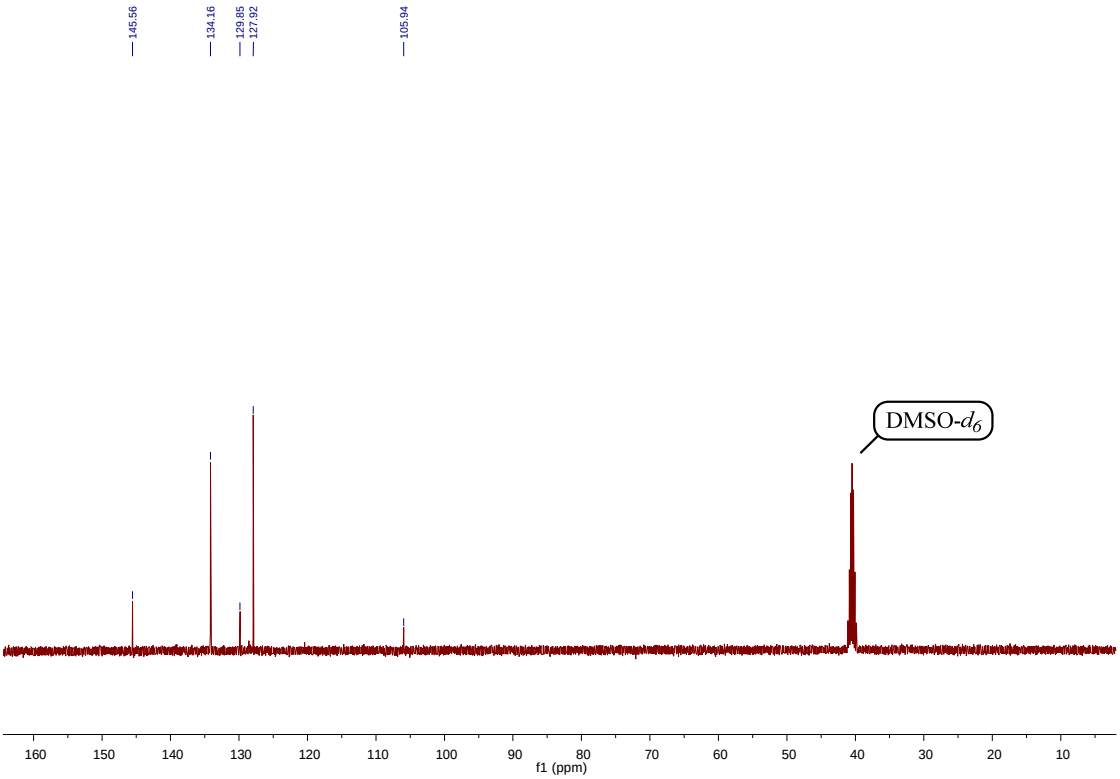
8. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.[2] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mMHepes (pH 7.5) as buffer, and 20 mM Na2SO4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO2 hydration reaction for a period of 10–100 s. The CO2 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 at room temperature 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 and the Cheng–Prusoff equation, as reported earlier,[1-6] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.[2-7]

