Supplementary Note 2 for Shimada *et al*.

Contents

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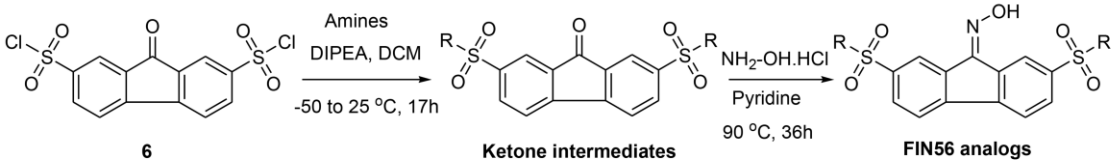
Total synthesis of FIN56 and analogs

We synthesized structural analogs of CIL56 and FIN56 based on reported work[1]. The total synthesis of CIL56 and FIN56 is depicted in Scheme 1.



Scheme 1. Total synthesis of FIN56 and analogs.

General procedure A: preparation of ketone compounds

9-oxo-9H-fluorene-2,7-disulfonyl dichloride (**6**) (1 equiv.) was dissolved in 50mL of dichloromethane and the mixture was cooled to -50^{o}C. To this mixture was added a primary or secondary amine (e.g. piperidine or cyclohexylamine) (2.6 equiv.) and diisopropylethylamine (2-3 equiv.). The reaction mixture was stirred at room temperature for 17 hrs. The solvent was evaporated and the residue was purified by flash-column chromatography on silica gel to provide the desired ketone compounds. The purity of the ketones were confirmed by different spectroscopic methods such as ^{1}H NMR and mass spectrometry.

General procedure B: preparation of oxime compounds

A mixture of the ketone from the **general procedure A** (1.0 equiv.) and hydroxylamine hydrochloride (10 equiv.) were dissolved in pyridine (10 mL). The mixture was stirred at 95^{o}C for 36 hrs. The pyridine was evaporated and the residue was stirred with 1 N hydrogen chloride (HCl) (10 mL) for several mins. White product was

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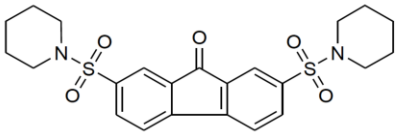
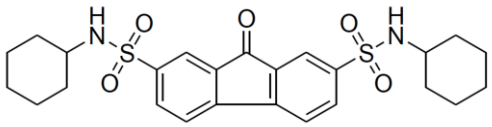
collected by filtration, washed with water and dried. The crude material was either crystallized from ethanol or purified by flash-column chromatography on silica gel to provide the desired oxime compounds. The purity of the oximes was confirmed by different spectroscopic methods such as ^{1}H NMR and mass spectrometry.



SRS1-63 (**7**)

Synthesis of 2,7-bis(piperidin-1-ylsulfonyl)-9H-fluoren-9-one (SRS1-63)

General procedure A was followed, using 9-oxo-9H-fluorene-2,7-disulfonyl dichloride (**6**) (400 mg, 1.060 mmol), piperidine (273 mL, 2.757 mmol) and diisopropylethylamine (270 mL, 2.120 mmol). The crude reaction mixture was purified by column chromatography (dichloromethane:methanol = 40:1) to give the desired 2,7-bis(piperidin-1-ylsulfonyl)-9H-fluoren-9-one (SRS1-63) (**7**) (420 mg, 0.886 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 2H), 7.99 (d, *J* = 7.4 Hz, 2H), 7.79 (d, *J* = 7.8 Hz, 2H), 3.04 (d, *J* = 4.8 Hz, 8H), 1.66 (s, 8H), 1.44 (s, 4H). MS (APCI+, *M+1*) 475.16.



