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Electronic Supplementary Information

Potent and selective HDAC6 inhibitory activity of *N*-(4-hydroxycarbamoylbenzyl)-1,2,4,9-tetrahydro-3-thia-9-azafluorenes as novel sulfur analogues of Tubastatin A

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Bioassay results (performed by Cerep – www.cerep.com)

Table. % inhibition of control values with regard to HDAC1 and HDAC6 inhibitory activity[a,b]

Compound	% inhibition HDAC1	% inhibition HDAC6	Compound	% inhibition HDAC1	% inhibition HDAC6
5a	26	99	**8a**	51	99
5b	0	38	**8b**	2	73
5c	17	99	**8c**	53	99
5d	0	51	**8d**	8	75

[a] Test concentration: 10 μM; [b] Mean value of two screening sessions

HDAC6 (*h*)

Compound	Test Concentration (M)	% of Control Values		
		1st	2nd	mean
5a	3.0E-10	100.8	100.8	100.8
5a	3.0E-09	94.4	85.8	90.1
5a	3.0E-08	28.2	29.8	29.0
5a	3.0E-07	1.2	3.2	2.2
5a	3.0E-06	-1.5	-0.2	-0.9
5a	3.0E-05	-1.2	-1.9	-1.5
5c	3.0E-10	106.1	105.8	106.0
5c	3.0E-09	93.9	103.9	98.9
5c	3.0E-08	44.4	38.0	41.2
5c	3.0E-07	3.7	-1.3	1.2
5c	3.0E-06	-0.2	-0.4	-0.3
5c	3.0E-05	-0.2	1.1	0.4
8a	3.0E-10	121.7	99.2	110.5
8a	3.0E-09	46.3	44.7	45.5
8a	3.0E-08	4.1	5.7	4.9
8a	3.0E-07	1.5	-1.1	0.2
8a	3.0E-06	-1.0	-0.5	-0.8
8a	3.0E-05	-0.7	0.9	0.1
8b	1.0E-08	109.7	109.7	109.7
8b	1.0E-07	98.5	99.9	99.2
8b	1.0E-06	74.8	69.2	72.0
8b	3.0E-06	47.2	41.0	44.1
8b	1.0E-05	19.3	16.0	17.7
8b	1.0E-04	3.3	3.3	3.3
8c	3.0E-10	92.0	93.9	93.0
8c	3.0E-09	53.0	57.0	55.0
8c	3.0E-08	6.9	5.5	6.2
8c	3.0E-07	0.2	1.0	0.6
8c	3.0E-06	-0.6	-0.3	-0.4

8c	3.0E-05	0.0	-0.4	-0.2
8d	1.0E-08	107.4	100.2	103.8
8d	1.0E-07	98.2	95.7	97.0
8d	1.0E-06	58.9	57.9	58.4
8d	3.0E-06	40.6	37.6	39.1
8d	1.0E-05	13.1	12.0	12.5
8d	1.0E-04	5.8	6.7	6.3

HDAC1 (*h*)

Compound	Test Concentration (M)	% of Control Values		
		1st	2nd	mean
8a	3.0E-08	108.9	103.8	106.3
8a	3.0E-07	106.1	103.5	104.8
8a	1.0E-06	98.6	94.4	96.5
8a	3.0E-06	81.2	81.5	81.4
8a	1.0E-05	55.9	60.2	58.0
8a	1.0E-04	10.3	11.5	10.9
8c	3.0E-08	108.2	104.9	106.6
8c	3.0E-07	102.4	103.2	102.8
8c	1.0E-06	97.0	99.1	98.0
8c	3.0E-06	80.5	86.8	83.7
8c	1.0E-05	55.2	63.6	59.4
8c	1.0E-04	10.8	11.6	11.2

HDAC4 (*h*)

Compound	Test Concentration (M)	% of Control Values		
		1st	2nd	mean
8a	3.0E-08	100.7	102.9	**101.8**
8a	3.0E-07	85.4	90.9	**88.2**
8a	1.0E-06	62.3	60.8	**61.5**
8a	3.0E-06	38.4	41.4	**39.9**
8a	1.0E-05	26.2	21.3	**23.8**
8a	1.0E-04	3.9	4.7	**4.3**
8c	3.0E-08	99.3	98.0	**98.6**
8c	3.0E-07	91.2	82.3	**86.7**
8c	1.0E-06	56.5	56.0	**56.3**
8c	3.0E-06	46.3	48.1	**47.2**
8c	1.0E-05	20.5	27.5	**24.0**
8c	1.0E-04	3.6	4.2	**3.9**

HDAC8 (*h*)

Compound	Test Concentration (M)	% of Control Values		
		1st	2nd	mean
8a	3.0E-08	96.5	82.1	**89.3**
8a	3.0E-07	77.6	83.7	**80.7**
8a	1.0E-06	80.0	51.5	**65.7**
8a	3.0E-06	23.4	18.7	**21.1**
8a	1.0E-05	-2.7	2.8	**0.0**
8a	1.0E-04	-12.0	-12.7	**-12.4**
8c	3.0E-08	92.4	93.4	**92.9**
8c	3.0E-07	71.9	64.2	**68.0**
8c	1.0E-06	51.5	47.4	**49.5**
8c	3.0E-06	29.2	26.9	**28.0**
8c	1.0E-05	10.9	22.2	**16.5**
8c	1.0E-04	14.7	16.0	**15.3**