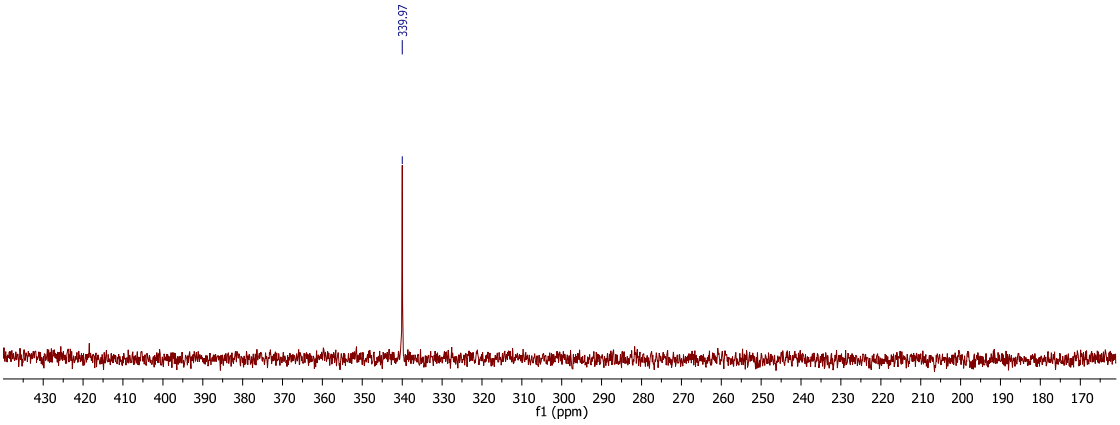
9. NMR Spectra

^{1}H and ^{13}C NMR Spectra of compound **2**

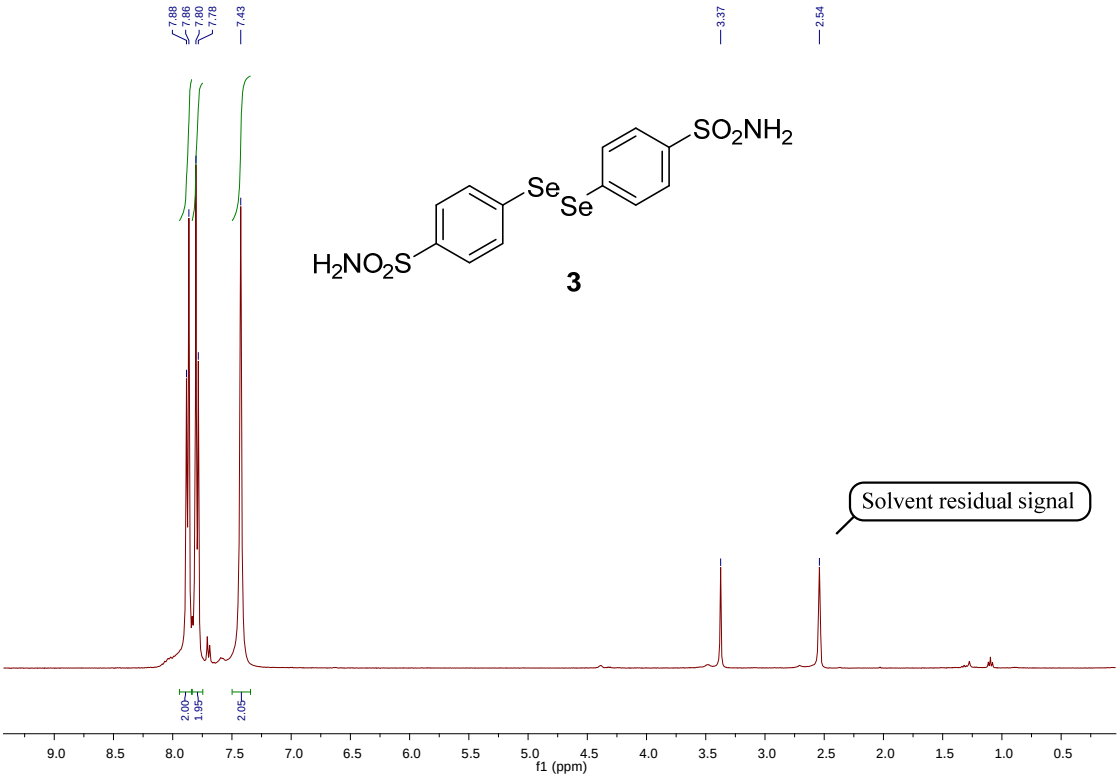


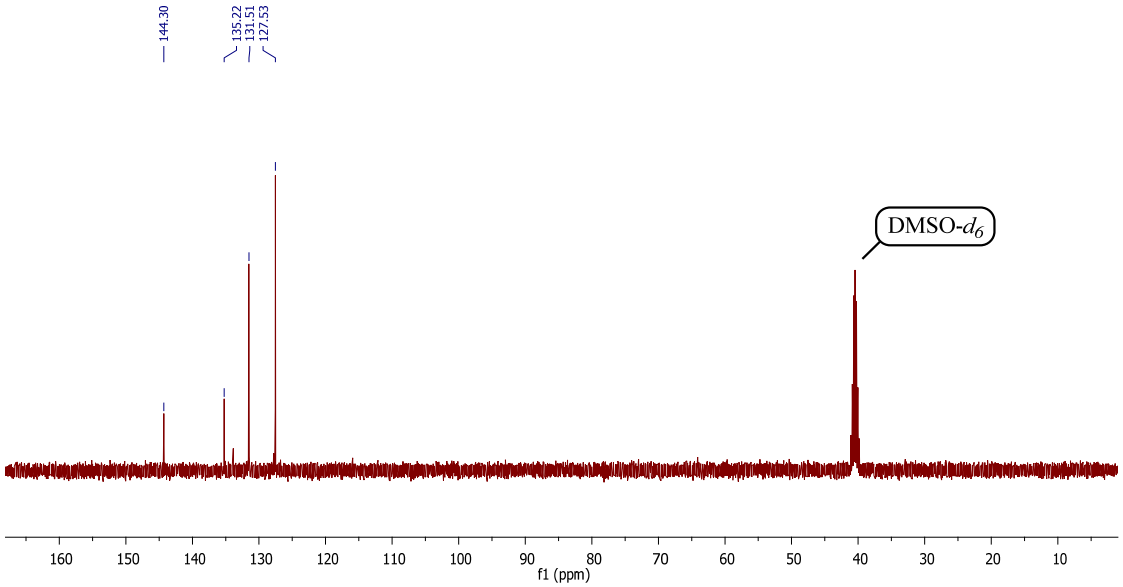


^{77}Se NMR Spectra of compound **2**

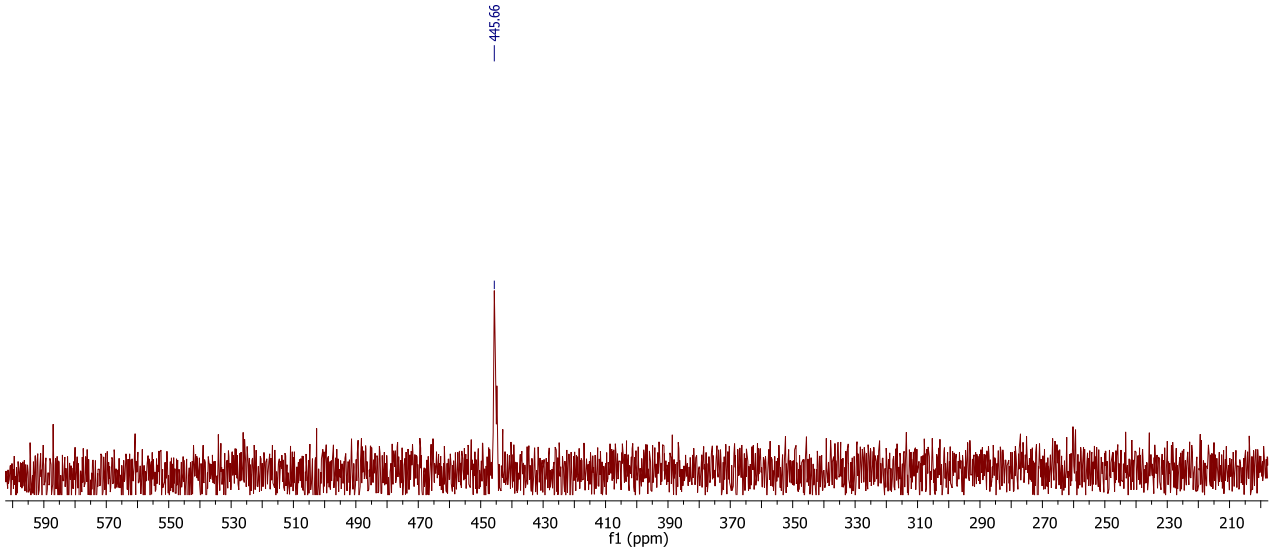


^{1}H and ^{13}C NMR Spectra of compound **3**

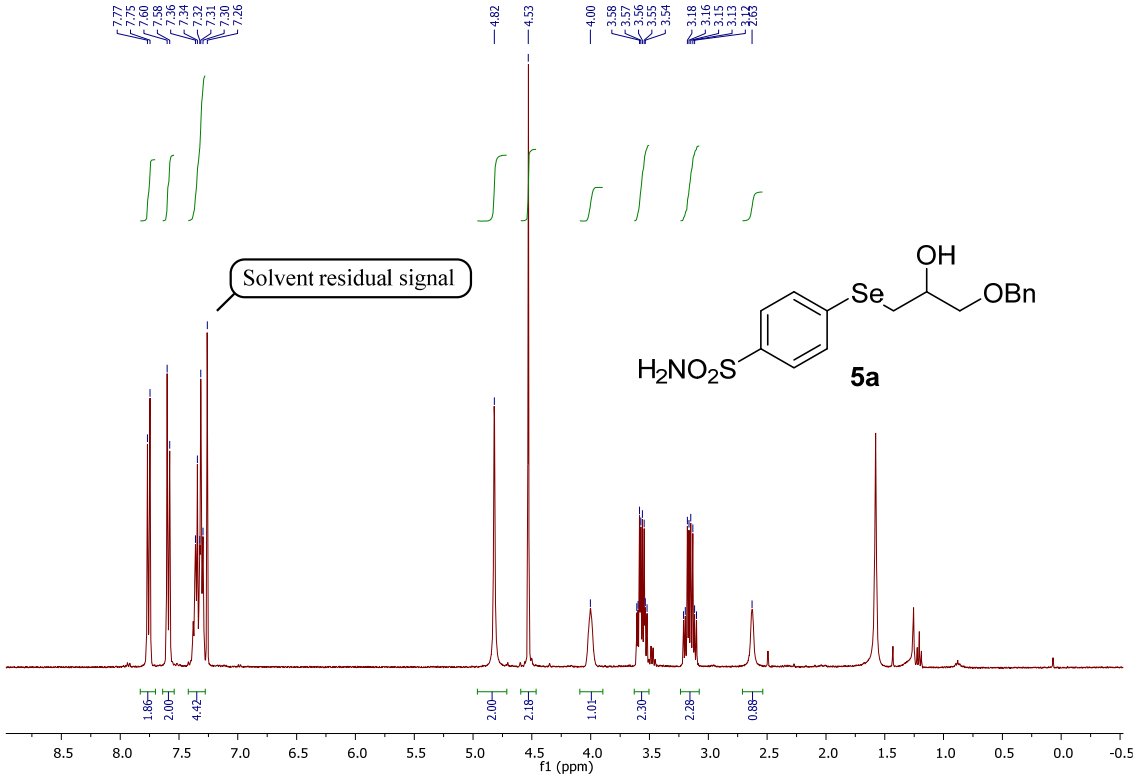


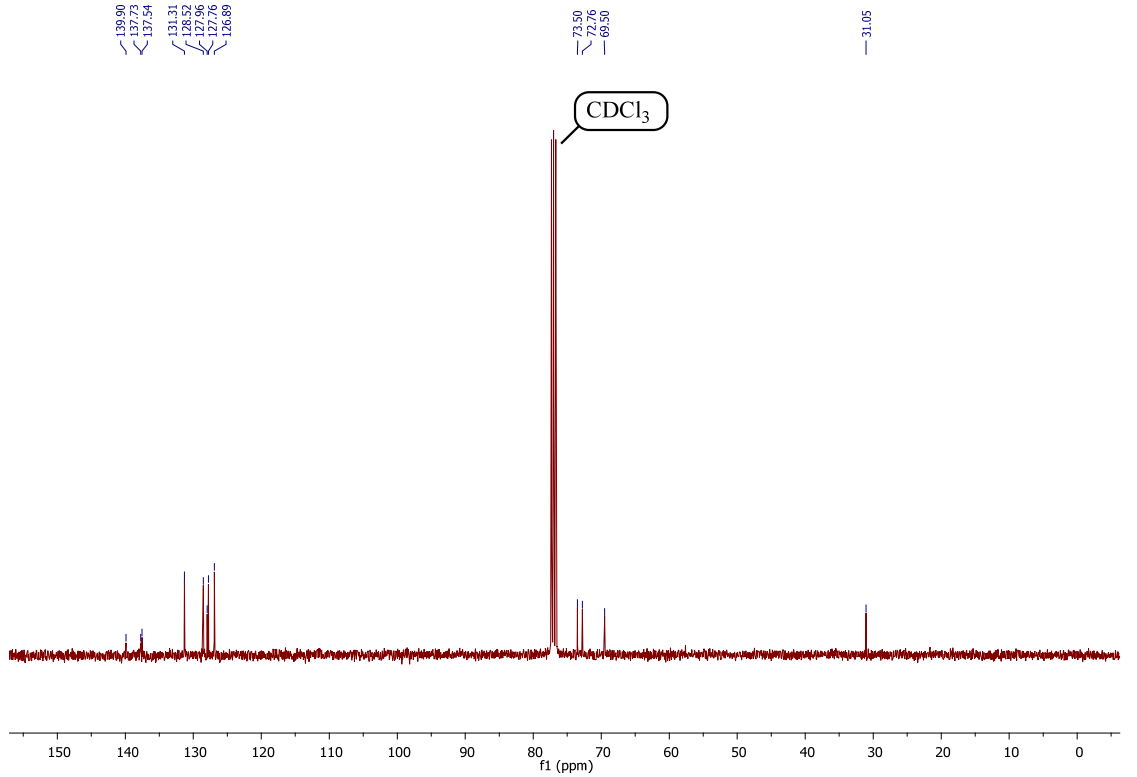


^{77}Se NMR Spectra of compound **3**

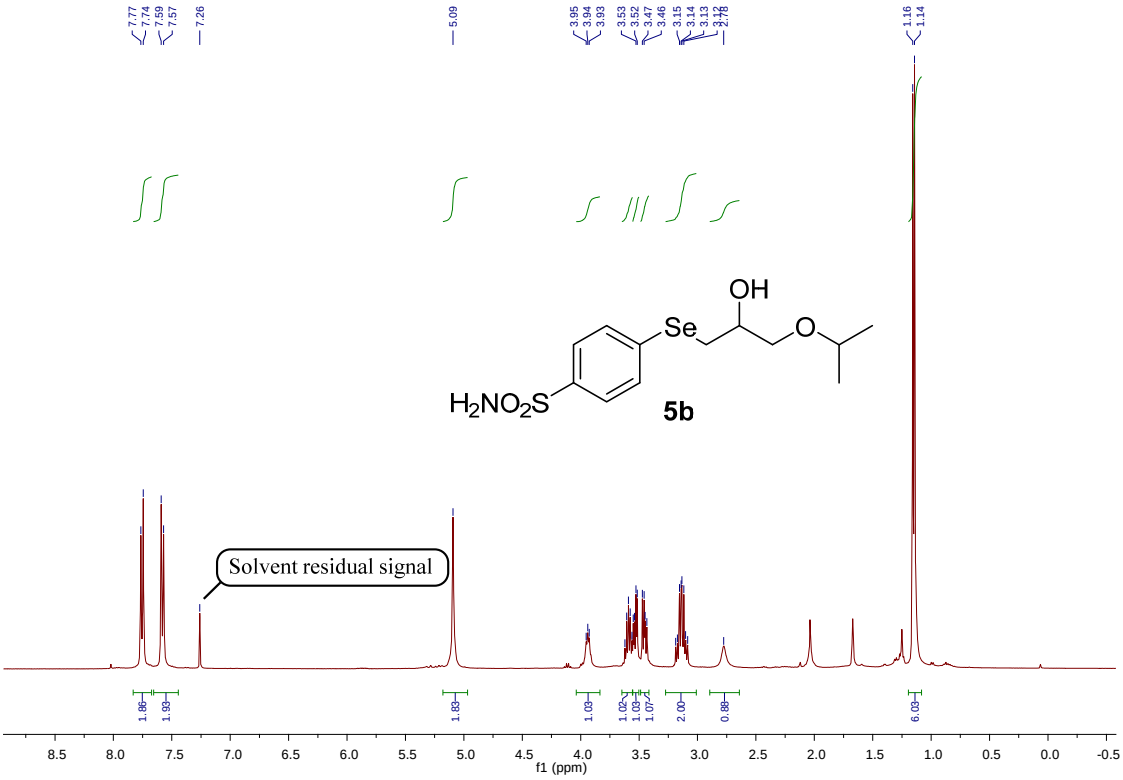


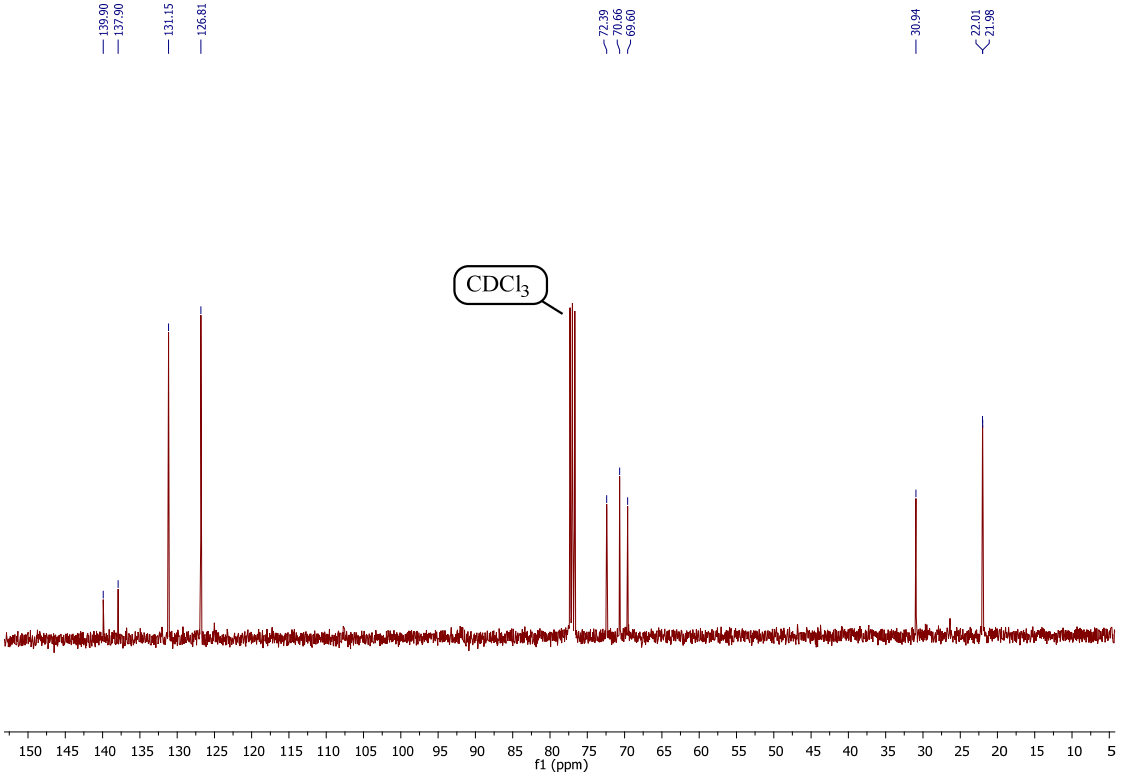
^{1}H and ^{13}C NMR Spectra of compound **5a**



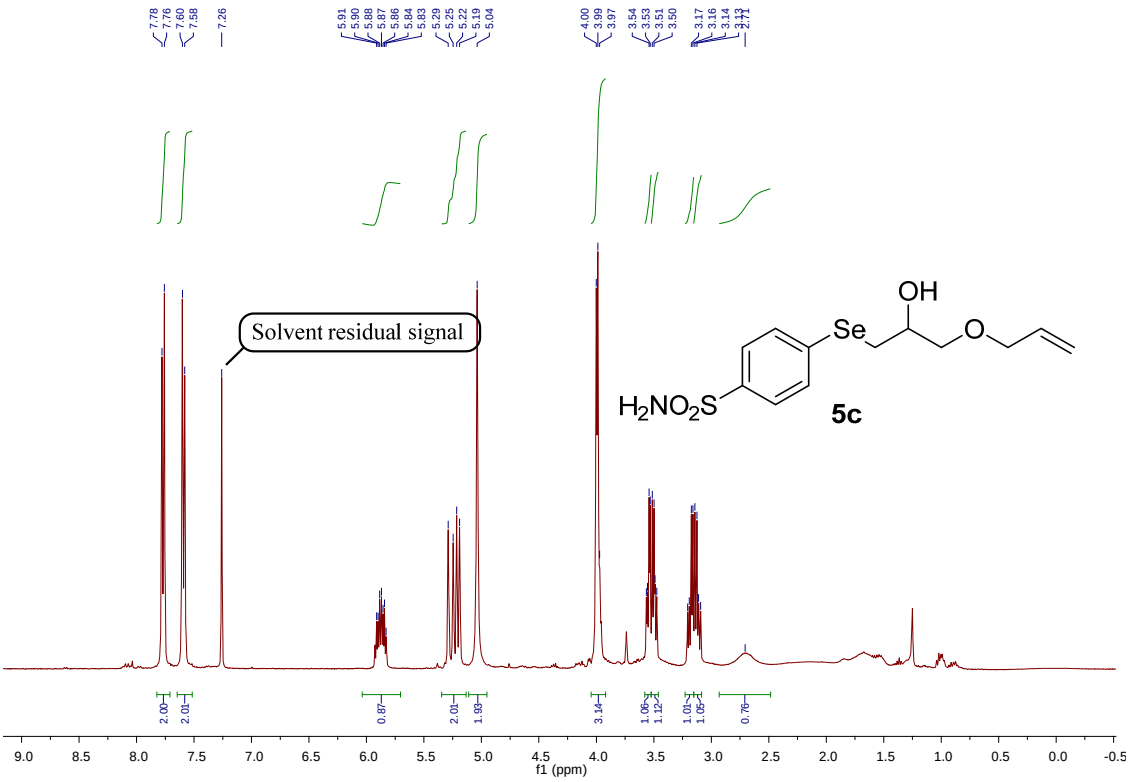


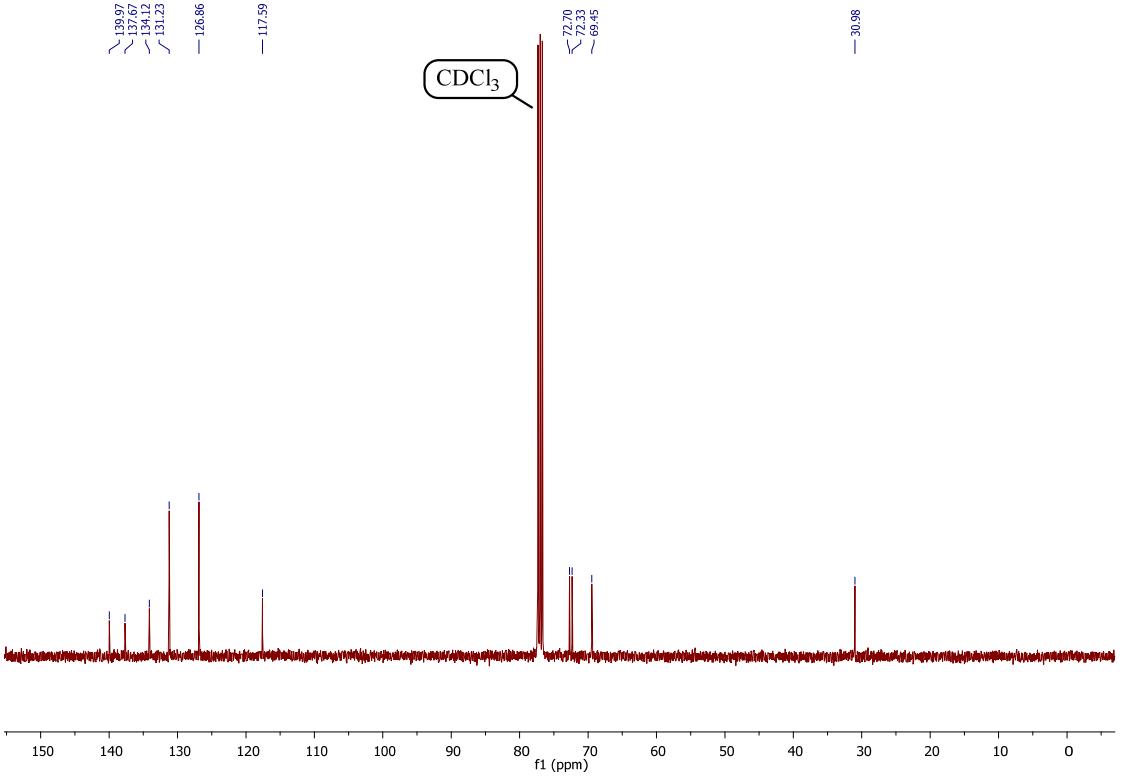
^{1}H and ^{13}C NMR Spectra of compound **5b**



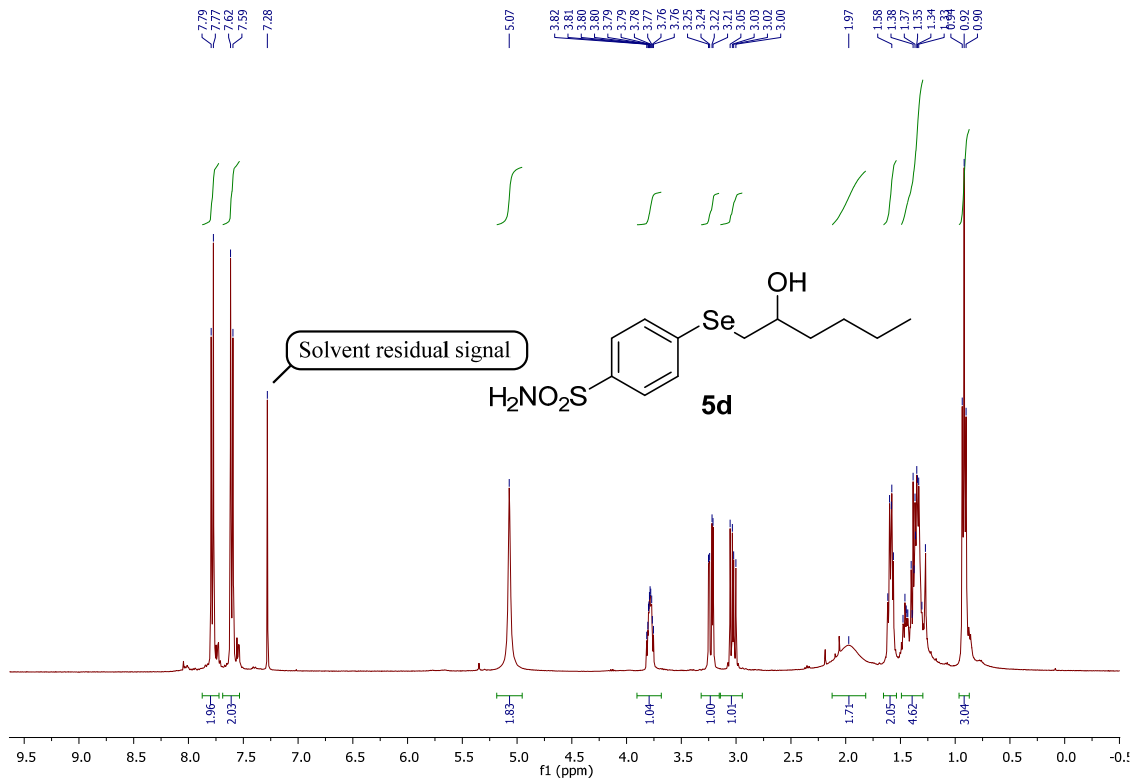


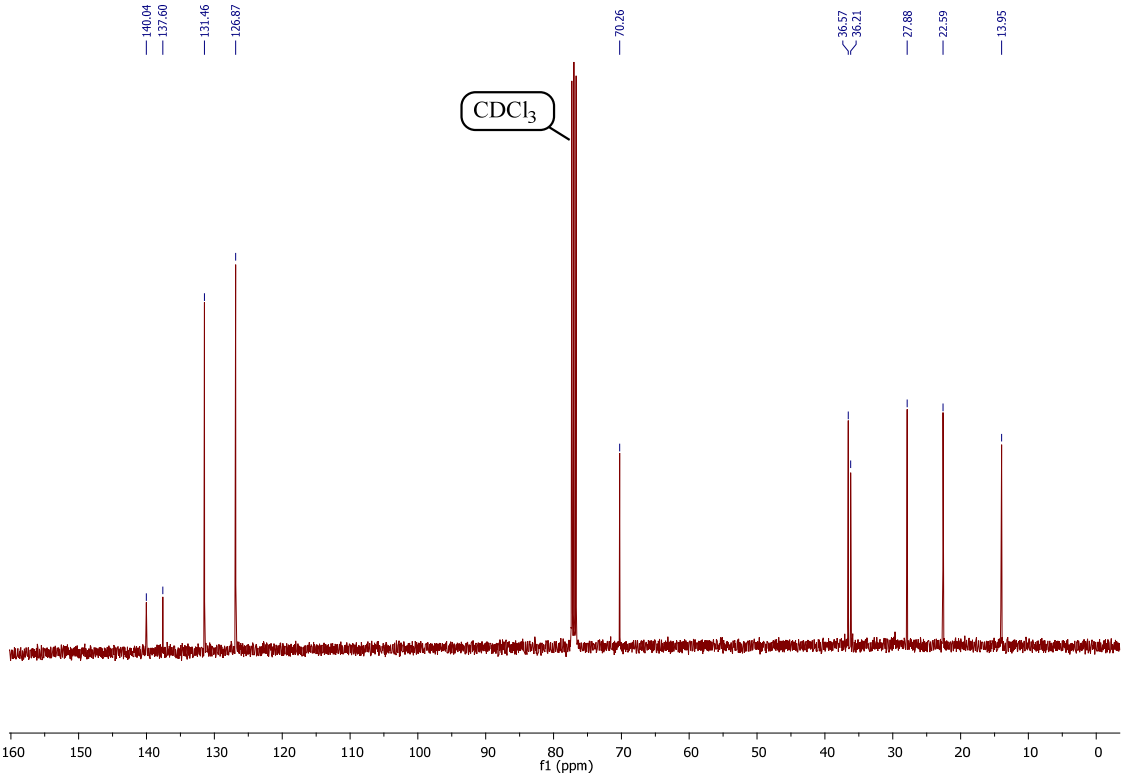
^{1}H and ^{13}C NMR Spectra of compound **5c**