SRS7-25 (**8**)

Synthesis of N2,N7-dicyclohexyl-9-oxo-9H-fluorene-2,7-disulfonamide (SRS7-25)

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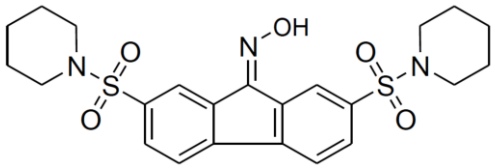
General procedure A was followed, with 9-oxo-9H-fluorene-2,7-disulfonyl dichloride (**1**) (500 mg, 1.326 mmol), cyclohexylamine (394 mL, 3.448 mmol) and diisopropylethylamine (692.9 mL, 3.978 mmol). The crude reaction mixture was purified by column chromatography (dichloromethane: methanol = 20:1) to give the desired N2,N7-dicyclohexyl-9-oxo-9H-fluorene-2,7-disulfonamide (SRS7-25) (**8**) (532 mg, 1.059mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 2H), 8.13 (dd, *J* = 7.8, 1.6 Hz, 2H), 7.77 (d, *J* = 7.8 Hz, 2H), 4.57 (d, *J* = 7.7 Hz, 2H), 3.22 (d, *J* = 3.8 Hz, 2H), 1.81 (d, *J* = 9.8 Hz, 4H), 1.65 (s, 4H), 1.32 – 1.13 (m, 12H).; MS (APCI+, *M+1*) 503.01.



CIL56 (**1**)

Synthesis of 2,7-bis(piperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (CIL56)

General procedure B was followed, with 2,7-bis(piperidin-1-ylsulfonyl)-9H-fluoren-9-one (**SRS1-63**) (350 mg, 0.738 mmol), hydroxylamine hydrochloride (509 mg, 7.384 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the N2,N7-dicyclohexyl-9-oxo-9H-fluorene-2,7-disulfonamide (**CIL56**) (**1**) (306 mg, 0.627 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ12.50 (s, 1H), 8.78 (s, 1H), 8.13 (s, 1H), 7.88 – 7.77 (m, 3H), 7.75 (d, *J* = 1.4 Hz, 1H), 2.96 (d, *J* = 4.8 Hz, 8H), 1.58 (s, 8H), 1.35 (s, 4H); MS (APCI+, *M+1*) 490.16.

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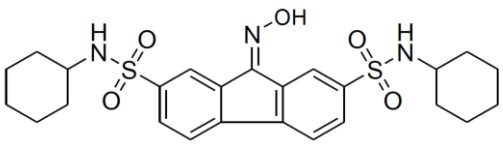
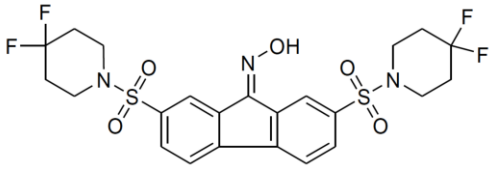


FIN56 (**2**)

Synthesis of N2,N7-dicyclohexyl-9-(hydroxyimino)-9H-fluorene-2,7-disulfonamide (FIN56)

General procedure B was followed, with N2,N7-dicyclohexyl-9-oxo-9H-fluorene-2,7-disulfonamide (SRS7-25) (500 mg, 0.996 mmol), hydroxylamine hydrochloride (687.2 mg, 9.960 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the N2,N7-dicyclohexyl-9-oxo-9H-fluorene-2,7-disulfonamide (**FIN56**) (**2)** (437 mg, 0.845 mmol, 85%). ^{1}H NMR (400 MHz, DMSO) δ 13.28 (s, 1H), 8.82 (s, 1H), 8.25 – 8.14 (m, 3H), 8.03 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.95 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.85 (d, *J* = 7.4 Hz, 1H), 7.81 (d, *J* = 7.3 Hz, 1H), 3.00 (s, 2H), 1.59 (s, 8H), 1.44 (d, *J* = 11.5 Hz, 2H), 1.22 – 0.98 (m, 10H); MS (APCI+, *M+1*) 517.92.