HDAC11 (*h*)

Compound	Test Concentration (M)	% of Control Values		
		1st	2nd	mean
8a	3.0E-08	101.3	99.7	**100.5**
8a	3.0E-07	100.6	99.2	**99.9**
8a	1.0E-06	98.3	99.3	**98.8**
8a	3.0E-06	98.4	99.2	**98.8**
8a	1.0E-05	99.5	100.0	**99.8**
8a	1.0E-04	98.6	99.0	**98.8**
8c	3.0E-08	99.7	100.1	**99.9**
8c	3.0E-07	100.3	99.3	**99.8**
8c	1.0E-06	100.6	99.6	**100.1**
8c	3.0E-06	99.1	99.5	**99.3**
8c	1.0E-05	98.8	98.9	**98.8**
8c	1.0E-04	77.3	77.5	**77.4**

Ligand docking – experimental details

All manipulations were performed with the molecular modelling program YASARA and the YASARA/WHATIF twinset. [1,2] The HDAC6 sequence was obtained from the UniProt database (www.uniprot.org; UniProt entry Q9UBN7). To increase the accuracy of the model, the sequence was limited to the major functional domain of HDAC6 (Gly482-Gly800). Possible templates were identified by running 3 PSI-BLAST iterations to extract a position specific scoring matrix (PSSM) from UniRef90, and then searching the PDB for a match. To aid the alignment of the HDAC6 sequence and templates, and the modelling of the loops, a secondary structure prediction was performed, followed by multiple sequence alignments. All side chains were ionised or kept neutral according to their predicted pKa values. Initial models were created from different templates, each with several alignment variations and up to hundred conformations tried per loop. After the side-chains had been built, optimised and fine-tuned, all newly modelled parts were subjected to a combined steepest descent and simulated annealing minimisation, i.e. the backbone atoms of aligned residues were kept fixed to preserve the folding, followed by a full unrestrained simulated annealing minimisation for the entire model. The final model was obtained as a hybrid model of the best parts of the initial models, and checked once more for anomalies like incorrect configurations or colliding side chains. Furthermore, it was structurally aligned with known HDAC crystal structures to check if the chelating residues and the zinc atom were arranged correctly.

The HDAC inhibitor structures were created with YASARA Structure1 and energy minimised with the AMBER03 force field.[3] The grid box used for docking had a dimension of 25 x 25 x 25 angstrom with a grid spacing of 0.2 Å, and comprised the entire catalytic cavity including the Zn ion and the outer surface of the active site entrance. Docking was performed with AutoDock 4.2[4] using the AMBER03 force field3 and default parameters. Ligands were allowed to freely rotate during docking. The figure was created with PyMol v1.3.[5]

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Synthetic procedures and spectral data of compounds 3b, 4a-d, 5a-d, 6b, 7a-d and 8a-d

General

^{1}H NMR spectra were recorded at 300 MHz (JEOL ECLIPSE+) with $CDCl_3$ or d_6-DMSO as solvent and tetramethylsilane as internal standard. ^{13}C NMR spectra were recorded at 75 MHz (JEOL ECLIPSE+) with $CDCl_3$ or d_6-DMSO as solvent and tetramethylsilane as internal standard. Mass spectra were obtained with a mass spectrometer Agilent 1100, 70 eV. IR spectra were measured with a Spectrum One FT-IR spectrophotometer. High resolution electron spray (ES) mass spectra were obtained with an Agilent Technologies 6210 series time-of-flight instrument. Melting points of crystalline compounds were measured with a Büchi 540 apparatus. The purity of all tested compounds was assessed by HRMS analysis and/or HPLC analysis, confirming a purity of ≥95%.

a) General procedure for the preparation of 1,2,4,9-tetrahydro-3-thia-9-azafluorenes 3

To a solution of phenyl hydrazine hydrochloride **1** (12 mmol) and tetrahydrothiopyran-4-one **2** (12 mmol) in methanol (50 mL) was added $Bi(NO_3)_3{\cdot}5H_2O$ (2.4 mmol). After being stirred for 2 h under reflux, the reaction mixture was poured into water (100 mL), and bismuth nitrate was removed trough filtration. The crude product was extracted with ethyl acetate (100 mL), washed with saturated $NaHCO_3$ (100 mL), brine (100 mL) and dried over anhydrous $MgSO_4$. Filtration of the drying agent and removal of the solvent *in vacuo* afforded the crude thioether **3**, which was purified by means of recrystallization from ethanol to provide pure 1,2,4,9-tetrahydro-3-thia-9-azafluorene **3** (10.2 mmol, 85%).