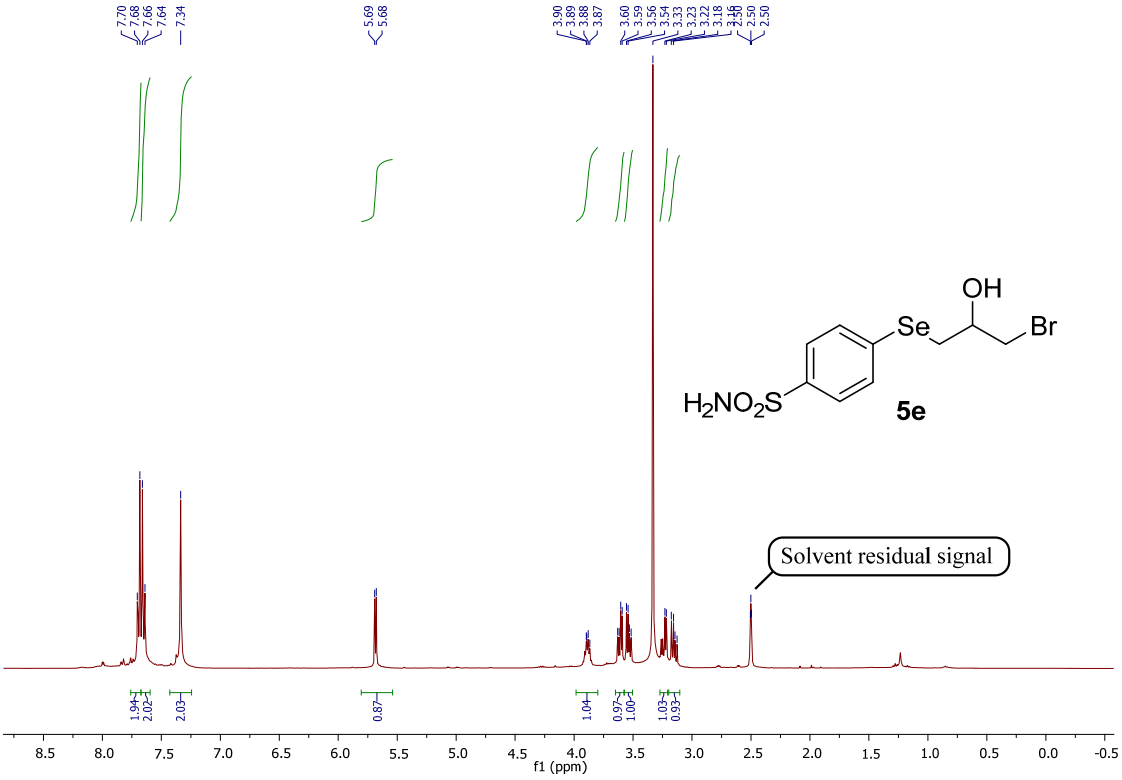


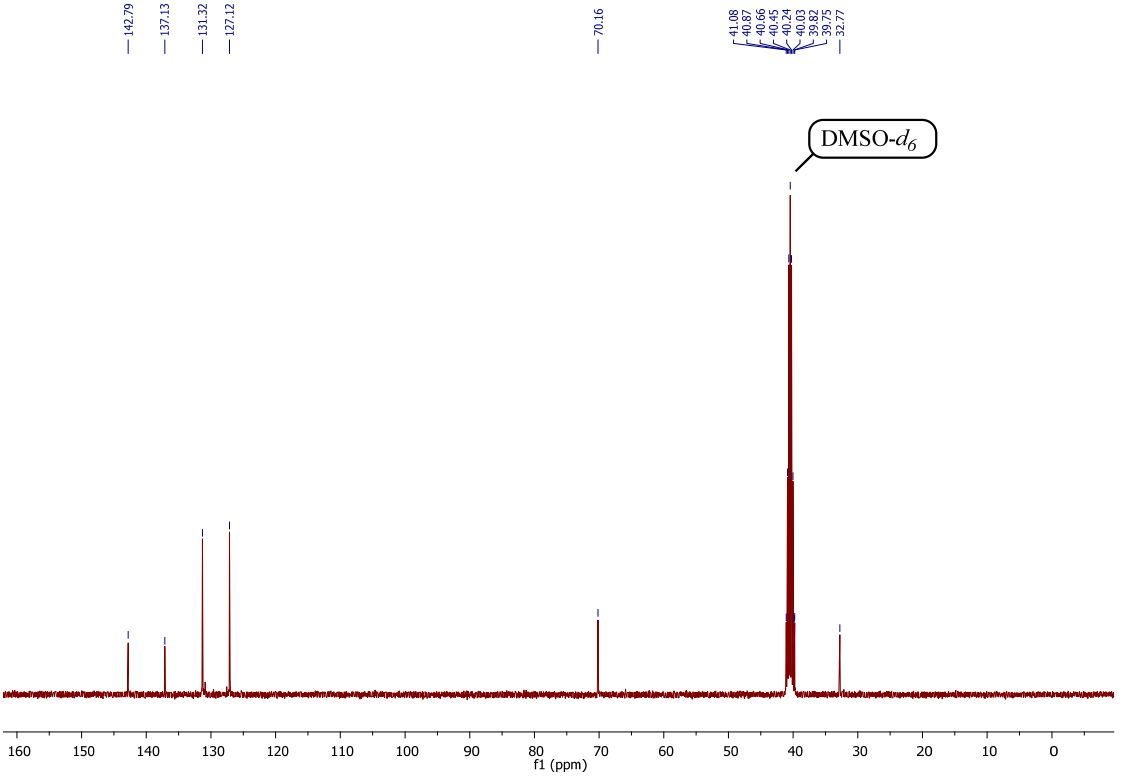
^{1}H and ^{13}C NMR Spectra of compound **5d**



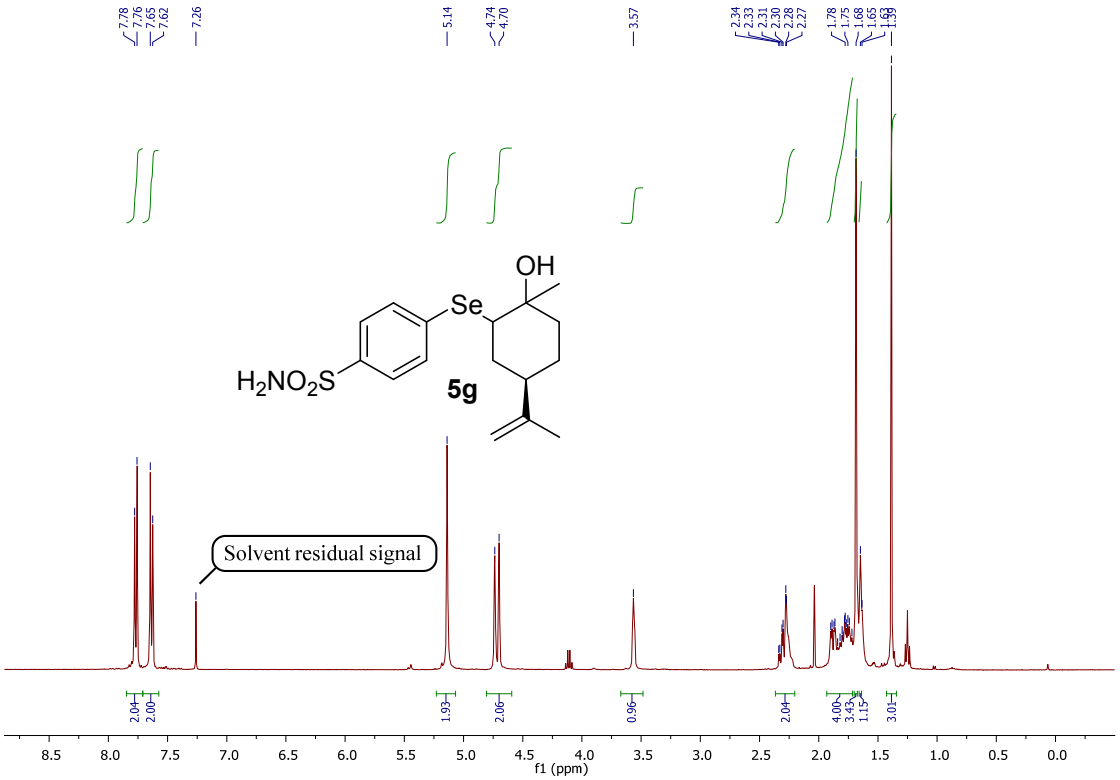


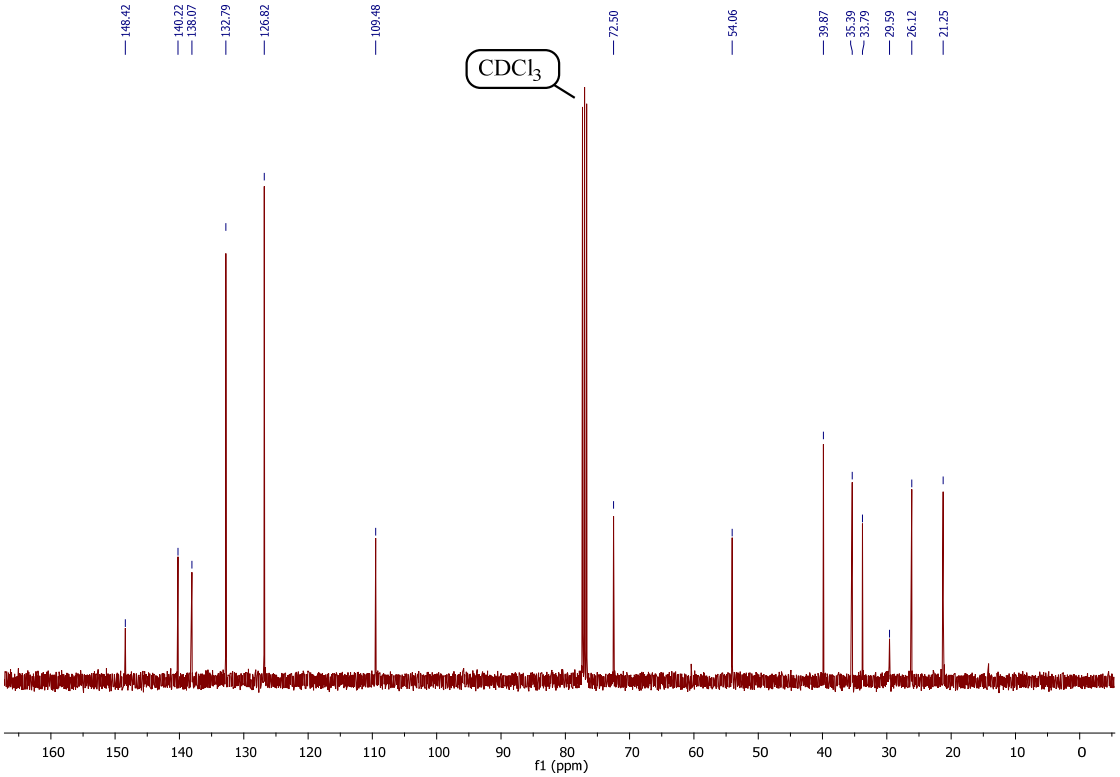
^{1}H and ^{13}C NMR Spectra of compound **5e**



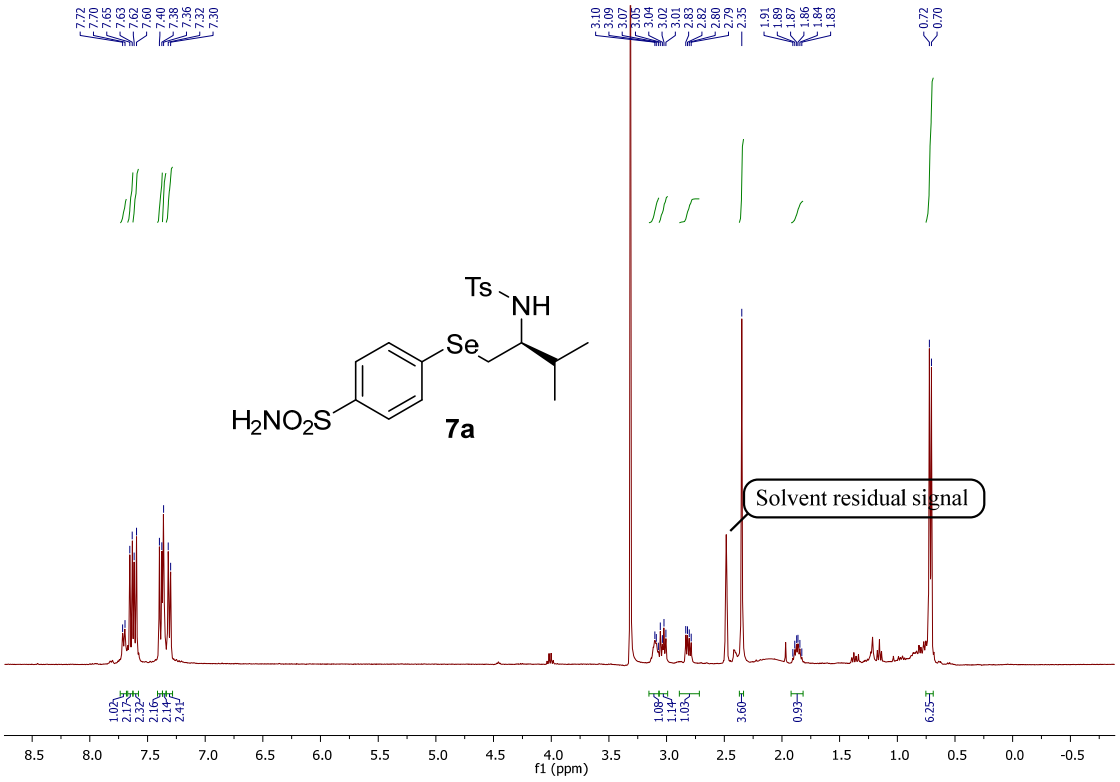


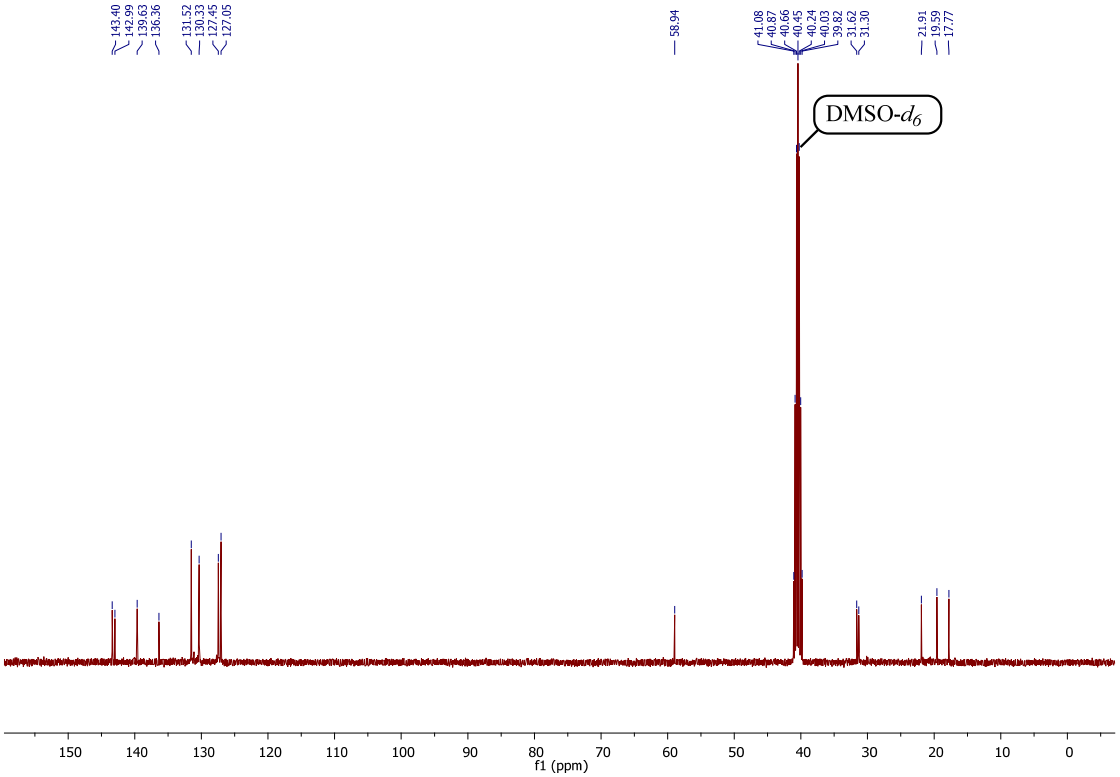
^{1}H and ^{13}C NMR Spectra of compound **5g**