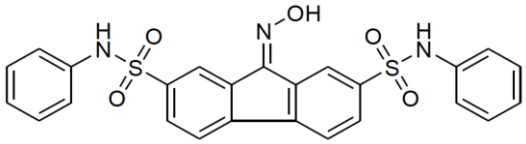
SRS5-19

Synthesis 2,7-bis(4,4-difluoropiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS5-19)

General procedure B was followed, with 2,7-bis(4,4-difluoropiperidin-1-ylsulfonyl)-9H-fluoren-9-one (25 mg, 0.0458 mmol), hydroxylamine hydrochloride (32.0 mg, 0.458 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give

the desired 2,7-bis(4,4-difluoropiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS5-19) (**10**) (15 mg, 0.026 mmol, 58%). ^{1}H NMR (400 MHz, DMSO) δ 13.42 (s, 1H), 8.68 (s,1H), 8.34 (dd, *J* = 17.8, 7.9 Hz, 2H), 8.09 – 7.87 (m, 13H), 3.15 (s, 8H), 2.08 (s, 8H); MS

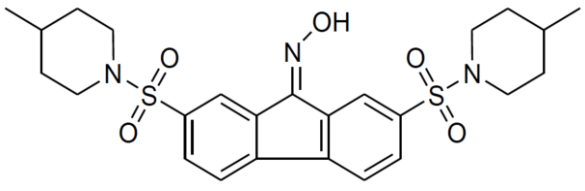
(APCI+, *M+1*) 561.95.



SRS5-55

Synthesis of 9-(hydroxyimino)-N2,N7-diphenyl-9H-fluorene-2,7-disulfonamide (SRS5-55)

General procedure B was followed**,** with 9-oxo-N2,N7-diphenyl-9H-fluorene-2,7-disulfonamide (25 mg, 0.051 mmol), hydroxylamine hydrochloride (35.2 mg, 0.510 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the desired 2,7-bis(4,4-difluoropiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS5-55) (**11**) (23 mg, 0.045 mmol, 89%). ^{1}H NMR (400 MHz, DMSO) δ 13.32 (s, 1H), 10.36 (d, *J* =25.6 Hz, 2H), 8.73 (s, 1H), 8.10 (dd, *J* = 14.8, 8.0 Hz, 2H), 8.02 (s, 1H), 7.85 (dd, *J* =26.4, 8.0 Hz, 2H), 7.29 – 6.98 (m, 10H); MS (APCI+, *M+1*) 505.98.



SRS5-57

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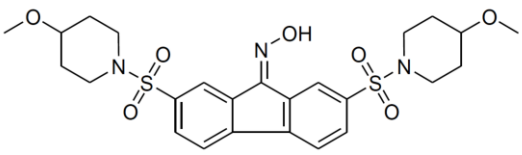
Synthesis of 2,7-bis(4-methylpiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS5-57)

General procedure B was followed, with 2,7-bis(4-methylpiperidin-1-ylsulfonyl)-9H-fluoren-9-one (25 mg, 0.050 mmol), hydroxylamine hydrochloride (35 mg, 0.5 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the desired 2,7-bis(4-methylpiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS5-57) (**12**) (23 mg, 0.044 mmol, 89%). ^{1}H NMR (400 MHz, DMSO) δ 13.36 (s, 1H), 8.65 (s, 1H), 8.31 (dd, *J* = 17.4, 7.9 Hz, 2H), 7.92 (dd, *J* = 35.8, 10.2 Hz, 3H), 3.66 (s, 4H), 3.48 (s,4H), 2.28 (t, *J* = 11.1 Hz, 3H), 1.66 (d, *J* = 11.7 Hz, 3H), 1.23 – 1.09 (m, 4H), 0.84 (d, *J* = 5.6 Hz, 6H); MS (APCI+, *M+1*) 518.08.



SRS6-13

Synthesis of 2,7-bis(4-methoxypiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS6-13)

General procedure B was followed, with 2,7-bis(4-methoxypiperidin-1-ylsulfonyl)-9H-fluoren-9-one (25 mg, 0.047 mmol), hydroxylamine hydrochloride (32 mg, 0.47 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the desired 2,7-bis(4-methoxypiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS6-13) (**13**) (19 mg, 0.034 mmol, 74%). ^{1}H NMR (400 MHz, DMSO) δ 13.37 (s, 1H), 8.65 (s, 1H),

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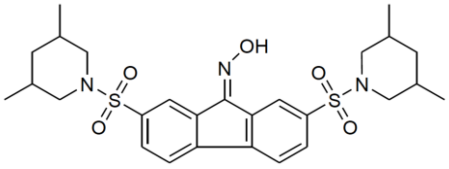
8.40 – 8.24 (m, 2H), 7.94 (d, *J* = 34.2 Hz, 3H), 3.23 (s, 2H), 3.12 (s, 10H), 2.88 (s, 4H), 1.83 (s, 4H), 1.54 (s, 4H); MS (APCI+, *M+1*) 550.01.