b) General procedure for the preparation of sulfones 6

To a solution of 1,2,4,9-tetrahydro-3-thia-9-azafluorene **3** (5 mmol) in tetrahydrofuran (50 mL) was added *m*-chloroperbenzoic acid in tetrahydrofuran (>70%, 15 mmol) at 0°C. The mixture was stirred at room temperature for 2 h. The solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate (100 mL). The solution was washed with saturated aqueous sodium sulfite (30 mL), water (30 mL), brine (2 × 30 mL), and dried over anhydrous $MgSO_4$. Filtration of the drying agent and removal of the solvent *in vacuo* afforded the crude sulfone **6**, which was purified by recrystallization from EtOH to provide pure 1,2,4,9-tetrahydro-3-thia-9-azafluorene-3,3-dioxide **6** (3.85 mmol, 77%).

c) General procedure for the preparation of esters 4 and 7

1,2,4,9-Tetrahydro-3-thia-9-azafluorene **3** (6 mmol) and sodium hydride (60 wt % in mineral oil, 6 mmol) were placed under nitrogen and dissolved in DMF (10 mL). After stirring for 30 minutes, methyl 4-(bromomethyl)benzoate (6 mmol) and potassium iodide (10 mg) were added to the reaction. The reaction was heated to 80 °C for 2 h, after which the reaction was quenched with water (30 mL) followed by addition of ethyl acetate (30 mL). The aqueous layer was extracted with ethyl acetate (2 × 10 mL) and the

combined organic layers were washed with water (2 × 20 mL), brine (15 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Recrystallization from ethanol afforded pure *N*-(4-methoxycarbonylbenzyl)-1,2,4,9-tetrahydro-3-thia-9-azafluorene **4** (3.12 mmol, 52%).

d) General procedure for the preparation of hydroxamic acids 5 and 8

To a solution of ester **4** (0.6 mmol) and hydroxylamine hydrochloride (3.6 mmol) in DMF (5 mL) under nitrogen atmosphere was added NaOMe/MeOH (4M, 1.2 mL, 4.8 mmol). The reaction was stirred for 16 h at room temperature and a white precipitate was formed. The reaction mixture was diluted with ethyl acetate (20 mL) and extracted with saturated $NaHCO_3$ (10 mL), brine (2 × 10mL), and dried with anhydrous $MgSO_4$. Filtration of the drying agent and removal of the solvent *in vacuo* afforded the crude hydroxamic acid **5**, which was recrystallized from ethanol to afford pure *N*-(4-hydroxycarbamoylbenzyl)-1,2,4,9-tetrahydro-3-thia-9-azafluorene **5** (0.23 mmol, 38%).

Table. Synthesis of tetrahydro-3-thia-9-azafluorenes **3**-**5** and their oxidized analogues **6**-**8**

Entry	R^1	R^2	Compound (yield)[a]
1	H	-	**3a** (85%)
2	F	-	**3b** (90%)
3	H	H	**4a** (52%)
4	H	MeO	**4b** (57%)
5	F	H	**4c** (69%)
6	F	MeO	**4d** (78%)
7	H	H	**5a** (38%)
8	H	MeO	**5b** (65%)
9	F	H	**5c** (70%)
10	F	MeO	**5d** (66%)
11	H	-	**6a** (77%)
12	F	-	**6b** (80%)
13	H	H	**7a** (48%)
14	H	MeO	**7b** (60%)
15	F	H	**7c** (47%)
16	F	MeO	**7d** (40%)
17	H	H	**8a** (51%)
18	H	MeO	**8b** (30%)
19	F	H	**8c** (69%)
20	F	MeO	**8d**(72%)

[a] Yields after purification by column chromatography (SiO_2) or recrystallization

6-Fluoro-1,2,4,9-tetrahydro-3-thia-9-azafluorene (3b).

Recrystallization from EtOH. Mp = 137.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.98 (4H, s); 3.79 (2H, s); 6.87 (1H, t × d, *J* = 9.1, 2.8 Hz); 7.07 (1H, d × d, *J* = 9.1, 2.8 Hz); 7.16 (1H, d × d, *J* = 9.1, 4.4 Hz); 7.80 (1H, s(br)). ^{19}F NMR (282 MHz, $CDCl_3$): δ (-124.46) – (-124.37) (m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.7, 25.3, 25.7, 102.9 (d, *J* = 24.2 Hz), 107.1 (d, *J* = 4.6 Hz), 109.7 (d, *J* = 26.6 Hz), 111.1 (d, *J* = 10.4 Hz), 127.4 (d, *J* = 9.2 Hz), 131.0, 135.3, 158.0 (d, *J* = 234.2 Hz). IR (ATR, cm^{-1}): ν_{NH} = 3336. MS (70eV): m/z (%) 206 (M^{-}-1, 100). HRMS (ESI) Anal. Calcd. for $C_{11}H_{11}FNS$ 208.0596 [M+H]+, Found 208.0595.

N-(4-Methoxycarbonylbenzyl)-1,2,4,9-tetrahydro-3-thia-9-azafluorene (4a).

