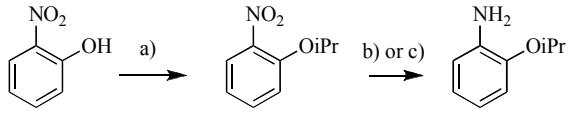
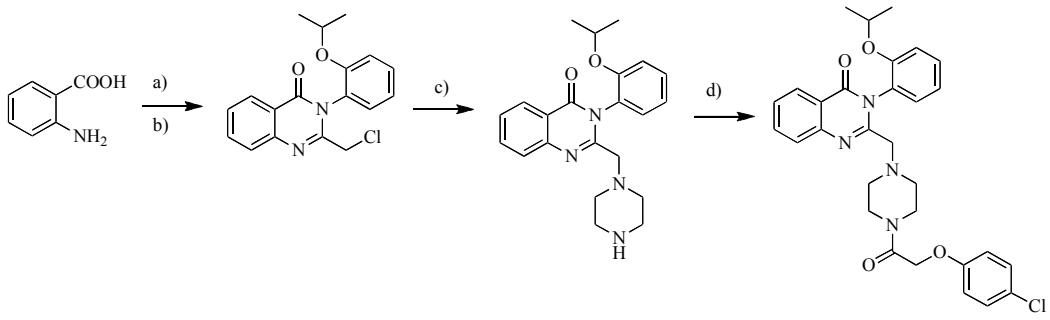
Data S1. Synthesis of Erastin Analogs and Synthesis of RSL3 Analogs, Related to Experimental Procedures.

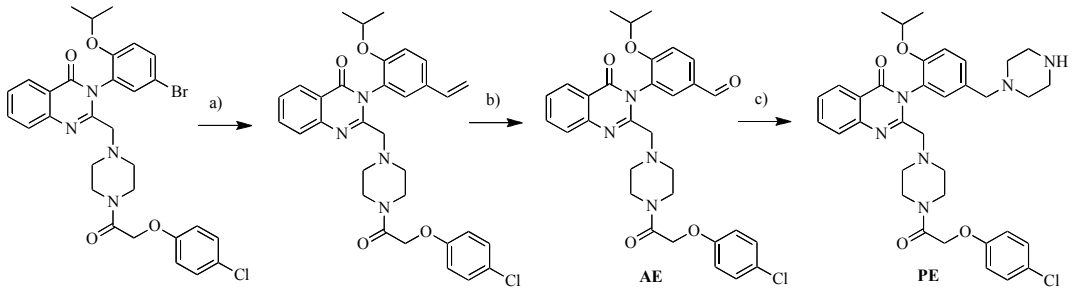
Synthesis of Erastin Analogs



Scheme 1 - Aniline synthesis. (a) 2-iodopropane (2.0 eq), K_2CO_3 (1.2 eq), DMF, 50°C, 12 hr; (b) $SnCl_2$ (4.0 eq), HCl (1M, 3.0 eq), THF, 50°C, 24 hr (c) H2 (1 atm) Pd/C (10%), MeOH, 12-72 hrs.



Scheme 2 - General erastin analog synthetic route. (a) TEA (1.1 eq), chloroacetyl chloride (1.1 eq), THF, 0°C to 25°C, 6 hr; (b) PCl3 (1.2 eq), EDIPA (1.0 eq), then 2-isoproxyaniline (1.1 eq), dioxane, 25°C to 70°C, 6 hr; (c) piperazine (3.0 eq), THF, 25°C, 14 hr; (e) EDIPA, 4-DMAP, 4-chlorophenoxy acetylchloride, CH_2Cl_2 0°C to 25°C, 3 hr.



Scheme 3 - AE and PE synthesis. (a) tributylvinyl tin (1.5 eq), $PdCl_2(PPh_3)_2$ (5%) (c) piperazine (6.0 eq), $ZnCl_2$ (0.1 eq), 1,2-dichloroethane, 25^{o}C, 3 hr, then $NaBH_3CN$ in MeOH, 40^{o}C, 4 hr.