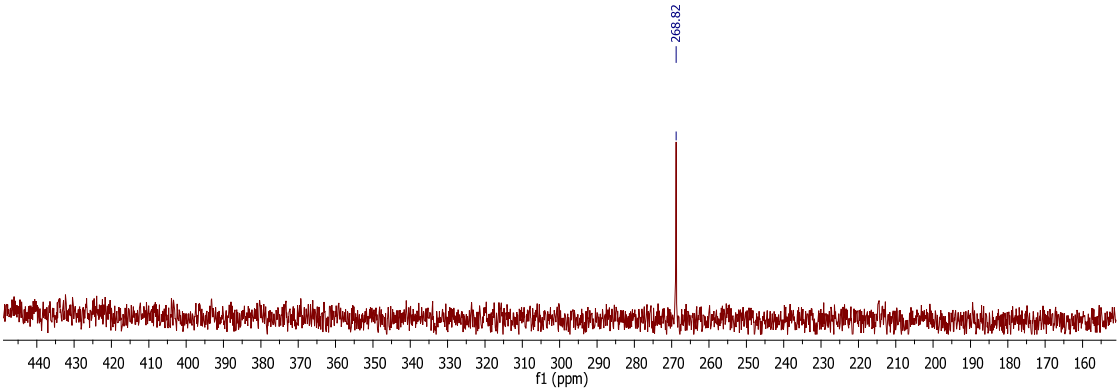


^{1}H and ^{13}C NMR Spectra of compound **7a**

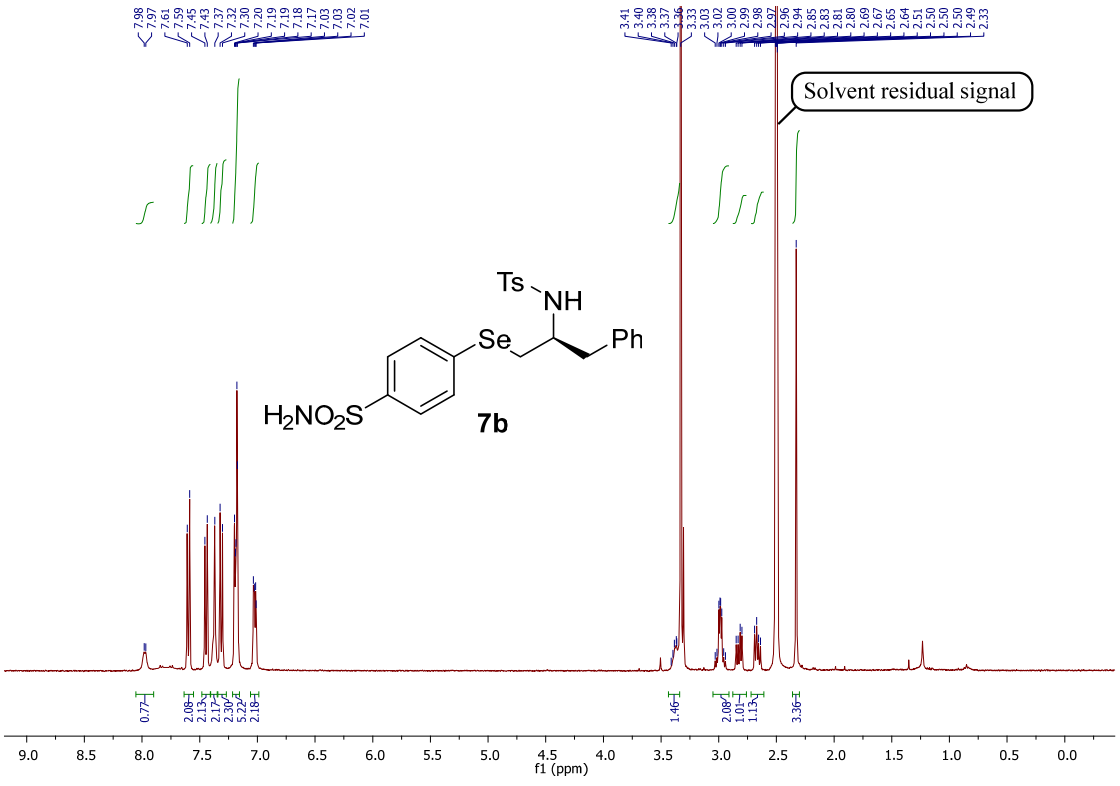


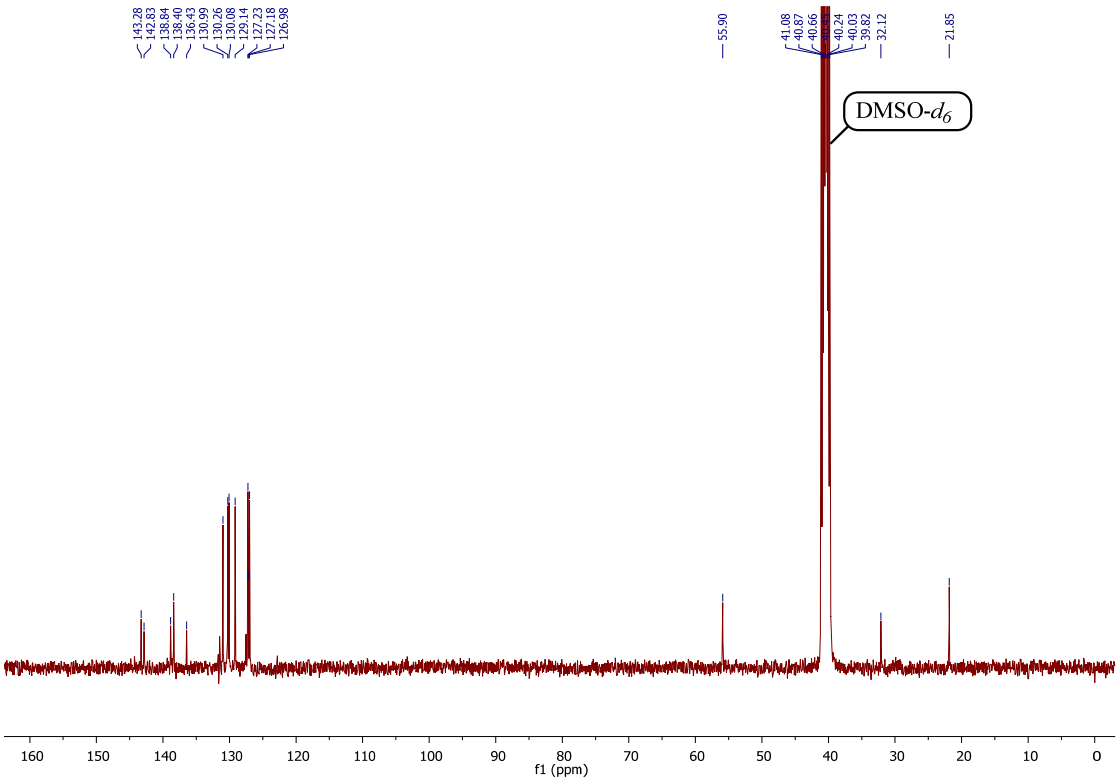


^{77}Se NMR Spectra of compound **7a**

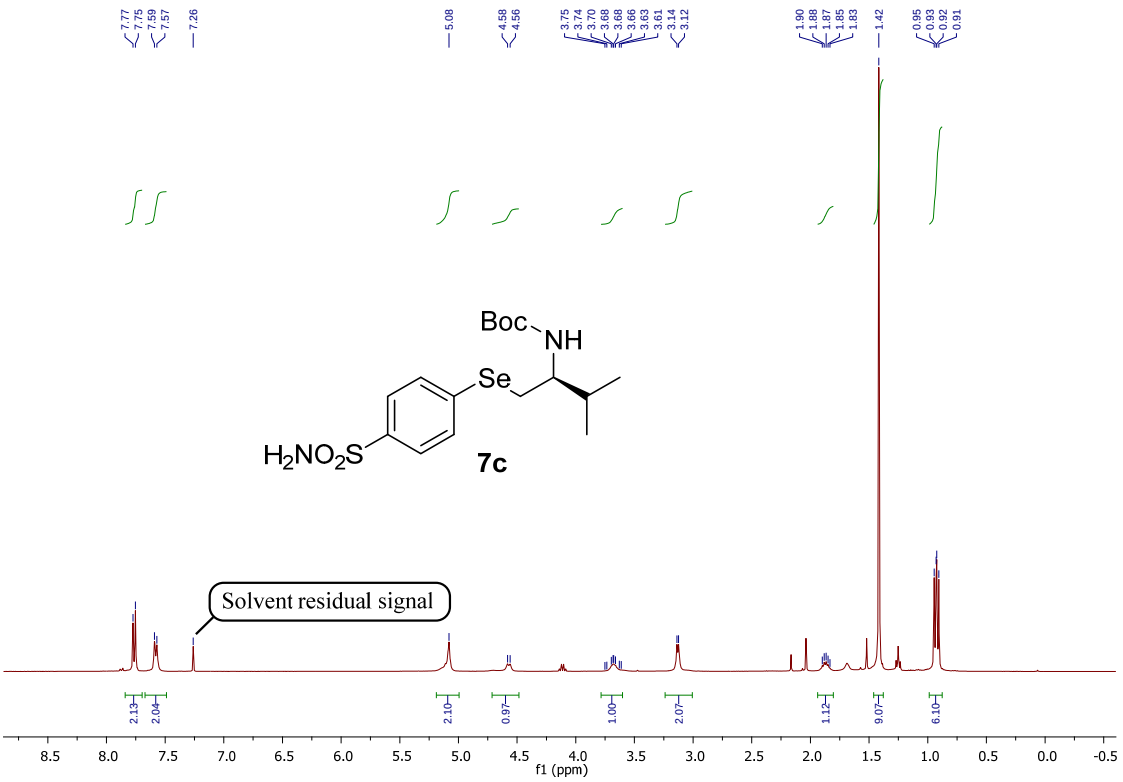


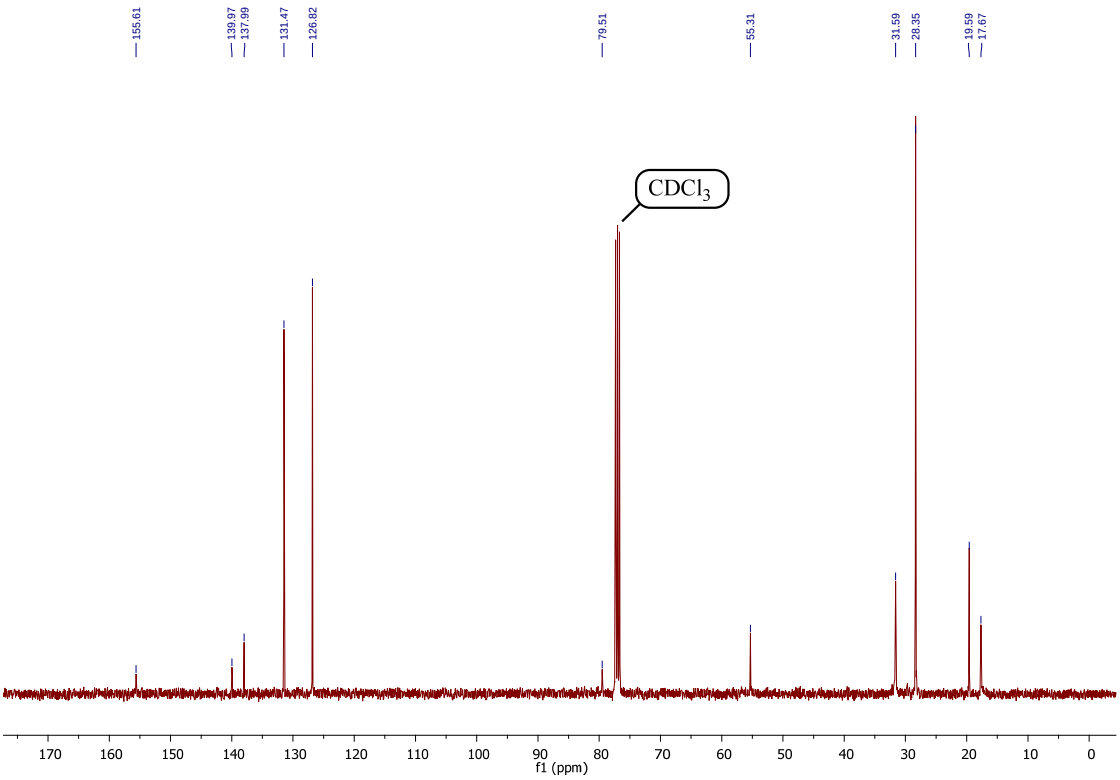
^{1}H and ^{13}C NMR Spectra of compound **7b**