Recrystallization from EtOH. Mp = 120.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.81 and 2.96 (2 × 2H, 2 × t, *J* = 5.7 Hz); 3.85 (3H, s); 3.88 (2H, s); 5.23 (2H, s); 6.99 (2H, d, *J* = 8.2 Hz); 7.10-7.15 and 7.47-7.50 (3H and 1H, 2 × m); 7.91 (2H, d, *J* = 8.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.2, 24.1, 26.0, 46.1, 52.3, 107.4, 109.1, 117.9, 119.7, 121.8, 126.2, 126.9, 129.5, 130.3, 134.7, 135.8, 143.1, 166.8. IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1717. MS (70eV): m/z (%) 338 (M^{+}+1, 65). HRMS (ESI) Anal. Calcd. for $C_{20}H_{20}NO_2S$ 338.1215 $[M+H]^{+}$, Found 338.1218.

N-(4-Methoxycarbonyl-2-methoxybenzyl)-1,2,4,9-tetrahydro-3-thia-9-azafluorene (4b).

Recrystallization from EtOH/EtOAc (1/1). Mp = 155.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.86 and 3.00 (2 × 2H, 2 × t, *J* = 5.8 Hz); 3.88 (3H, s); 3.94 (2H, s); 3.98 (3H, s); 5.27 (2H, s); 6.34 (1H, d, *J* = 7.9 Hz); 7.08-7.19 (3H, m); 7.42 (1H, d × d, *J* = 7.9, 1.4 Hz); 7.50-7.54 (1H, m); 7.56 (1H, d, *J* = 1.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.2, 23.9, 26.0, 41.6, 52.3, 55.7, 107.1, 109.1, 110.7, 117.7, 119.5, 121.6, 122.4, 126.3, 126.9, 130.4, 131.3, 134.8, 135.8, 156.3, 166.9. IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1716. MS (70eV): m/z (%) 368 (M^{+}+1, 67). HRMS (ESI) Anal. Calcd. for $C_{21}H_{22}NO_3S$ 368.1320 $[M+H]^{+}$, Found 368.1318.

N-(4-Methoxycarbonylbenzyl)-6-fluoro-1,2,4,9-tetrahydro-3-thia-9-azafluorene (4c).

Recrystallization from EtOH. Mp = 125.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.86 and 2.99 (2 × 2H, 2 × t, *J* = 5.5 Hz); 3.85 (2H, s); 3.88 (3H, s); 5.28 (2H, s); 6.87 (1H, t × d, *J* = 9.1, 2.8 Hz); 7.00 (2H, d, *J* = 8.3 Hz); 7.07 (1H, d × d, *J* = 9.1, 4.4 Hz); 7.14 (1H, d × d, *J* = 9.1, 2.8 Hz); 7.94 (2H, d, *J* = 8.3 Hz). ^{19}F NMR (282 MHz, $CDCl_3$): δ (-124.42) – (-124.34) (m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.0, 24.3, 25.9, 46.3, 52.3, 103.1 (d, *J* = 24.2 Hz), 107.5 (d, *J* = 4.6 Hz), 109.6 (d, *J* = 9.2 Hz), 109.8 (d, *J* = 26.5 Hz), 126.0, 127.2 (d, *J* = 10.4 Hz), 129.6, 130.3, 132.3, 136.4, 142.6, 158.0 (d, *J* = 235.4 Hz), 166.7. IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1712. MS (70eV): m/z (%) 356 (M^{+}+1, 30). HRMS (ESI) Anal. Calcd. for $C_{20}H_{19}FNO_2S$ 356.1121 $[M+H]^{+}$, Found 356.1121.

N-(4-Methoxycarbonyl-2-methoxybenzyl)-6-fluoro-1,2,4,9-tetrahydro-3-thia-9-azafluorene (4d).

Recrystallization from EtOH. Mp = 139.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.86 and 3.00 (2 × 2H, 2 × t, *J* = 5.7 Hz); 3.87 (2H, s); 3.89 and 3.98 (2 × 3H, 2 × s); 5.25 (2H, s); 6.31 (1H, d, *J* = 7.7 Hz); 6.86 (1H, t × d, *J* = 9.1, 2.8 Hz); 7.06 (1H, d × d, *J* = 9.1, 4.4 Hz); 7.16 (1H, d × d, *J* = 9.1, 2.8 Hz); 7.43 (1H, d × d, *J* = 7.7, 1.1 Hz); 7.56 (1H, d, *J* = 1.1 Hz). ^{19}F NMR (282 MHz, $CDCl_3$): δ (-124.68) – (-124.60) (m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.1, 24.1, 25.9, 41.8, 52.3, 55.7, 103.0 (d, *J* = 24.2 Hz), 107.2 (d, *J* = 4.6 Hz), 109.6 (d, *J* = 26.5 Hz), 109.7 (d, *J* = 10.4 Hz), 110.7, 122.4, 126.2, 127.1 (d, *J* = 9.3 Hz), 130.5, 131.0, 132.3, 136.6, 156.3, 158.0 (d, *J* = 235.4 Hz), 166.8. IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1714. MS (70eV): m/z (%) 386 (M^{+}+1, 100). HRMS (ESI) Anal. Calcd. for $C_{21}H_{21}FNO_3S$ 386.1226 $[M+H]^{+}$, Found 386.1218.