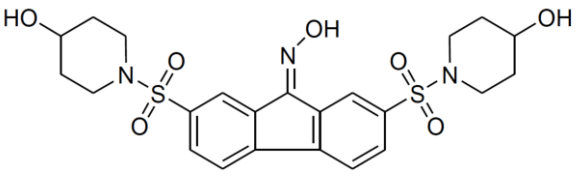


SRS6-15

Synthesis of 2,7-bis(3,5-dimethylpiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS6-15)

General procedure B was followed, with 2,7-bis(3,5-dimethylpiperidin-1-ylsulfonyl)-9H-fluoren-9-one (25 mg, 0.047 mmol), hydroxylamine hydrochloride (32.5 mg, 0.47 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the desired 2,7-bis(3,5 dimethyl-piperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS6-15) (**14**) (21 mg, 0.038 mmol, 82%). ^{1}H NMR (400 MHz, DMSO) δ 13.37 (s, 1H), 8.64 (d, *J* =

6.2 Hz, 1H), 8.30 (dd, *J* = 17.5, 8.1 Hz, 2H), 8.01 – 7.83 (m, 3H), 2.18 (t, *J* = 7.2 Hz, 4H), 1.77 (d, *J* = 5.2 Hz, 4H), 1.66 (d, *J* = 7.4 Hz, 4H), 1.47 (s, 4H), 1.23 – 1.20 (m, 12H); MS (APCI+, *M+1*) 546.09.



SRS6-23

Synthesis of 2,7-bis(4-hydroxypiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS6-23)

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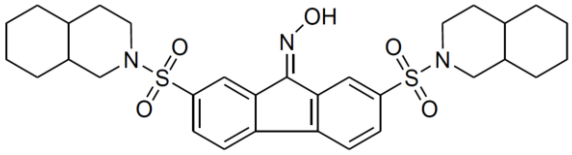
General procedure B was followed with 2,7-bis(4-hydroxypiperidin-1-ylsulfonyl)-9H-fluoren-9-one (25 mg, 0.049 mmol), hydroxylamine hydrochloride (34.1 mg, 0.49 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the desired 2,7-bis(4-hydroxypiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS6-23) (**15**) (14 mg, 0.027 mmol, 55%). ^{1}H NMR (400 MHz, DMSO) δ 13.36 (s, 1H), 8.65 (s, 1H),

8.31 (dd, *J* = 17.6, 8.1 Hz, 2H), 7.93 (dd, *J* = 34.3, 8.6 Hz, 2H), 3.50 (s, 45H), 3.22 (s, 95H), 2.79 (s, 92H), 1.74 (s, 92H), 1.45 (s, 87H); MS (APCI+, *M+1*) 522.03.



SRS6-25

Synthesis of 2,7-bis(octahydroisoquinolin-2(1H)-ylsulfonyl)-9H-fluoren-9-one oxime (SRS6-25)

General procedure B was followed, with 2,7-bis(octahydroisoquinolin-2(1H)-ylsulfonyl)-9H-fluoren-9-one (25 mg, 0.043 mmol), hydroxylamine hydrochloride (29.6 mg, 0.43 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the desired 2,7-bis(octahydroisoquinolin-2(1H)-ylsulfonyl)-9H-fluoren-9-one oxime (SRS6-25) (**16**) (17 mg, 0.028 mmol, 66%). ^{1}H NMR (400 MHz, DMSO) δ 13.35 (s, 1H), 8.64

(s, 1H), 8.30 (d, *J* = 9.4 Hz, 2H), 7.92 (d, *J* = 34.3 Hz, 3H), 3.72 (s, 2H), 3.57 (s, 2H), 2.26 (s, 4H), 1.91 (s, 4H), 1.59 (d, *J* = 23.0 Hz, 12H), 1.19 (s, 12H); MS (APCI+, *M+1*) 598.13.