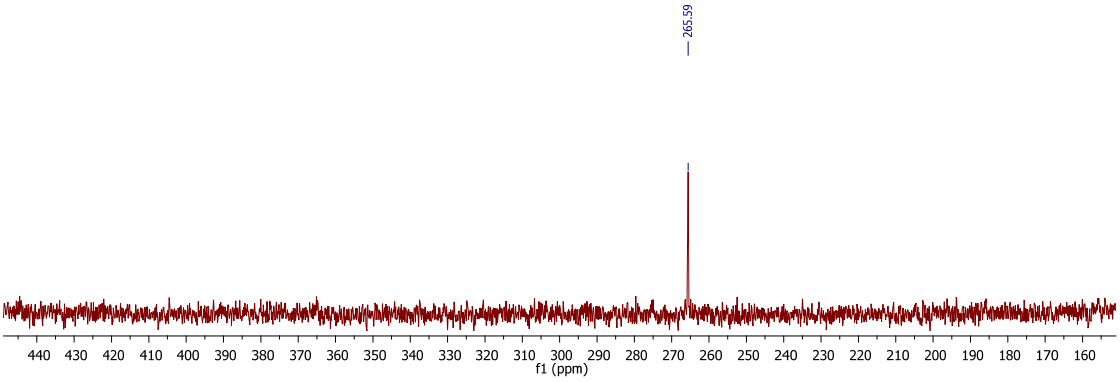


^{1}H and ^{13}C NMR Spectra of compound **7c**



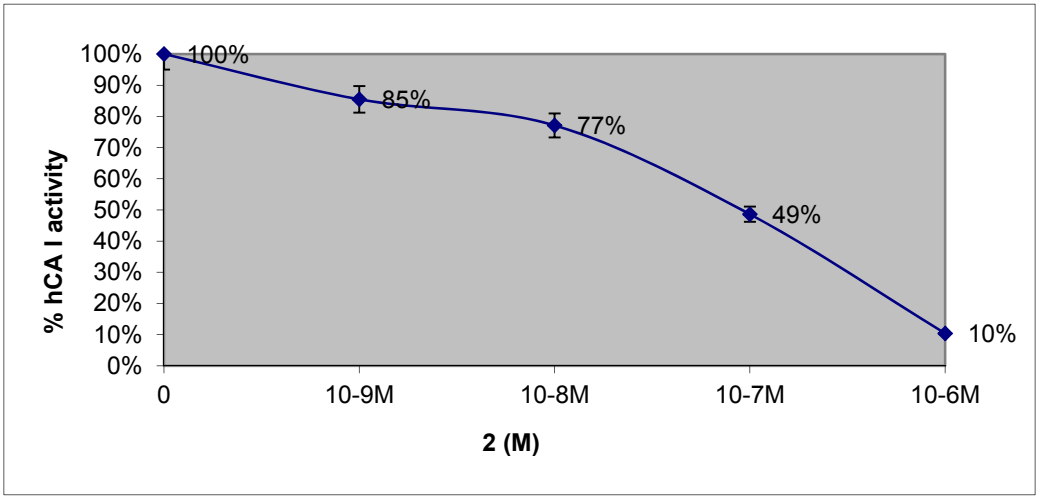


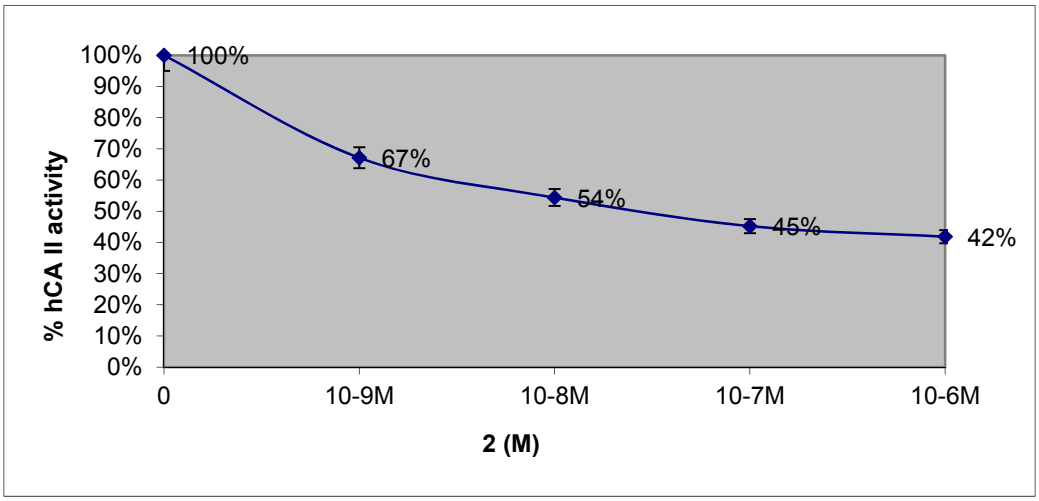
^{77}Se NMR Spectra of compound **7c**

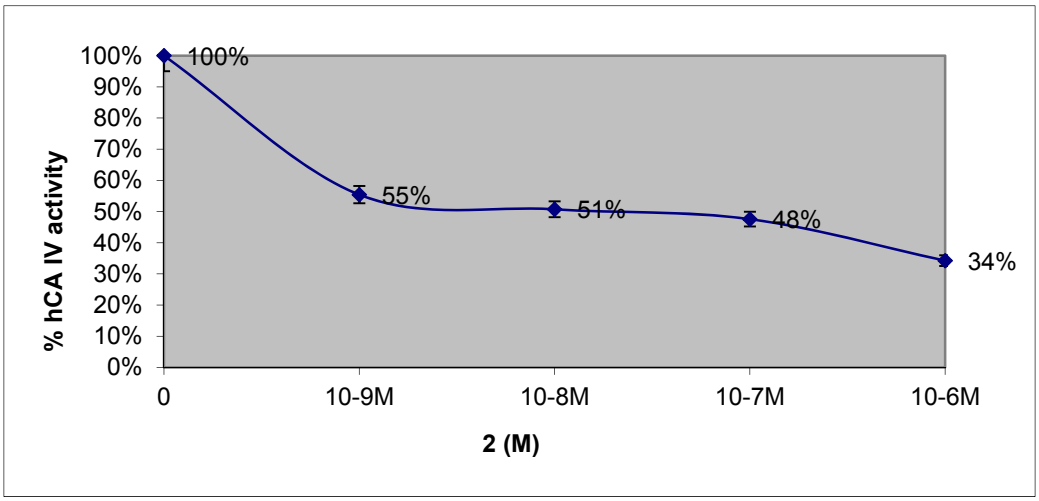


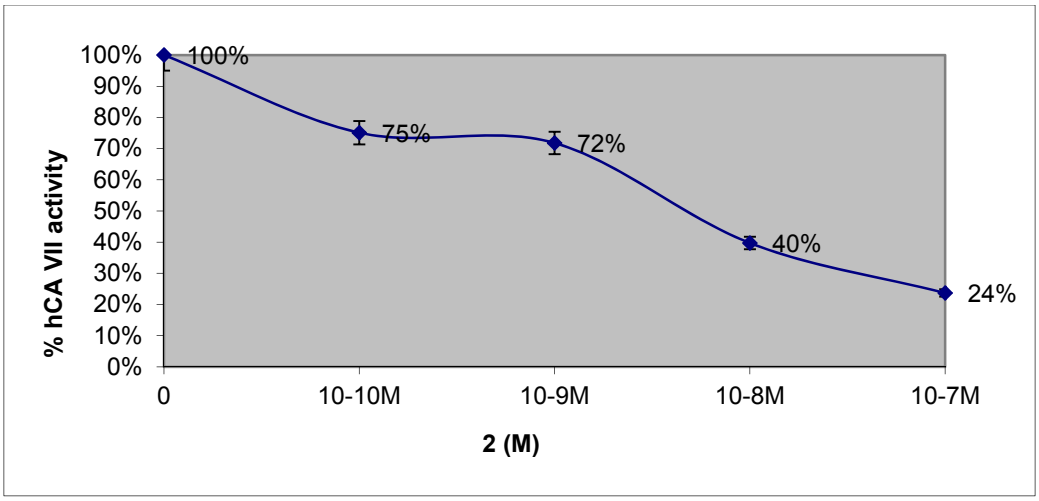
10. Human Carbonic Anhydrase activity

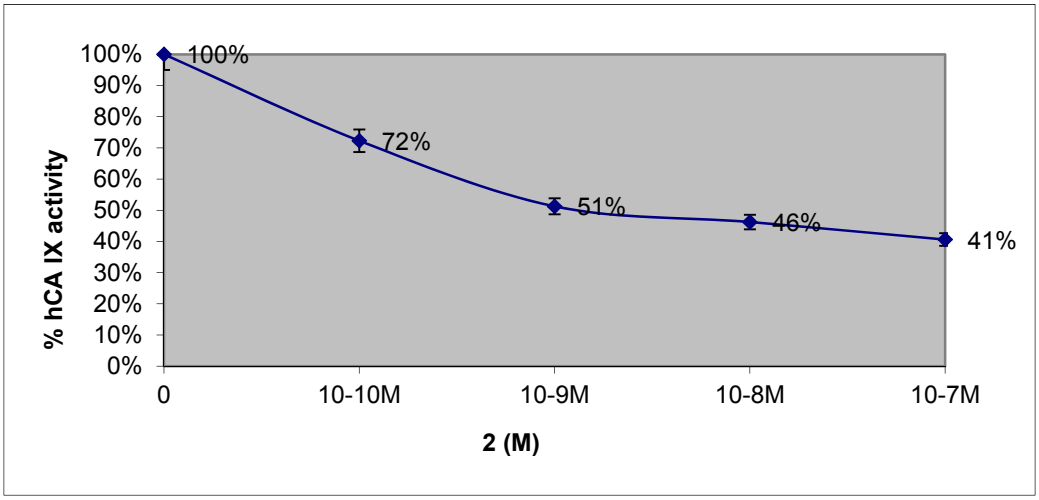
Compound 2:



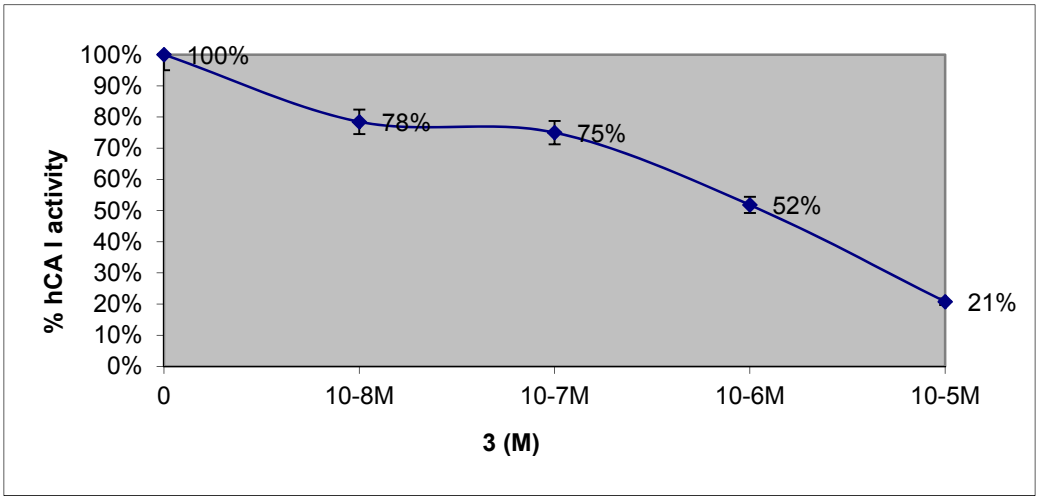


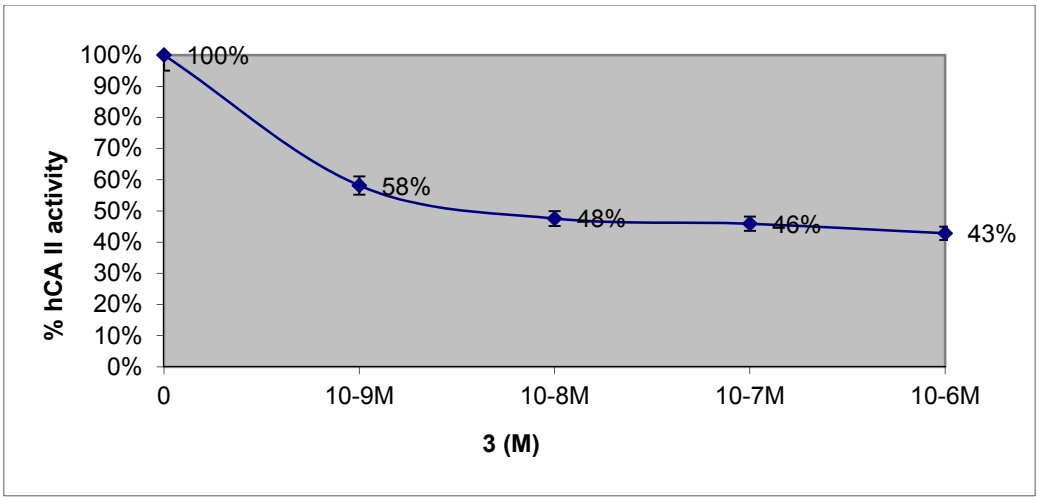


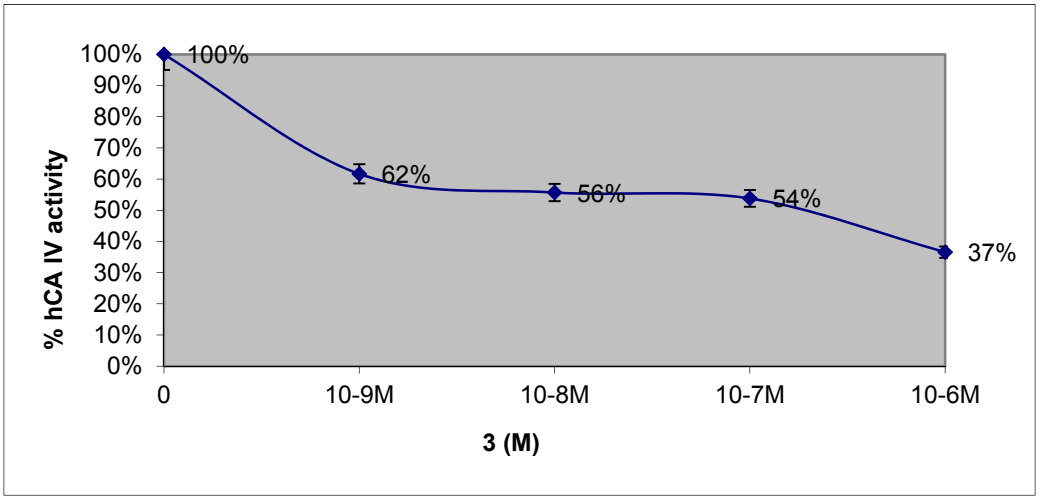

% hCA VII activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
75%
72%
40%
24%
0
10-10M
10-9M
10-8M
10-7M
2 (M)

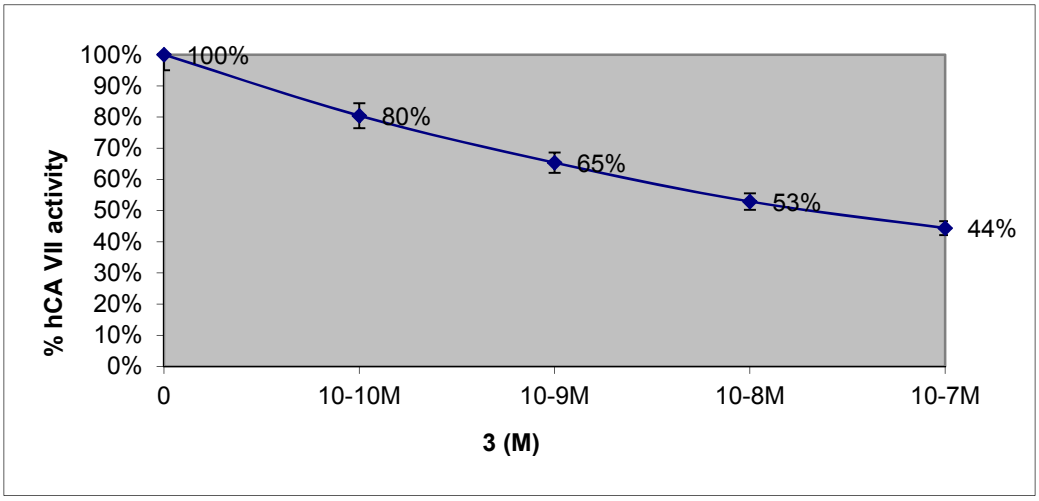

% hCA IX activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
72%
51%
46%
41%
0
10-10M
10-9M
10-8M
10-7M
2 (M)

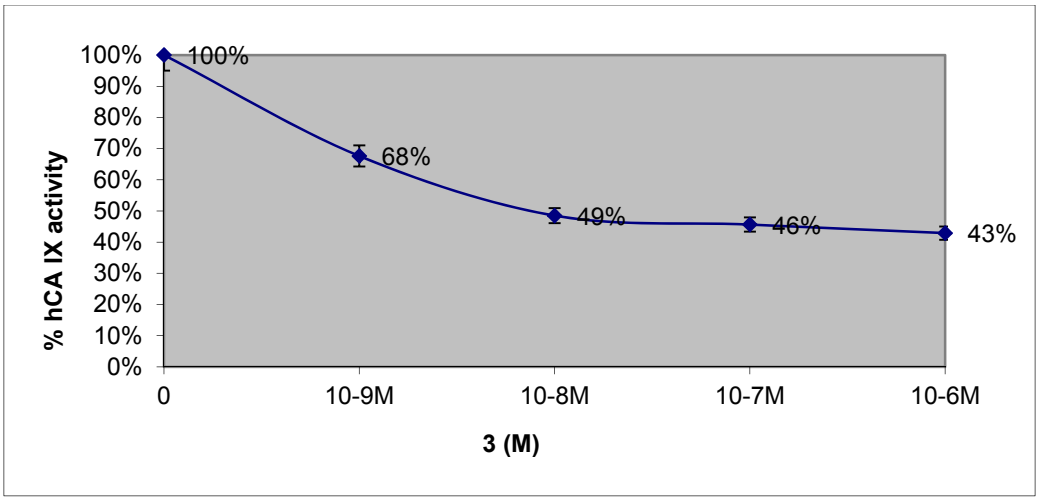
Compound 3:


% hCA I activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
78%
75%
52%
21%
0
10-8M
10-7M
10-6M
10-5M
3 (M)

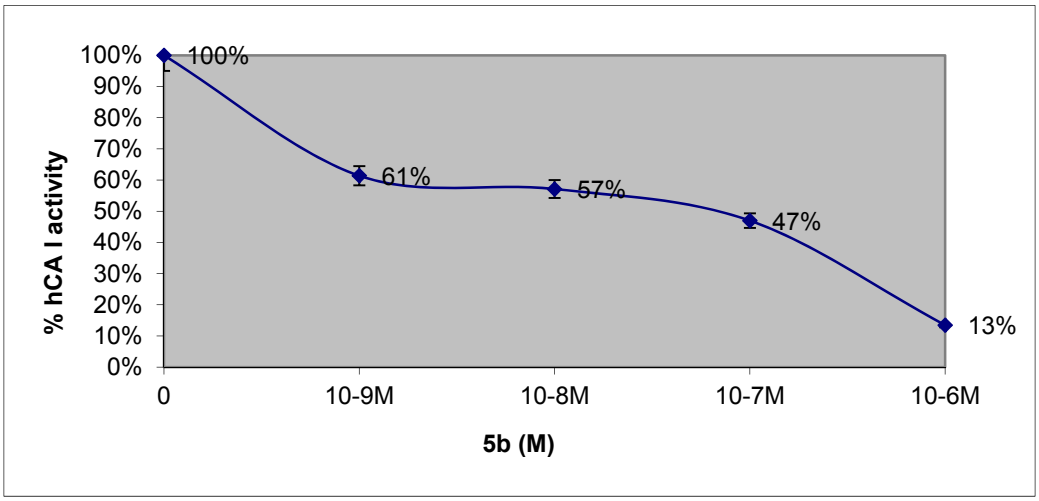

% hCA II activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
58%
48%
46%
43%
0
10-9M
10-8M
10-7M
10-6M
3 (M)

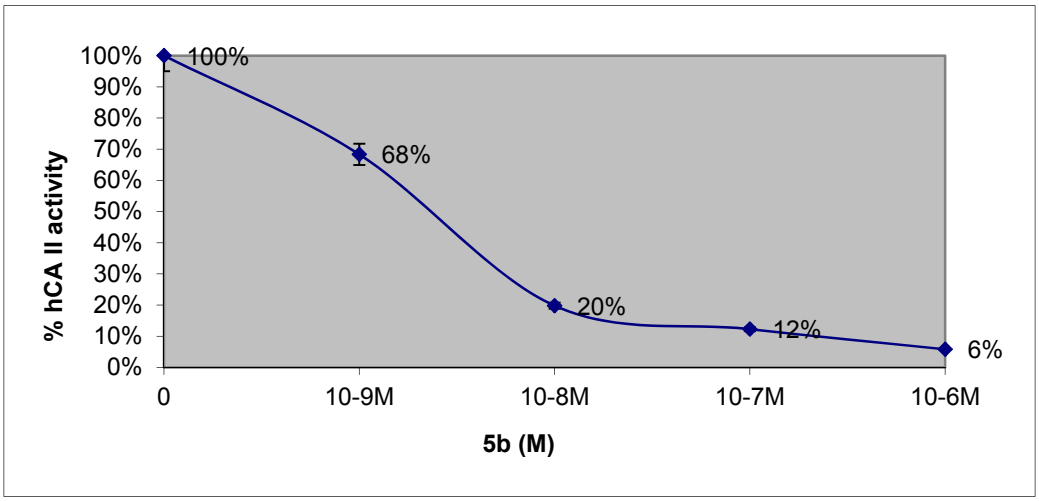

% hCA IV activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
62%
56%
54%
37%
0
10-9M
10-8M
10-7M
10-6M
3 (M)

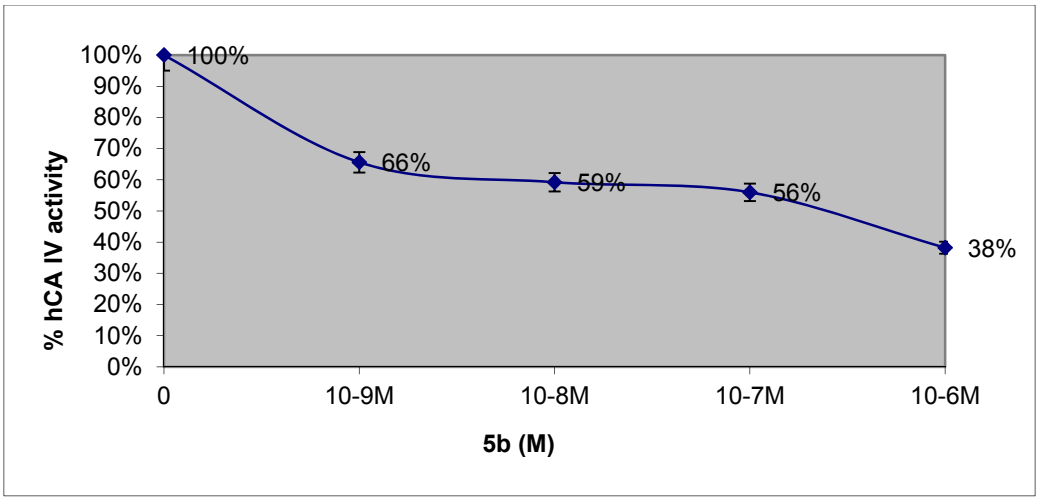

% hCA VII activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
80%
65%
53%
44%
0
10-10M
10-9M
10-8M
10-7M
3 (M)