N-(4-Hydroxycarbamoylbenzyl)-1,2,4,9-tetrahydro-3-thia-9-azafluorene (5a).

Recrystallization from EtOH. Mp = 149.6 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 2.87 and 2.98 (2 × 2H, 2 × t, *J* = 5.4 Hz); 3.83 (2H, s); 5.42 (2H, s); 6.99-7.10 and 7.39-7.41 (4H and 1H, 2 × m); 7.47 (1H, d × d, *J* = 6.9, 1.4 Hz); 7.65 (2H, d, *J* = 8.3 Hz); 9.02 (1H, s(br)); 11.14 (1H, s(br)). ^{13}C NMR (75 MHz, d_6-DMSO): δ 22.8, 24.1, 25.7, 45.7, 107.0, 110.0, 118.1, 119.5, 121.7, 126.8, 126.9, 127.8, 132.4, 135.5, 135.8, 142.1, 164.5. IR (ATR, cm^{-1}): $\nu_{NH/OH}$ = 3201; $\nu_{C=O}$ = 1636. MS (70eV): m/z (%) 339 (M^++1, 100). HRMS (ESI) Anal. Calcd. for $C_{19}H_{19}N_2O_2S$ 339.1167 $[M+H]^+$, Found 339.1164.

N-(4-Hydroxycarbamoyl-2-methoxybenzyl)-1,2,4,9-tetrahydro-3-thia-9-azafluorene (5b).

Recrystallization from EtOH. Mp = 191.5 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 2.82 and 2.96 (2 × 2H, 2 × t, *J* = 5.4 Hz); 3.84 (2H, s); 3.92 (3H, s); 5.30 (2H, s); 6.22 (1H, d, *J* = 7.7 Hz); 6.99-7.08 (2H, m); 7.15 (1H, d × d, *J* = 7.7, 1.1 Hz); 7.28-7.30 (1H, m); 7.39 (1H, d, *J* = 1.1 Hz); 7.46-7.49 (1H, m); 9.04 (1H, s(br)); 11.16 (1H, s(br)). ^{13}C NMR (75 MHz, d_6-DMSO): δ 22.9, 23.9, 25.7, 41.4, 56.2, 106.9, 109.7, 109.9, 118.1, 119.5, 121.6, 126.5, 126.8, 129.5, 133.6, 135.7, 135.8, 156.6, 164.3. IR (ATR, cm^{-1}): $\nu_{NH/OH}$ = 3220; $\nu_{C=O}$ = 1619. MS (70eV): m/z (%) 369 (M^++1, 100). HRMS (ESI) Anal. Calcd. for $C_{20}H_{21}N_2O_3S$ 369.1273 $[M+H]^+$, Found 369.1280.

N-(4-Hydroxycarbamoylbenzyl)-6-fluoro-1,2,4,9-tetrahydro-3-thia-9-azafluorene (5c).

Recrystallization from ethanol. Mp = 194.5 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 2.86 and 2.97 (2 × 2H, 2 × t, *J* = 5.7 Hz); 3.80 (2H, s); 5.42 (2H, s); 6.91 (1H, t × d, *J* = 9.1, 2.8 Hz); 7.03 (2H, d, *J* = 8.3 Hz); 7.26 (1H, d × d, *J* = 9.1, 2.8 Hz); 7.41 (1H, d × d, *J* = 9.1, 4.4 Hz); 7.65 (2H, d, *J* = 8.3 Hz), 9.01 (1H, s(br)); 11.13 (1H, s(br)). ^{19}F NMR (282 MHz, d_6-DMSO): δ (-124.75) – (-124.66) (m). ^{13}C NMR (75 MHz, d_6-DMSO): δ 22.7, 24.3, 25.6, 45.9, 103.3 (d, *J* = 23.1 Hz), 107.3 (d, *J* = 4.6 Hz), 109.4 (d, *J* = 26.5 Hz), 111.0 (d, *J* = 10.4 Hz), 126.8, 127.1 (d, *J* = 10.4 Hz), 127.8, 132.4, 132.5, 137.6, 141.9, 157.6 (d, *J* = 230.7 Hz), 164.5. IR (ATR, cm^{-1}): $\nu_{NH/OH}$ = 3224; $\nu_{C=O}$ = 1613. MS (70eV): m/z (%) 355 (M^--1, 100). HRMS (ESI) Anal. Calcd. for $C_{19}H_{16}FN_2O_2S$ 355.0922 $[M-H]^-$, Found 355.0924.

N-(4-Hydroxycarbamoyl-2-methoxybenzyl)-6-fluoro-1,2,4,9-tetrahydro-3-thia-9-azafluorene (5d).