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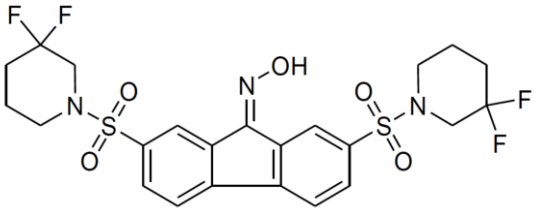
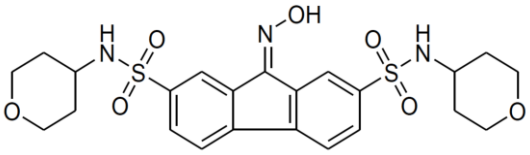


SRS6-27

Synthesis of 2,7-bis(3,3-difluoropiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS6-27)

General procedure B was followed, with 2,7-bis(3,3-difluoropiperidin-1-ylsulfonyl)-9H-fluoren-9-one (25 mg, 0.045 mmol), hydroxylamine hydrochloride (31.5 mg, 0.457 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the desired 2,7-bis(3,3-difluoropiperidin-1-ylsulfonyl)-9H-fluoren-9-one oxime (SRS6-27) (**17**) (14 mg, 0.025 mmol, 55%). ^{1}H NMR (400 MHz, DMSO) δ 13.41 (s, 1H), 8.68 (s,

1H), 8.35 (dd, *J* = 18.0, 8.0 Hz, 2H), 8.00 (dd, *J* = 34.5, 8.1 Hz, 3H), 3.32 – 3.29 (m, 4H), 3.12 (s, 4H), 1.95 (s, 4H), 1.71 (s, 4H); MS (APCI+, *M+1*) 562.01.



SRS8-18

Synthesis of 9-(hydroxyimino)-N2,N7-bis(tetrahydro-2H-pyran-4-yl)-9H-fluorene-2,7-disulfonamide (SRS8-18)

General procedure B was followed with 9-oxo-N2,N7-bis(tetrahydro-2H-pyran-4-yl)-9H-fluorene-2,7-disulfonamide (**9**) (25 mg, 0.049 mmol), hydroxylamine

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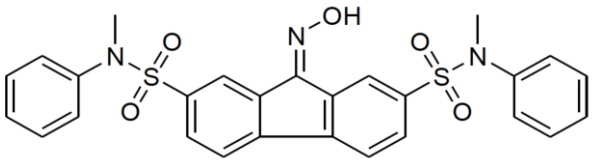
hydrochloride (34 mg, 0.494 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the desired 9-(hydroxyimino)-N2,N7-bis(tetrahydro-2H-pyran-4-yl)-9H-fluorene- 2,7-disulfonamide (SRS8-18) (**3)** (17 mg, 0.033 mmol, 66%). ^{1}H NMR (400 MHz, DMSO) δ 8.77 (s, 1H), 8.22 – 8.10 (m, 3H), 7.96 (dd, *J* = 33.0, 8.0 Hz, 4H), 3.66 (d, *J* = 11.5 Hz,

4H), 3.17 (dd, *J* = 21.0, 9.6 Hz, 10H), 2.46 (d, *J* = 1.5 Hz, 3H), 1.49 (d, *J* = 11.9 Hz, 5H), 1.41 – 1.25 (m, 6H); MS (APCI+, *M+1*) 522.03.



SRS6-51

Synthesis of 9-(hydroxyimino)-N2,N7-dimethyl-N2,N7-diphenyl-9H-fluorene-2,7-disulfonamide (SRS6-51)

General procedure B was followed with N2,N7-dimethyl-9-oxo-N2,N7-diphenyl-9H-fluorene-2,7-disulfonamide (25 mg, 0.048 mmol), hydroxylamine hydrochloride (33 mg, 0.48 mmol) and pyridine (30 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the desired 9-(hydroxyimino)-N2,N7-dimethyl-N2,N7-diphenyl-9H-fluorene-2,7-disulfonamide (SRS6-51) **(18)** (21 mg, 0.039 mmol, 82%). ^{1}H NMR

(400 MHz, DMSO) δ 13.17 (s, 5H), 8.46 (s, 4H), 8.21 (dd, *J* = 14.4, 8.1 Hz, 10H), 7.73 – 7.62 (m, 16H), 7.34 (d, *J* = 7.6 Hz, 32H), 7.16 (d, *J* = 7.0 Hz, 23H), 3.19 (s, 40H); MS

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(APCI+, *M+1*) 534.01.