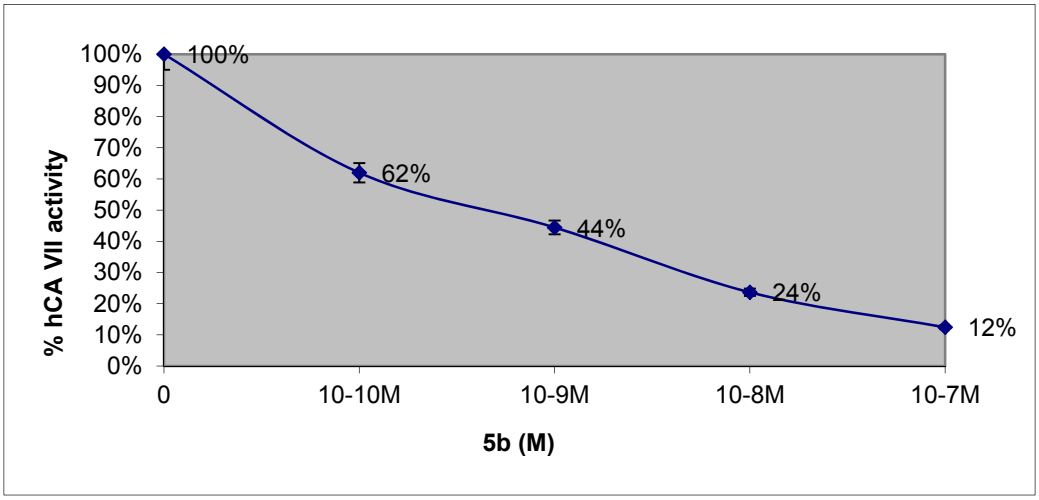

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% hCA IX activity
100%
68%
49%
46%
43%
0
10-9M
10-8M
10-7M
10-6M
3 (M)

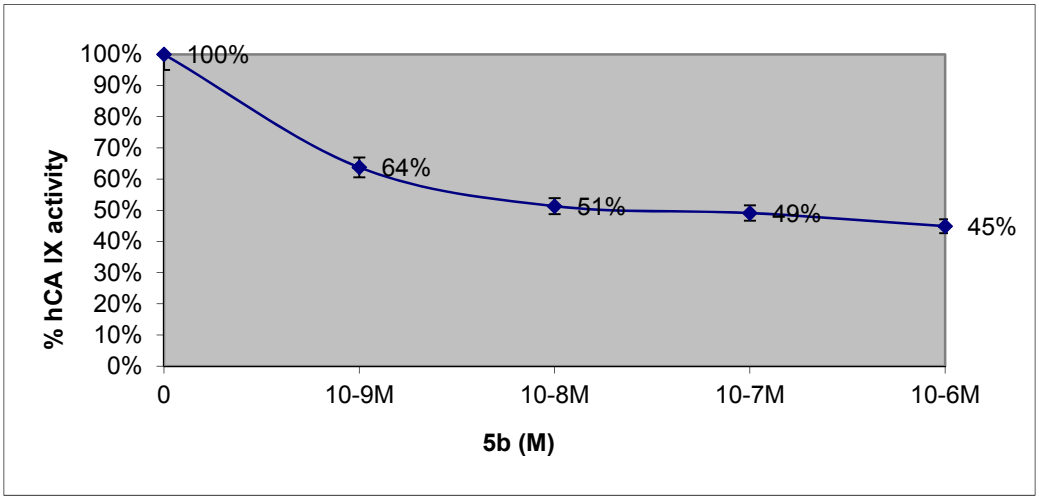
Compound 5b:


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% hCA I activity
100%
61%
57%
47%
13%
0
10-9M
10-8M
10-7M
10-6M
5b (M)

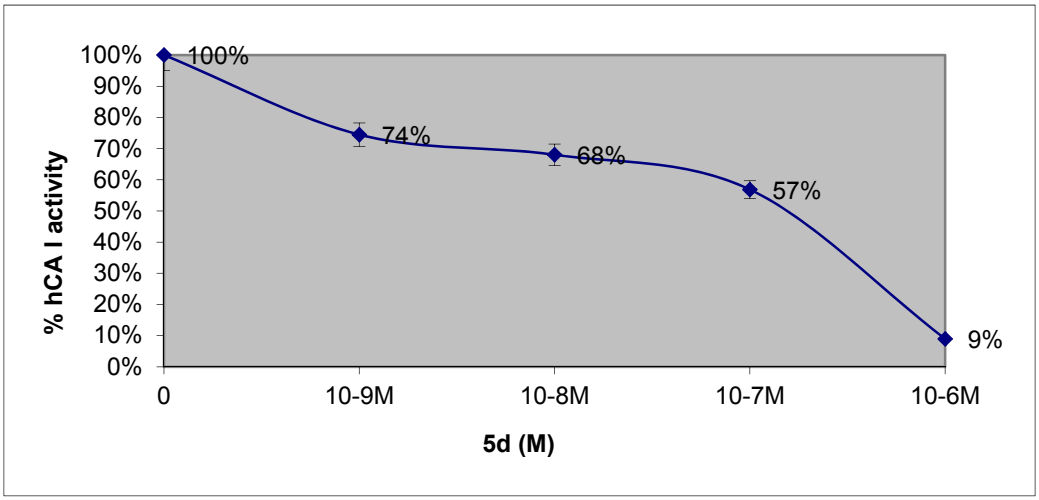

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% hCA II activity
100%
68%
20%
12%
6%
0
10-9M
10-8M
10-7M
10-6M
5b (M)

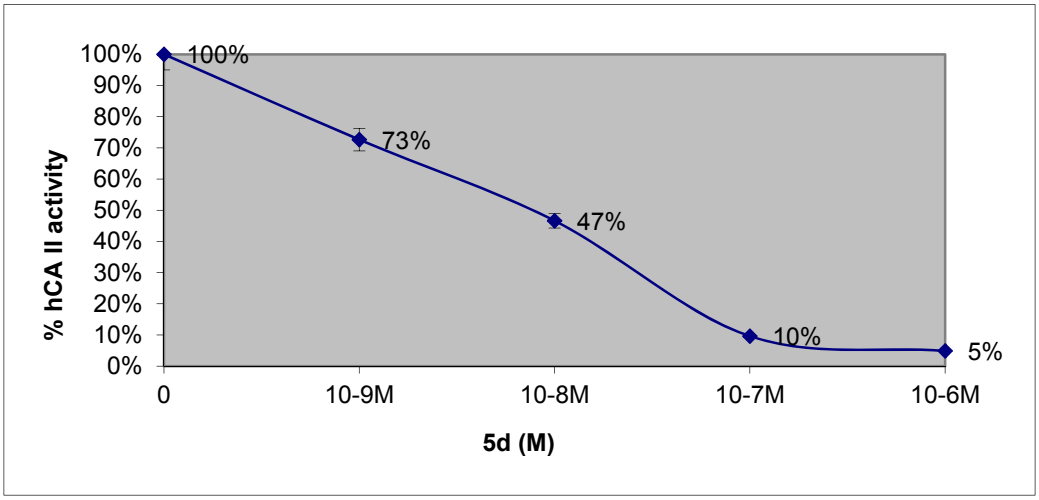

% hCA IV activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
66%
59%
56%
38%
0
10-9M
10-8M
10-7M
10-6M
5b (M)

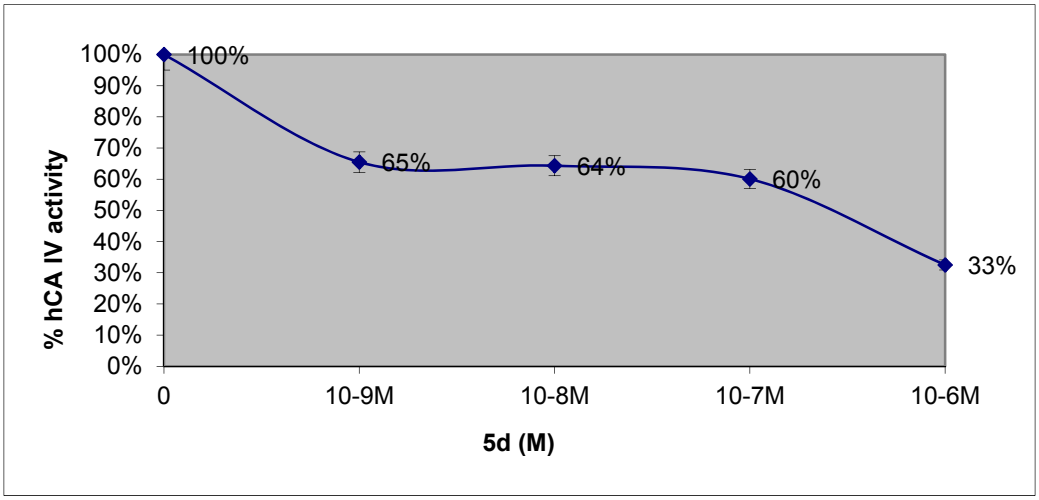

% hCA VII activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
62%
44%
24%
12%
0
10-10M
10-9M
10-8M
10-7M
5b (M)

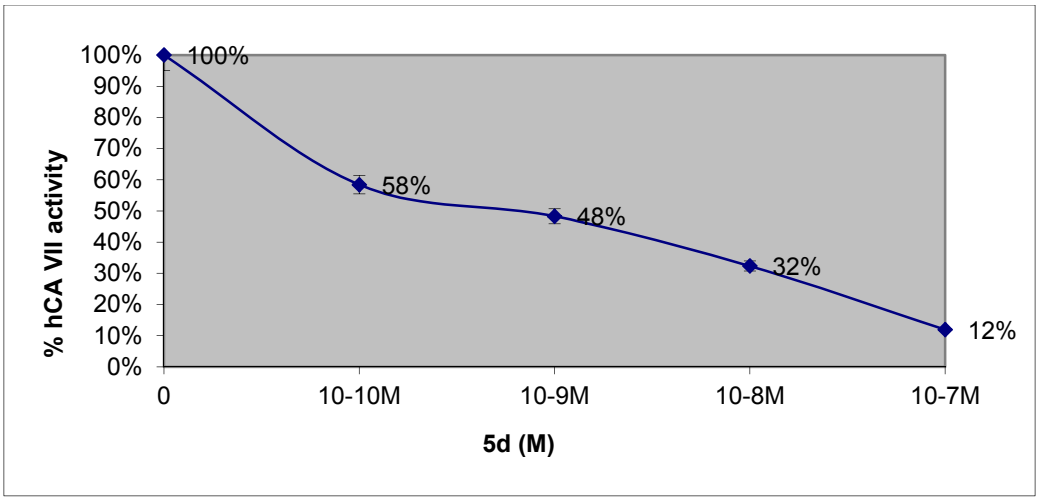

% hCA IX activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
64%
51%
49%
45%
0
10-9M
10-8M
10-7M
10-6M
5b (M)

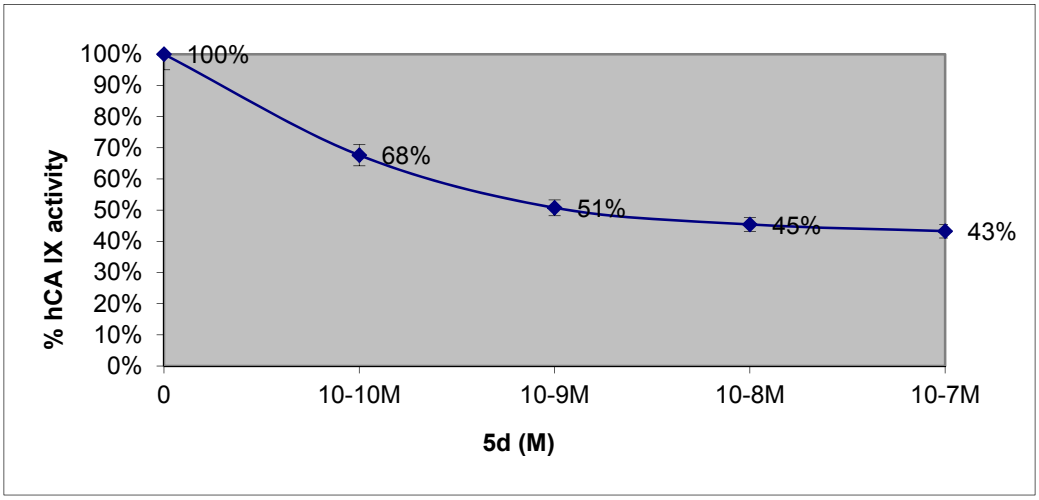
Compound 5d:


% hCA I activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
74%
68%
57%
9%
0
10-9M
10-8M
10-7M
10-6M
5d (M)

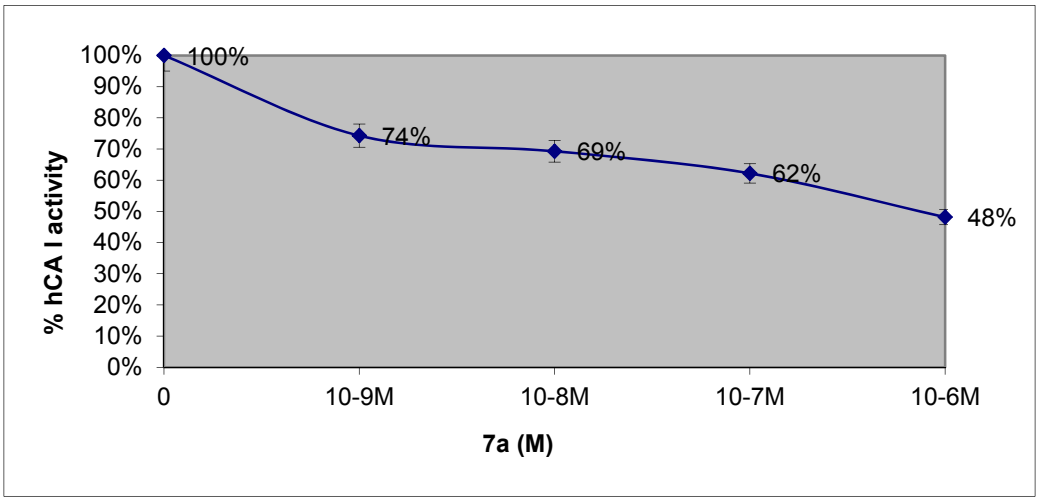

% hCA II activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
73%
47%
10%
5%
0
10-9M
10-8M
10-7M
10-6M
5d (M)

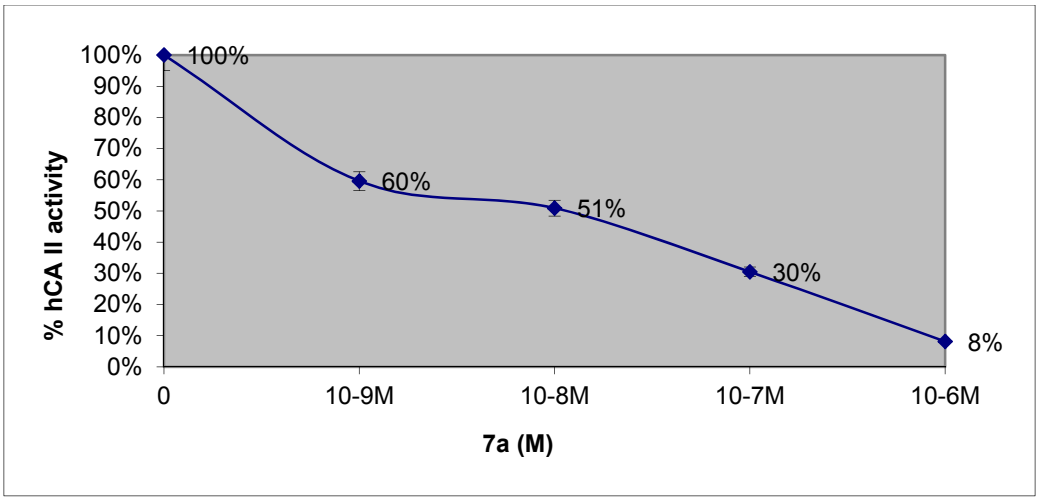

% hCA IV activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
65%
64%
60%
33%
0
10-9M
10-8M
10-7M
10-6M
5d (M)