Recrystallization from EtOH. Mp = 148.1 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 2.82 and 2.96 (2 × 2H, 2 × t, *J* = 5.5 Hz); 3.80 (2H, s); 3.90 (3H, s); 5.30 (2H, s); 6.24 (1H, d, *J* = 7.7 Hz); 6.87 (1H, t × d, *J* = 9.1, 2.8 Hz); 7.15 (1H, d, *J* = 7.7 Hz); 7.26 (1H, d × d, *J* = 9.1, 2.8 Hz); 7.31 (1H, d × d, *J* = 9.1, 4.4 Hz); 7. 38 (1H, s); 9.03 (1H, s(br)); 11.15 (1H, s(br)). ^{19}F NMR (282 MHz, d_6-DMSO): δ (-124.81) – (-124.73) (m). ^{13}C NMR (75 MHz, d_6-DMSO): δ 22.7, 24.1, 25.6, 41.7, 56.2, 103.2 (d, *J* = 24.3 Hz), 107.2 (d, *J* = 4.7 Hz), 109.3 (d, *J* = 26.5 Hz), 109.7, 111.0 (d, *J* = 10.4 Hz), 119.6, 126.6, 127.0 (d, *J* = 10.3 Hz), 129.3, 132.5, 133.7, 137.8, 156.7, 157.6 (d, *J* = 231.9 Hz), 164.3. IR (ATR, cm^{-1}): $\nu_{NH/OH}$ = 3209; $\nu_{C=O}$ = 1636. MS (70eV): m/z (%) 385 (M^--1, 100). HRMS (ESI) Anal. Calcd. for $C_{20}H_{20}FN_2O_3S$ 387.1179 $[M+H]^+$, Found 387.1180.

6-Fluoro-1,2,4,9-tetrahydro-3-thia-9-azafluorene-3,3-dioxide (6b).

Recrystallization from EtOH. Mp = 260.4 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 3.24 and 3.46 (2 × 2H, 2 × t, *J* = 6.1 Hz); 4.40 (2H, s); 6.90 (1H, t × d, *J* = 9.1, 2.8 Hz); 7.21 (1H, d × d, *J* = 9.1, 2.8 Hz); 7.30 (1H, d × d, *J* = 9.1, 4.4 Hz); 11.23 (1H, s(br)). ^{19}F NMR (282 MHz, d_6-DMSO): δ (-124.67) – (-124.58) (m). ^{13}C NMR (75 MHz, d_6-DMSO): δ 23.3, 47.2, 48.9, 102.4 (d, *J* = 4.6 Hz), 103.0 (d, *J* = 24.2 Hz), 109.9 (d, *J* = 25.4 Hz), 112.5 (d, *J* = 10.4 Hz), 127.6 (d, *J* = 10.3 Hz), 132.9, 133.3, 157.4 (d, *J* = 231.9 Hz). IR (ATR, cm^{-1}): ν_{NH} = 3359; $\nu_{S=O}$ = 1119, 1102. MS (70eV): m/z (%) 238 (M^--1, 100). HRMS (ESI) Anal. Calcd. for $C_{11}H_9FNO_2S$ 238.0344 $[M-H]^-$, Found 238.0344.

N-(4-Methoxycarbonylbenzyl)-1,2,4,9-tetrahydro-3-thia-9-azafluorene-3,3-dioxide (7a).

Rf = 0.15 (EtOAc/PE 1/2). Mp = 163.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27 and 3.32 (2 × 2H, 2 × t, *J* = 4.7 Hz); 3.89 (3H, s); 4.44 (2H, s); 5.34 (2H, s); 7.04 (2H, d, *J* = 8.2 Hz); 7.15-7.24 and 7.43-7.46 (3H and 1H, 2 × m); 7.96 (2H, d, *J* = 8.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.3, 46.6, 47.5, 49.0, 52.3, 102.6, 109.6, 117.7, 120.6, 123.1, 126.0, 126.5, 129.9, 130.1, 130.5, 137.3, 142.0, 166.6. IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1723; $\nu_{S=O}$ = 1162, 1111. MS (70eV): m/z (%) 370 (M^++1, 100). HRMS (ESI) Anal. Calcd. for $C_{20}H_{20}NO_4S$ 370.1113 $[M+H]^+$, Found 370.1118.

N-(4-Methoxycarbonyl-2-methoxybenzyl)-1,2,4,9-tetrahydro-3-thia-9-azafluorene-3,3-dioxide (7b).

Recrystallization from EtOH. Mp = 208.2 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 3.17 and 3.47 (2 × 2H, 2 × t, *J* = 5.9 Hz); 3.81 and 3.92 (2 × 3H, 2 × s); 4.50 (2H, s); 5.38 (2H, s); 6.46 (1H, d, *J* = 7.9 Hz); 7.02-7.12 (2H, m); 7.33 (1H, d, *J* = 7.7 Hz); 7.40 (1H, d × d, *J* = 7.9, 1.1 Hz); 7.49 (1H, d, *J* = 7.2 Hz); 7.52 (1H, d, *J* = 1.1 Hz). ^{13}C NMR (75 MHz, d_6-DMSO): δ 22.3, 42.1, 46.9, 48.6, 52.8, 56.3, 102.8, 110.4, 111.4, 118.3, 120.1, 122.2, 122.5, 126.7, 127.3, 130.6, 131.6, 131.9, 137.1, 157.0, 166.5. IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1719; $\nu_{S=O}$ = 1113, 1105. MS (70eV): m/z (%) 400 (M^++1, 100). HRMS (ESI) Anal. Calcd. for $C_{21}H_{22}NO_5S$ 400.1219 $[M+H]^+$, Found 400.1216.