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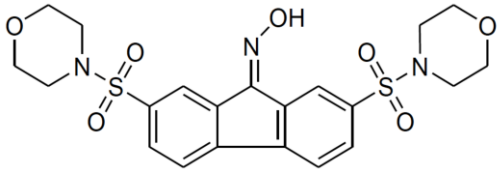

SRS2-95

Synthesis 2,7-bis(morpholinosulfonyl)-9H-fluoren-9-one oxime (SRS2-95)

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General procedure B was followed with 2,7-bis(morpholinosulfonyl)-9H-fluoren-9-one (25 mg, 0.052 mmol), hydroxylamine hydrochloride (36 mg, 0.52 mmol) and pyridine (10 mL). The crude reaction mixture was precipitated as a salt after 1 N HCl treatment and washed several times with cold water and ethanol to give the 2,7-bis(morpholinosulfonyl)-9H-fluoren-9-one oxime (SRS2-95) (**19)** (18 mg, 0.037 mmol, 70%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.4 Hz, 1H), 7.88 – 7.77 (m, 2H), 3.12 – 2.96 (m, 4H), 1.69 (d, *J* = 5.0 Hz, 4H); MS (APCI+, *M+1*) 494.09.

Synthesis of SRS11-31 and SRS11-66 for target identification study.

The N2,N7-dicyclohexyl-9-oxo-9H-fluorene-2,7-disulfonamide (FIN56) or 9-(hydroxyimino)-N2,N7-bis(tetrahydro-2H-pyran-4-yl)-9H-fluorene-2,7-disulfonamide (SRS8-18) were reacted 2-(2-(2-chloroethoxy)ethoxy)ethyl benzoate (1 equiv) in the presence of sodium iodide (0,5 equiv) and potassium carbonate (3 equiv.) in DMF. The mixture was heated at 50 ^{o}C for 17h then aqueous ammonium chloride was added. The compounds were extracted with ethyl acetate, dried with magnesium sulfate and the organic solvent was evaporated under vaccum. The mass of the desired ester intermediates were confirmed by LC/MS and used without further purification. To the ester intermediates were added sodium hydroxide (NaOH; 5 equiv.) in THF (2ml). The mixture were stirred for 17h at room temperature then acidified to pH = 5. The compounds were extracted with ethyl acetate, dried with magnesium sulfate and the solvent was evaporated under vaccum. The crud material was either crystallized from ethanol or purified by flash-column chromatography on silica gel to provide the desired

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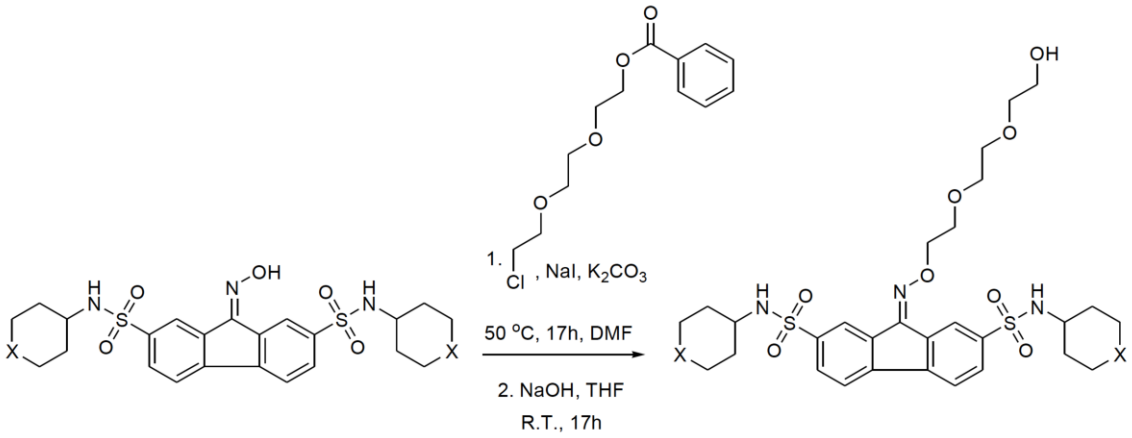
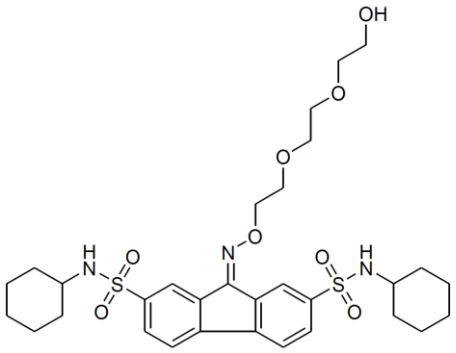
oxime compounds. The purity of the oxime alcohol linkers SRS11-31 (**4**) and SRS11-66 (**5**) were confirmed by ^{1}H NMR and Mass.