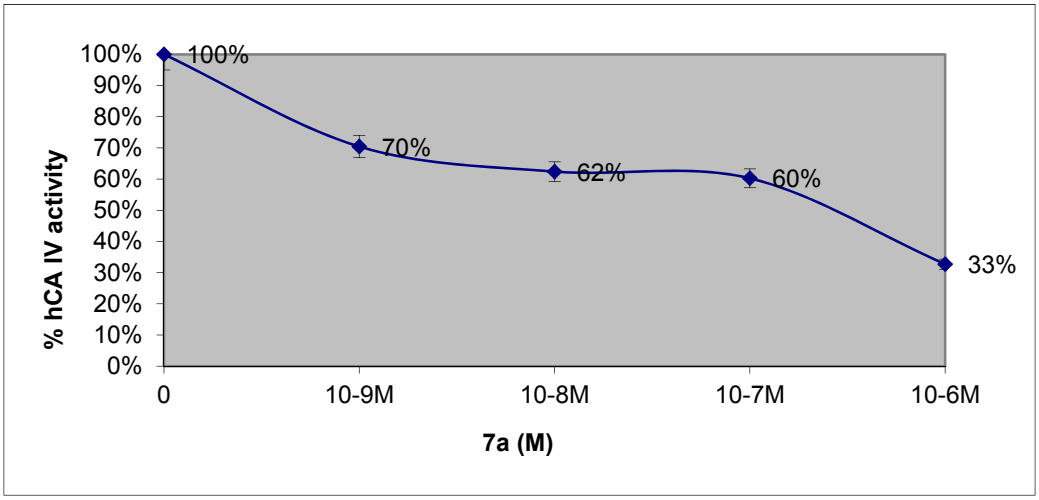

% hCA VII activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
58%
48%
32%
12%
0
10-10M
10-9M
10-8M
10-7M
5d (M)

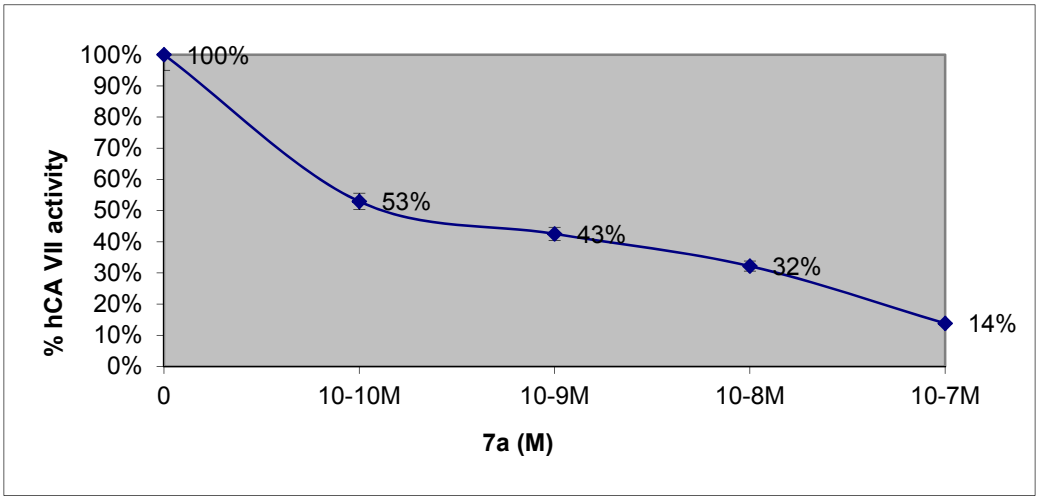

% hCA IX activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
68%
51%
45%
43%
0
10-10M
10-9M
10-8M
10-7M
5d (M)

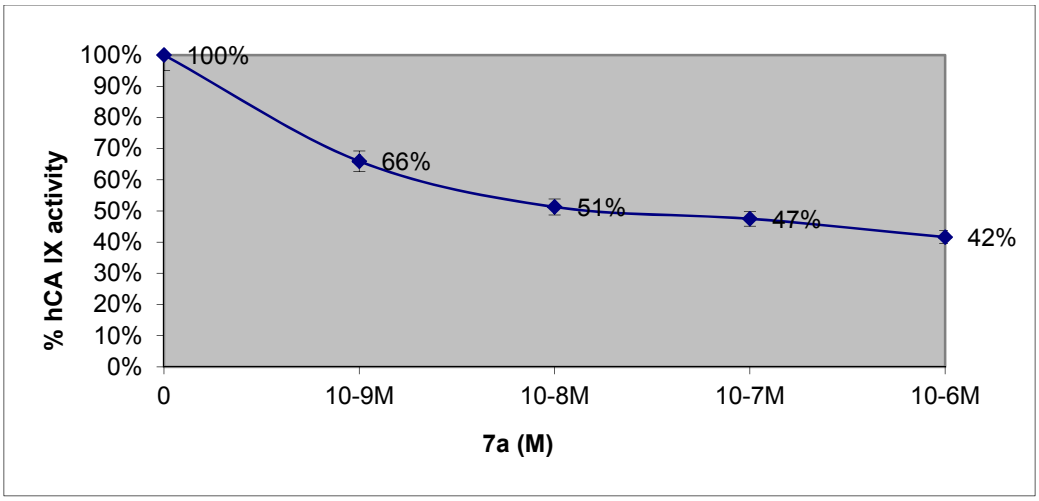
Compound 7a:


% hCA I activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
74%
69%
62%
48%
0
10-9M
10-8M
10-7M
10-6M
7a (M)

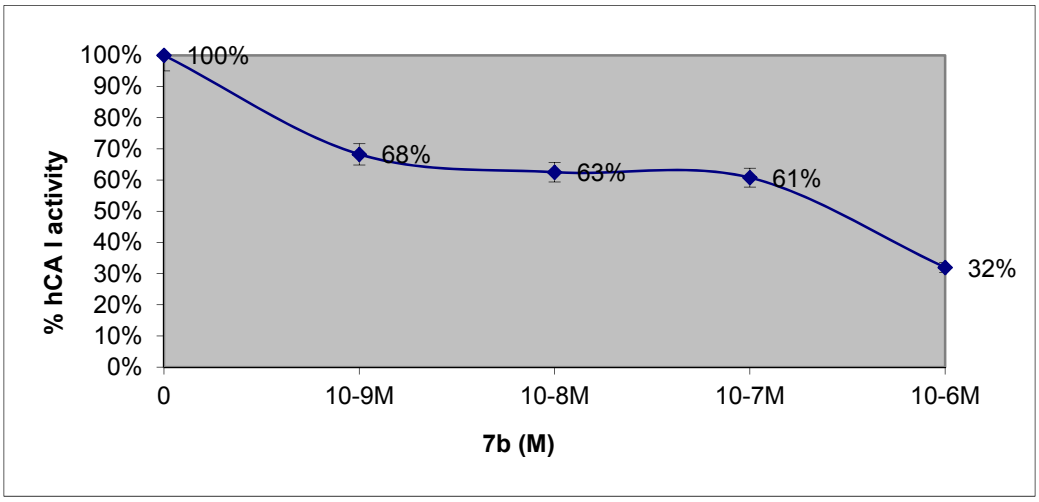

% hCA II activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
60%
51%
30%
8%
0
10-9M
10-8M
10-7M
10-6M
7a (M)

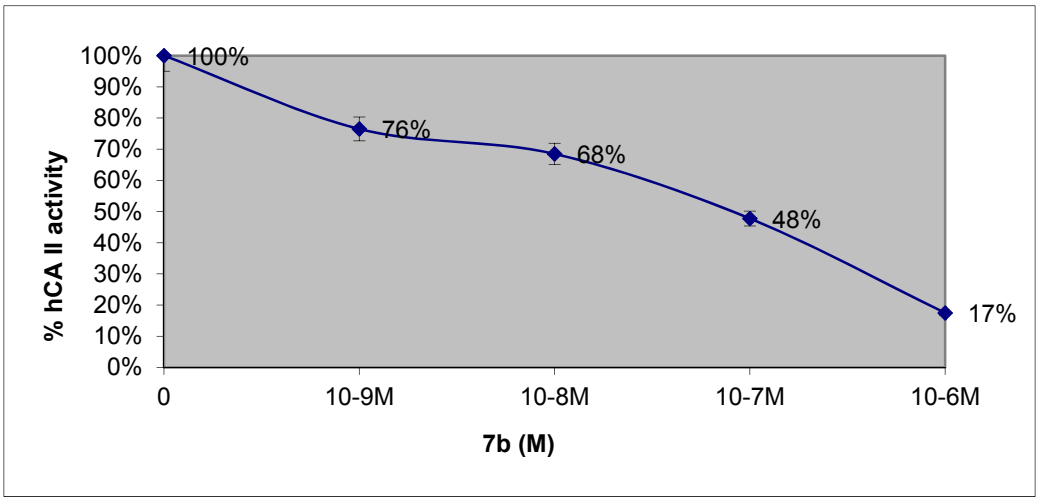

% hCA IV activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
70%
62%
60%
33%
0
10-9M
10-8M
10-7M
10-6M
7a (M)

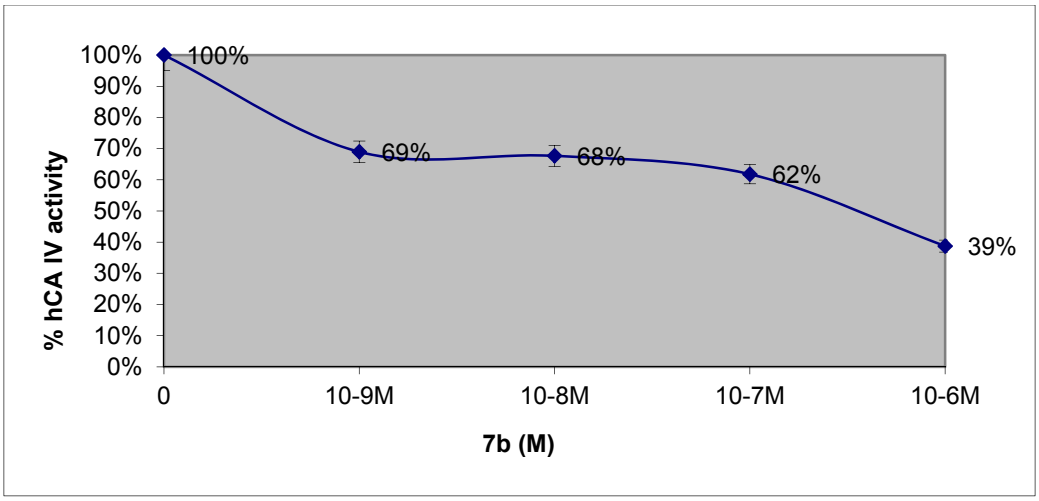

% hCA VII activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
53%
43%
32%
14%
0
10-10M
10-9M
10-8M
10-7M
7a (M)

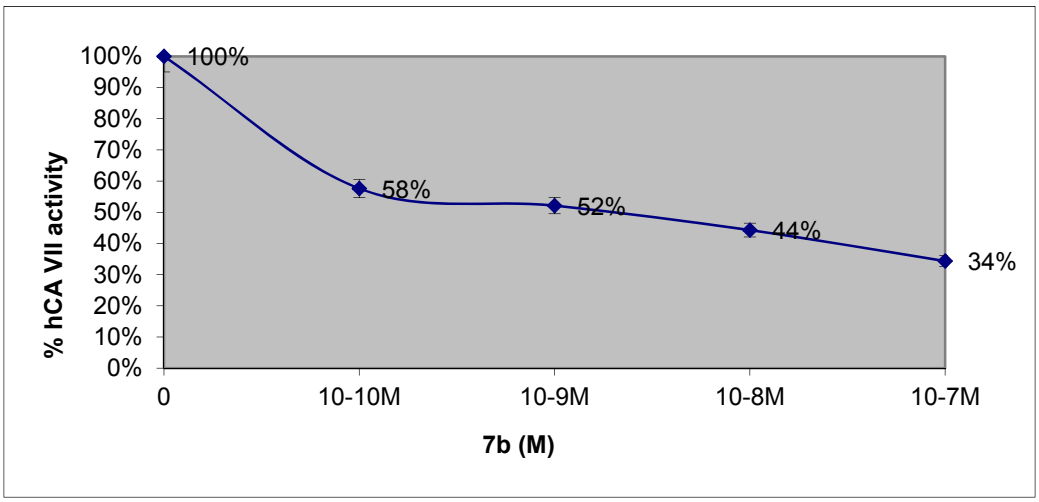

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% hCA IX activity
100%
66%
51%
47%
42%
0
10-9M
10-8M
10-7M
10-6M
7a (M)

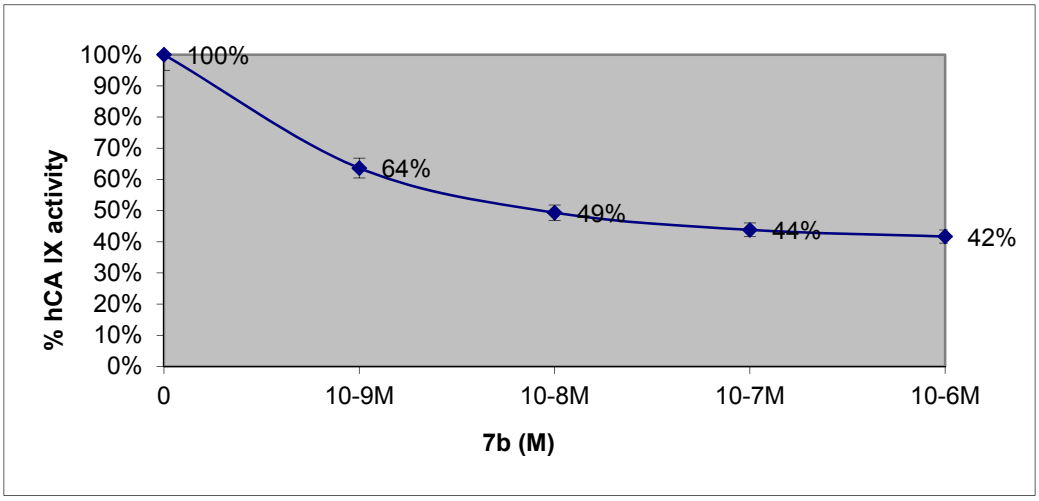
Compound 7b:


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% hCA I activity
100%
68%
63%
61%
32%
0
10-9M
10-8M
10-7M
10-6M
7b (M)

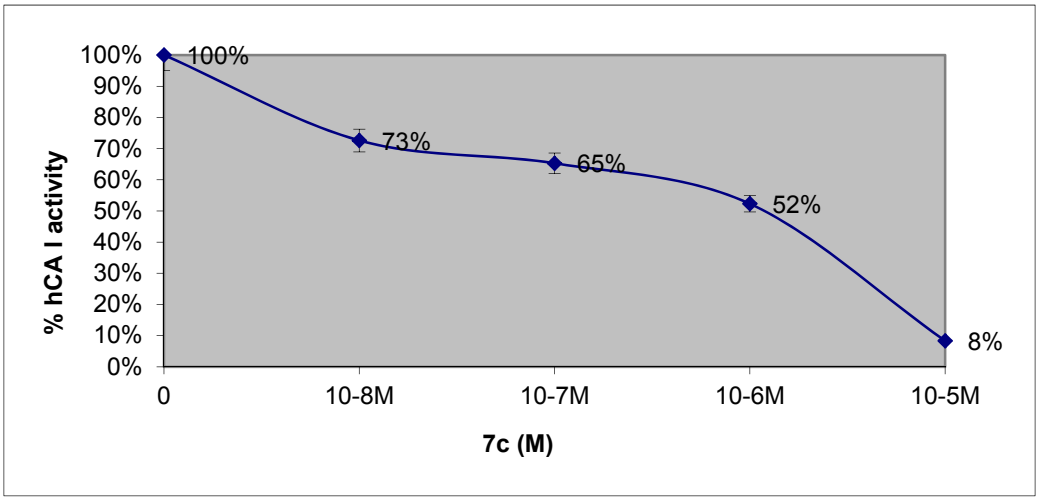

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% hCA II activity
100%
76%
68%
48%
17%
0
10-9M
10-8M
10-7M
10-6M
7b (M)

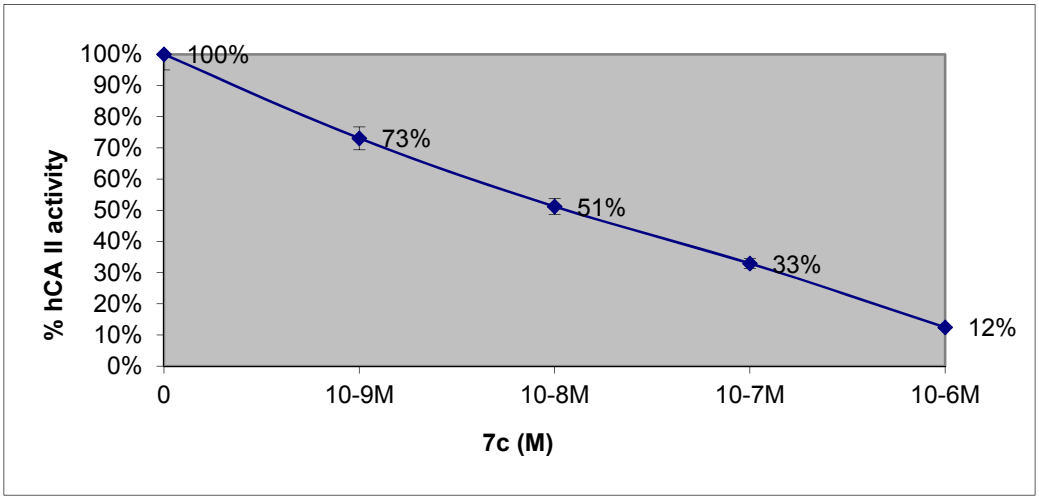

% hCA IV activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
69%
68%
62%
39%
0
10-9M
10-8M
10-7M
10-6M
7b (M)