N-(4-Methoxycarbonylbenzyl)-6-fluoro-1,2,4,9-tetrahydro-3-thia-9-azafluorene-3,3-dioxide (7c).

Rf = 0.13 (EtOAc/PE 1/2). Mp = 163.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27-3.33 (4H, m); 3.90 (3H, s); 4.38 (2H, s); 5.32 (2H, s); 6.96 (1H, t × d, *J* = 9.1, 2.8 Hz); 7.02 (2H, d, *J* = 8.8 Hz); 7.09 (1H, d × d, *J* = 9.1, 2.8 Hz); 7.12 (1H, d × d, *J* = 9.1, 4.4 Hz); 7.97 (2H, d, *J* = 8.8 Hz). ^{19}F NMR (282 MHz, $CDCl_3$): δ (-122.72) – (-122.64) (m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.4, 46.8, 47.3, 48.9, 52.4, 102.6 (d, *J* = 4.6 Hz), 103.1 (d, *J* = 24.2 Hz), 110.4 (d, *J* = 10.4 Hz), 111.4 (d, *J* = 26.5 Hz), 125.9, 126.8 (d, *J* = 10.4 Hz), 130.0, 130.5, 131.8, 133.7, 141.6, 158.3 (d, *J* = 237.7 Hz), 166.6. IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1704; $\nu_{S=O}$ = 1136, 1121. MS (70eV): m/z (%) 386 (M^--1, 100). HRMS (ESI) Anal. Calcd. for $C_{20}H_{19}FNO_4S$ 388.1019 $[M+H]^+$, Found 388.1014.

N-(4-Methoxycarbonyl-2-methoxybenzyl)-6-fluoro-1,2,4,9-tetrahydro-3-thia-9-azafluorene-3,3-dioxide (7d).

Recrystallization from EtOH. Mp = 259.9 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 3.17 and 3.47 (2 × 2H, 2 × t, *J* = 6.1 Hz); 3.81 and 3.91 (2 × 3H, 2 × s); 4.47 (2H, s); 5.39 (2H, s); 6.49 (1H, d, *J* = 7.7 Hz); 6.94 (1H, t × d, *J* = 9.1, 2.8 Hz); 7.30 (1H, d × d, *J* = 9.1, 2.8 Hz); 7.36 (1H, d × d, *J* = 9.1, 4.4 Hz); 7.41 (1H, d × d, *J* = 7.7, 1.6 Hz); 7.51 (1H, d, *J* = 1.6 Hz). ^{19}F NMR (282 MHz, d_6-DMSO): δ (-124.02) – (-123.94) (m). ^{13}C NMR (75 MHz, d_6-DMSO): δ 22.5, 42.5, 46.7, 48.4, 52.8, 56.3, 103.1 (d, *J* = 4.6 Hz), 103.5 (d, *J* = 23.1 Hz), 110.3 (d, *J* = 25.4 Hz), 111.4, 111.7 (d, *J* = 9.2 Hz), 122.2, 127.0 (d, *J* = 10.3 Hz), 127.4, 130.7, 131.4, 133.8, 133.9, 157.0, 157.8 (d, *J* = 233.1 Hz), 166.4. IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1716; $\nu_{S=O}$ = 1114, 1104. MS (70eV): m/z (%) 418 (M^++1, 100). HRMS (ESI) Anal. Calcd. for $C_{21}H_{21}FNO_5S$ 418.1124 $[M+H]^+$, Found 418.1110.

N-(4-Hydroxycarbamoylbenzyl)-1,2,4,9-tetrahydro-3-thia-9-azafluorene-3,3-dioxide (8a).

Recrystallization from EtOH. Mp = 200.2 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 3.21 and 3.50 (2 × 2H, 2 × t, *J* = 6.1 Hz); 4.49 (2H, s); 5.47 (2H, s); 7.04-7.16 (4H, m); 7.45 (1H, d, *J* = 8.3 Hz); 7.49 (1H, d, *J* = 7.7 Hz); 7.66 (2H, d, *J* = 8.2 Hz), 9.01 (1H, s(br)); 11.15 (1H, s(br)). ^{13}C NMR (75 MHz, d_6-DMSO): δ 22.5, 46.1, 46.8, 48.6, 102.9, 110.5, 118.3, 120.1, 122.5, 126.7, 126.9, 127.9, 131.6, 132.5, 137.1, 141.7, 164.5. IR (ATR, cm^{-1}): $\nu_{NH/OH}$ = 3192; $\nu_{C=O}$ = 1613; $\nu_{S=O}$ = 1126, 1114. MS (70eV): m/z (%) 371 (M^++1, 100). HRMS (ESI) Anal. Calcd. for $C_{19}H_{19}N_2O_4S$ 371.1066 $[M+H]^+$, Found 371.1062.

N-(4-Hydroxycarbamoyl-2-methoxybenzyl)-1,2,4,9-tetrahydro-3-thia-9-azafluorene-3,3-dioxide (8b).