X=C: FIN56
X=O: SRS8-18

X=C:SRS11-31
X=O:SRS11-66

Scheme 2. Total synthesis of **SRS11-31** and **SRS11-66** for target identification study.



SRS11-31

***N2,N7-dicyclohexyl-9-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxyimino)-9H-fluorene-2,7-* disulfonamide (SRS11-31) (5)**

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, *J* = 12.2 Hz, 1H), 8.29 (d, *J* = 7.8 Hz, 1H), 8.07 – 8.00 (m, 1H), 8.00 – 7.92 (m, 1H), 7.80 (dd, *J* = 12.1, 5.8 Hz, 2H), 5.90 (t, *J* =11.3 Hz, 1H), 5.10 (dd, *J* = 13.9, 9.9 Hz, 1H), 4.63 (s, 2H), 3.74 (d, *J* = 3.9 Hz,

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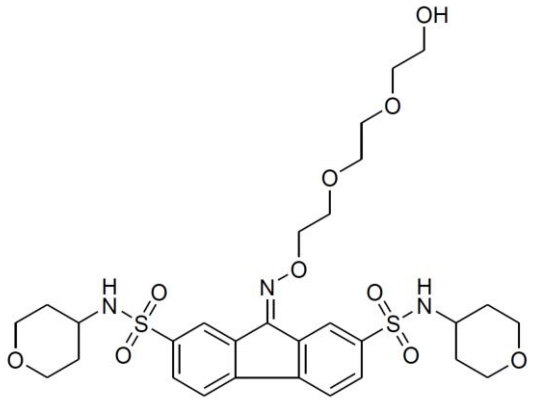
8H), 3.65 (d, *J* = 3.9 Hz, 2H), 3.16 (s, 2H), 1.82 (s, 8H), 1.62 (s, 4H), 1.51 (d, *J* = 10.4 Hz, 2H), 1.21 (s, 6H). MS (APCI+, M+1) 650.19



SRS11-66

9-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxyimino)-N2,N7-bis(tetrahydro-2H-pyran-4-yl)-9H-fluorene-2,7-disulfonamide (SRS11-66) (**4**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 8.31 (s, 1H), 8.06 (d, *J* = 7.3 Hz, 1H), 7.97 (d, *J* = 7.7 Hz, 1H), 7.82 (t, *J* = 7.6 Hz, 2H), 6.22 (d, *J* = 7.2 Hz, 1H), 4.92 (d, *J* =7.3 Hz, 1H), 4.66 (s, 2H), 3.96 (s, 4H), 3.76 (d, *J* = 9.2 Hz, 2H), 3.70 (s, 2H), 3.34 – 3.30 (m, 4H), 1.69 (s, 4H), 1.53 (d, *J* = 6.2 Hz, 2H), 1.25 (dd, *J* = 35.5, 16.2 Hz, 10H).; MS (APCI+, M+1) 654.01

1. Chellappan, S. *et al.* Derivatives of fluorene, anthracene, xanthene, dibenzosuberone and acridine and uses thereof. (2008).

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