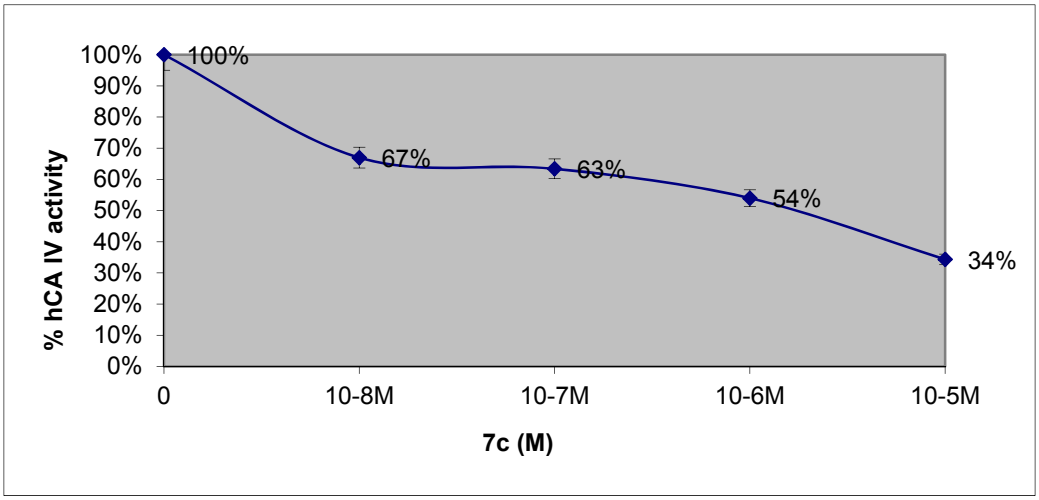

% hCA VII activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
58%
52%
44%
34%
0
10-10M
10-9M
10-8M
10-7M
7b (M)

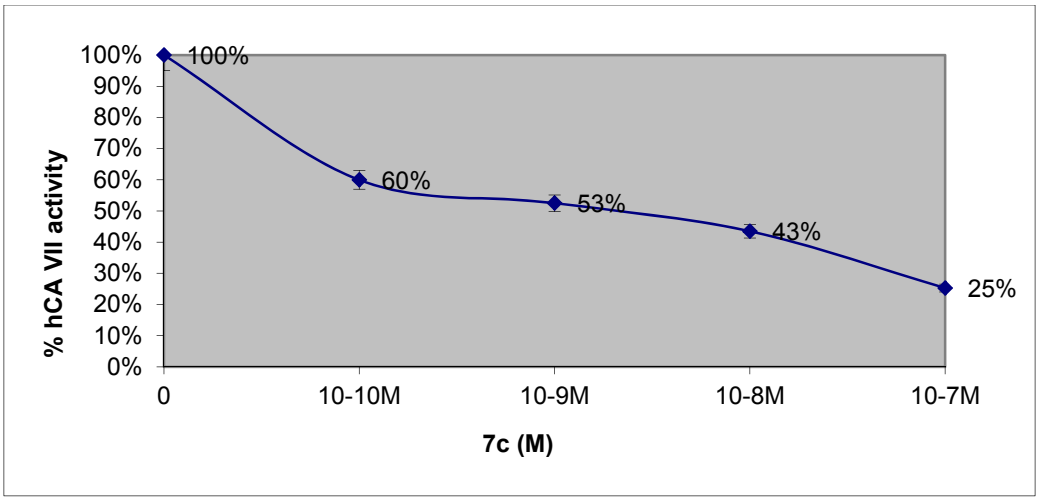

% hCA IX activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
64%
49%
44%
42%
0
10-9M
10-8M
10-7M
10-6M
7b (M)

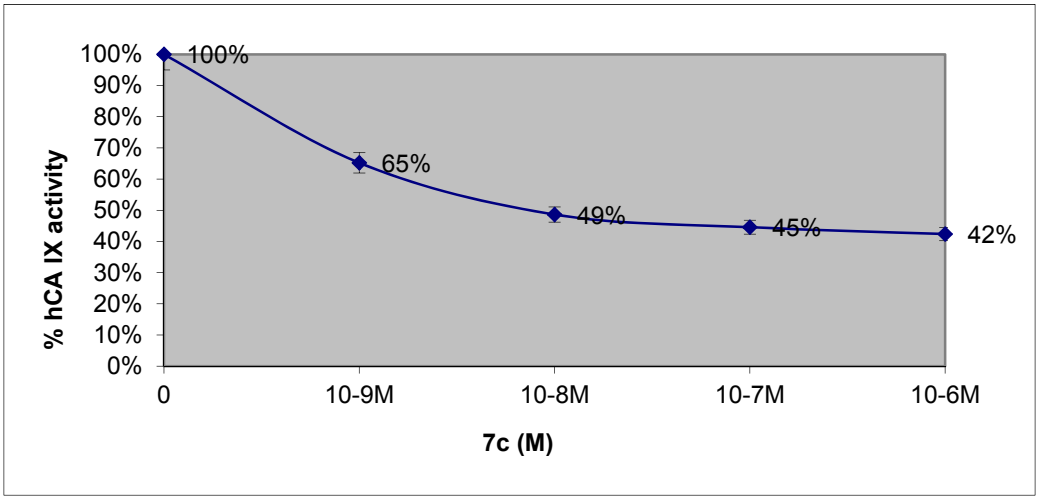
Compound 7c:


% hCA I activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
73%
65%
52%
8%
0
10-8M
10-7M
10-6M
10-5M
7c (M)

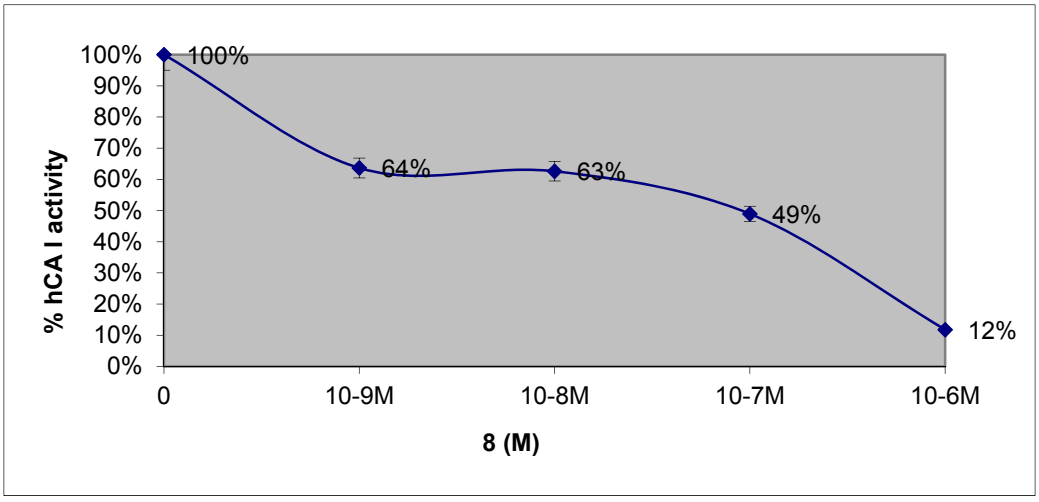

% hCA II activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
73%
51%
33%
12%
0
10-9M
10-8M
10-7M
10-6M
7c (M)

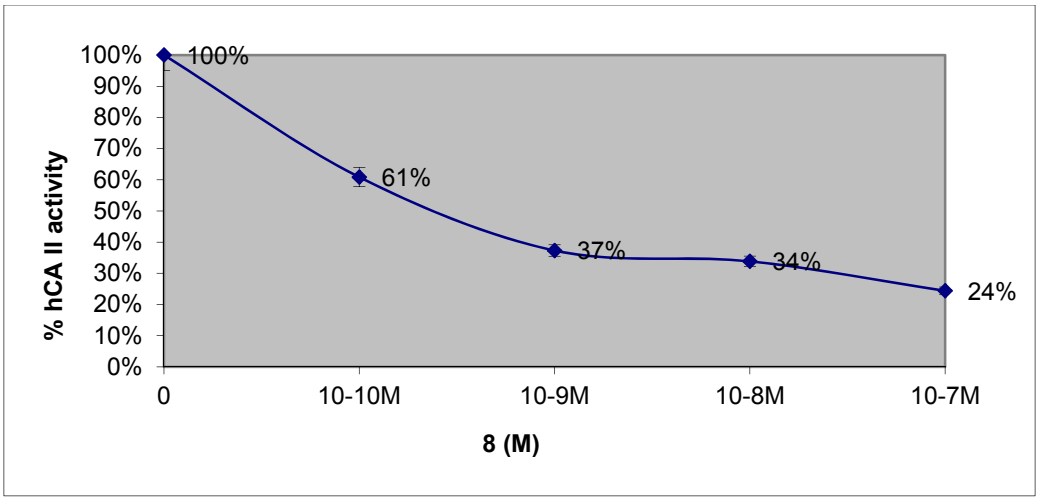

% hCA IV activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
67%
63%
54%
34%
0
10-8M
10-7M
10-6M
10-5M
7c (M)

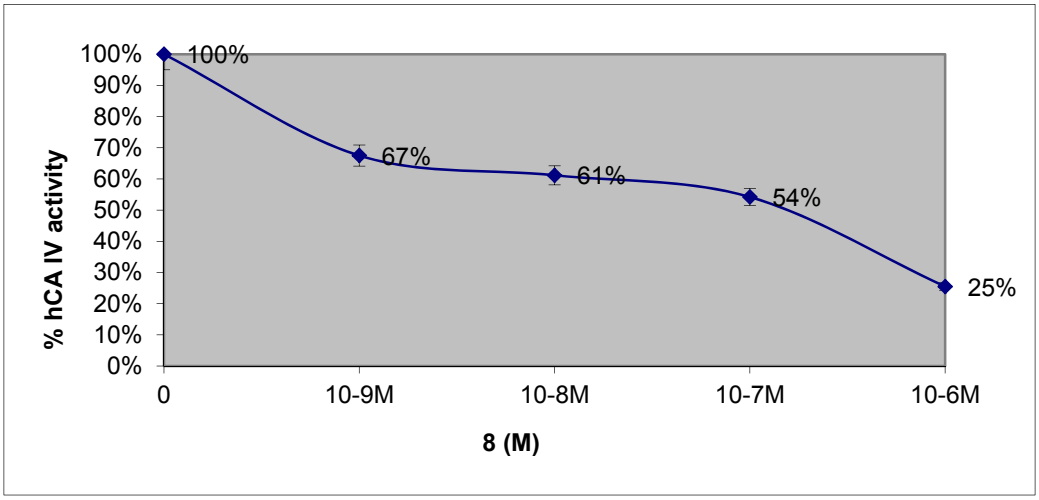

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% hCA VII activity
100%
60%
53%
43%
25%
0
10-10M
10-9M
10-8M
10-7M
7c (M)

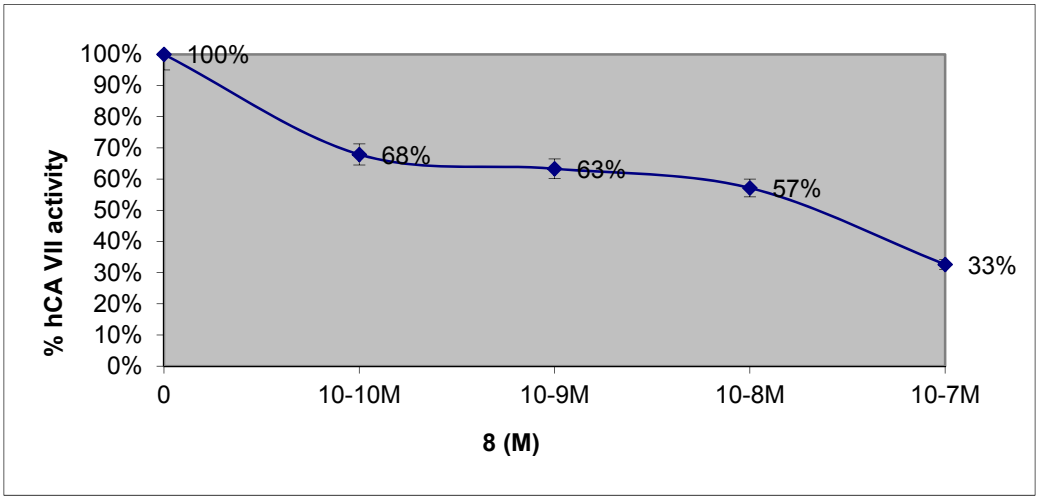

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% hCA IX activity
100%
65%
49%
45%
42%
0
10-9M
10-8M
10-7M
10-6M
7c (M)

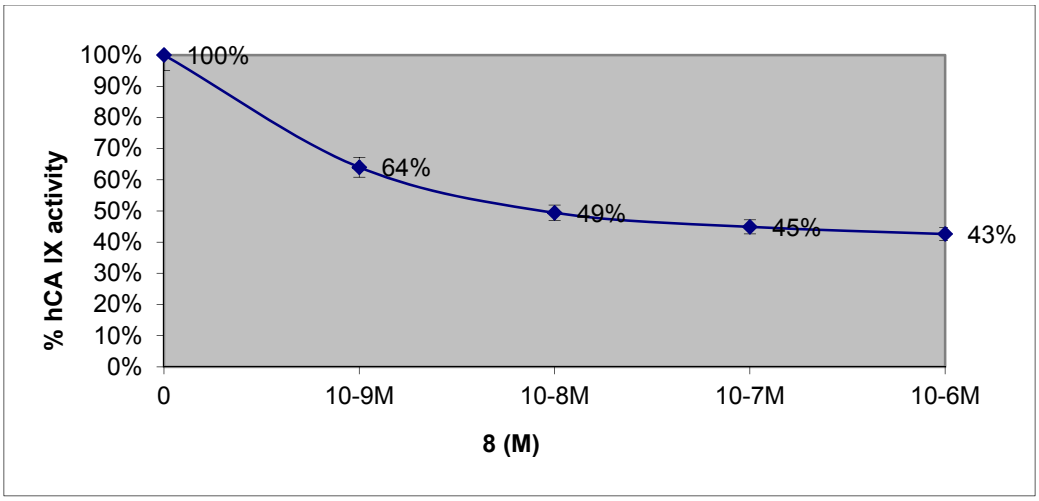
Compound 8:


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% hCA I activity
100%
64%
63%
49%
12%
0
10-9M
10-8M
10-7M
10-6M
8 (M)


% hCA II activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
61%
37%
34%
24%
0
10-10M
10-9M
10-8M
10-7M
8 (M)


% hCA IV activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
67%
61%
54%
25%
0
10-9M
10-8M
10-7M
10-6M
8 (M)


% hCA VII activity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
68%
63%
57%
33%
0
10-10M
10-9M
10-8M
10-7M
8 (M)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% hCA IX activity
100%
64%
49%
45%
43%
0
10-9M
10-8M
10-7M
10-6M
8 (M)

11. References

1. C. Charrat, A. Biscotti, G. Godeau, J. Greiner, P. Vierling, J. M. Guigonis, C. Di Giorgio, Formulation of highly functionalizable DNA nanoparticles based on 1,2-dithiolane derivatives, *ChemBioChem*, **2015**; *16*, 792-804.

2. A. Mollica, M. Locatelli, G. Macedonio, S. Carradori, A.P. Sobolev, R.F. De Salvador, S.M. Monti, M. Buonanno, G. Zengin, A. Angeli, C.T. Supuran. Microwave-assisted extraction, HPLC analysis, and inhibitory effects on carbonic anhydrase I, II, VA, and VII isoforms of 14 blueberry Italian cultivars, *J. Enzyme Inhib. Med. Chem*., **2016**; *31*, 1-6.

3. D. De Vita, A. Angeli, F. Pandolfi, M. Bortolami, R. Costi, R. Di Santo, E. Suffredini, M. Ceruso, S. Del Prete, C. Capasso, L. Scipione, C.T. Supuran, Inhibition of the α-carbonic anhydrase from Vibrio cholerae with amides and sulfonamide incorporating imidazole moieties, *J. Enzyme Inhib. Med. Chem*., **2017**; *32*, 798-804.

4. E. Bruno, M.R. Buemi, A. Di Fiore, L. De Luca, S. Ferro, A. Angeli, R. Cirilli, D. Sadutto, V. Alterio, S.M. Monti, C.T. Supuran, G. De Simone, R. Gitto, Probing Molecular Interactions between Human Carbonic Anhydrases (hCAs) and a Novel Class of Benzenesulfonamides, *J Med Chem.,* **2017**; *60*, 4316-4326.

5. M. Abdoli, A. Angeli, M. Bozdag, F. Carta, A. Kakanejadifard, H. Saeidian, C.T. Supuran, Synthesis and carbonic anhydrase I, II, VII, and IX inhibition studies with a series of benzo[d]thiazole-5- and 6-sulfonamides, *J Enzyme Inhib Med Chem.* **2017**; *32*, 1071-1078.

6. C.B. Mishra, S. Kumari, A. Angeli, S.M. Monti, M. Buonanno, M. Tiwari, C.T. Supuran, Discovery of Benzenesulfonamides with Potent Human Carbonic Anhydrase Inhibitory and Effective Anticonvulsant Action: Design, Synthesis, and Pharmacological Assessment, *J Med Chem.* **2017**; *60***,** 2456-2469.

7. A.A. Abdel-Aziz, A. Angeli, A.S. El-Azab, M.A. Abu El-Enin, C.T. Supuran, Synthesis and biological evaluation of cyclic imides incorporating benzenesulfonamide moieties as carbonic anhydrase I, II, IV and IX inhibitors, *Bioorg Med Chem.* **2017**; *25*, 1666-1671.