Recrystallization from EtOH. Mp = 174.9 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 3.20 and 3.48 (2 × 2H, 2 × t, *J* = 6.1 Hz); 3.89 (3H, s); 4.50 (2H, s); 5.34 (2H, s); 6.38 (1H, d, *J* = 7.7 Hz); 7.02-7.17 (3H, m); 7,34 (1H, d, *J* = 7.7 Hz); 7.39 (1H, d, *J* = 1.1 Hz); 7.49 (1H, d × d, *J* = 7.7, 1.1 Hz). ^{13}C NMR (75 MHz, d_6-DMSO): δ 22.3, 42.0, 46.9, 48.6, 56.2, 102.7, 109.8, 110.5, 118.3, 119.5, 120.0, 122.4, 126.7, 127.0, 129.0, 131.9, 133.8, 137.1, 156.8, 164.2. IR (ATR, cm^{-1}): $\nu_{NH/OH}$ = 3186; $\nu_{C=O}$ = 1631; $\nu_{S=O}$ = 1126, 1113. MS (70eV): m/z (%) 401 (M^{+}+1, 100). HRMS (ESI) Anal. Calcd. for $C_{20}H_{21}N_2O_5S$ 401.1171 $[M+H]^{+}$, Found 401.1164.

N-(4-Hydroxycarbamoylbenzyl)-6-fluoro-1,2,4,9-tetrahydro-3-thia-9-azafluorene-3,3-dioxide (8c).

Recrystallization from EtOH. Mp = 170.0 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 3.19 and 3.49 (2 × 2H, 2 × t, *J* = 6.1 Hz); 4.46 (2H, s); 5.47 (2H, s); 6.97 (1H, t × d, *J* = 9.1, 2.8 Hz); 7.06 (2H, d, *J* = 8.3 Hz); 7.30 (1H, d × d, *J* = 9.6, 2.8 Hz); 7.46 (1H, d × d, *J* = 9.1, 4.4 Hz); 7.65 (2H, d, *J* = 8.3 Hz), 9.01 (1H, s(br)); 11.14 (1H, s(br)). ^{19}F NMR (282 MHz, d_6-DMSO): δ (-123.94) – (-123.85) (m). ^{13}C NMR (75 MHz, d_6-DMSO): δ 22.6, 46.3, 46.7, 48.4, 103.1 (d, *J* = 4.6 Hz), 103.5 (d, *J* = 24.2 Hz), 110.4 (d, *J* = 25.4 Hz), 111.6 (d, *J* = 9.3 Hz), 126.9, 127.0 (d, *J* = 12.7 Hz), 127.9, 132.5, 133.7, 133.8, 141.5, 157.8 (d, *J* = 233.0 Hz), 164.4. IR (ATR, cm^{-1}): $\nu_{NH/OH}$ = 3200; $\nu_{C=O}$ = 1619; $\nu_{S=O}$ = 1146, 1123. MS (70eV): m/z (%) 387 (M^{-}-1, 100). HRMS (ESI) Anal. Calcd. for $C_{19}H_{16}FN_2O_4S$ 387.0820 $[M-H]^{-}$, Found 387.0824.

N-(4-Hydroxycarbamoyl-2-methoxybenzyl)-6-fluoro-1,2,4,9-tetrahydro-3-thia-9-azafluorene-3,3-dioxide (8d).

Recrystallization from EtOH. Mp = 236.3 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 3.20 and 3.49 (2 × 2H, 2 × t, *J* = 6.1 Hz); 3.88 (3H, s); 4.48 (2H, s); 5.35 (2H, s); 6.43 (1H, d, *J* = 8.3 Hz); 6.94 (1H, t × d, *J* = 9.1, 2.8 Hz); 7.17 (1H, d, *J* = 8.3 Hz) 7.31 (1H, d × d, *J* = 9.6, 2.8 Hz); 7.34-7.38 (2H, m); 9.04 (1H, s(br)); 11.17 (1H, s(br)). ^{19}F NMR (282 MHz, d_6-DMSO): δ (-124.07) – (-123.98) (m). ^{13}C NMR (75 MHz, d_6-DMSO): δ 22.5, 42.4, 46.7, 48.4, 56.2, 102.9 (d, *J* = 3.5 Hz), 103.5 (d, *J* = 24.3 Hz), 109.9, 110.3 (d, *J* = 25.4 Hz), 111.7 (d, *J* = 9.2 Hz), 119.5, 127.0 (d, *J* = 12.7 Hz), 127.1, 128.8, 133.8, 133.9, 134.0, 156.8, 157.7 (d, *J* = 233.1 Hz), 164.2. IR (ATR, cm^{-1}): $\nu_{NH/OH}$ = 3313; $\nu_{C=O}$ = 1662; $\nu_{S=O}$ = 1139, 1114. MS (70eV): m/z (%) 417 (M^{-}-1, 100). HRMS (ESI) Anal. Calcd. for $C_{20}H_{18}FN_2O_5S$ 417.0926 $[M-H]^{-}$, Found 417.0928.