1-isopropoxy-2-nitrobenzene (general procedure 1)

2-Iodopropane (14.4 mL, 143.8 mmol, 2.0 eq) was added to a stirred solution of 2-nitrophenol (10 g, 71.9 mmol) and potassium carbonate (14.9 g, 108 mmol, 1.5 eq) in DMF (160 mL) and the mixture was subsequently heated to 50^{0}C for 12 hrs. Upon completion the reaction contents were added to water and extracted twice with ethyl acetate. The combined organic layers were dried (Na_2SO_4), concentrated and purified by combiflash 0-20% EtOAc to afford 1-isopropoxy-2-nitrobenzene (11.7 g, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 p.p.m. (d app, *J*= 8.4 Hz, 1H), 7.46 (t app, *J*=8 Hz, 1H), 7.07 (d app, *J*=8.4 Hz, 1H), 6.97-6.93 (m, 1H), 4.66 (h, *J*=1.5 Hz, 1H), 1.35-1.33 (m, 6H); ^{13}C NMR (125 MHz): δ 141.0, 133.7, 125.2, 120.0, 116.1, 72.5, HRMS (*m/z*): [M+] cald for $C_9H_{11}NO_3$ 181.19, found 182.08

2-isopropoxyaniline (general procedure 2)

To a solution of 1-isopropoxy-2-nitrobenzene (11.7 g, 64.6 mmol) in methanol (300 mL) Pd/C (10%) (5% wt, 0.585 g) was added and stirred under hydrogen (1 atm) for 72 hr.

Upon completion the reaction was filtered over celite, concentrated, and purified by combiflash 0->30% EtOAc to afford 2-isopropoxyaniline (7.88 g, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.80-6.79 p.p.m. (m, 2H), 6.78-6.75 (m, 2H), 4.59 (h, 1.5 Hz, 1H), 3.85 (s, 1H), 1.42 (d, *J*=6 Hz, 2H); ^{13}C NMR (125 MHz): δ 145.4, 137.4, 121.1, 118.4, 115.4, 113.7, 70.6 HRMS (*m/z*): [M+] cald for $C_9H_{13}NO$ 151.21, found 151.47

Bromo-isopropoxy amine (general procedure 3)

To a solution of 4-bromo-1-isopropoxy-2-nitrobenzene (prepared using general procedure 1, 84%, 17.54 g, 67.4 mmol) in THF (270 mL), HCl (1 M aq, 270 mL, 270 mmol, 4.0 eq), and stannous chloride (38 g, 282 mmol, 3.0 eq) were added and heated to 50^0C for 24 hr. Upon completion the mixture was quenched with saturated aqueous sodium bicarbonate, filtered over celite and the crude product was extracted twice with ethyl acetate. The combined organic layers were dried (Na_2SO_4), concentrated and purified by combiflash 0->30% EtOAc to yield bromo-isopropoxy amine (9.93 g, 64% yield) ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.82 p.p.m. (d, *J*=2.4 Hz, 1H), 6.77 (dd, *J1*=8.5 Hz, *J2*=2.4 Hz, 1H), 6.63 (d, *J*=8.6 Hz, 1H), 4.46 (hept, *J*=1.5 Hz, 1H), 1.33 (d, *J*=6.0, 6H), ^{13}C NMR (125 MHz): δ 114.4, 138.9, 120.6, 117.6, 114.8, 113.2, 71.0 HRMS (*m/z*): [M+] cald for $C_9H_{13}NOBr$ 230.1, found 229.01

2-(2-chloroethanamido)benzoic acid (general procedure 4)

A solution of chloroacetyl chloride (2.09 mL, 26.25 mmol, 1.2 eq) in THF (40 mL) was added dropwise, over about 1 hr, to a solution of triethyl amine (3.05 mL, 21.9 mmol, 1.0 eq) and anthranillic acid (3.00 g, 21.9 mmol) in THF (120 mL) at 0^0C. The mixture was

slowly warmed 25^{0}C and stirred for an additional 4 hr. Upon completion, the reaction contents were diluted with EtOAc and washed with 1 M HCl and water. The organic layer was dried (Na_2SO_4), the solvent was removed, and the crude solid was triturated with dichloromethane to afford 2-(2-chloroethanamido)benzoic acid (3.20 g, 68% yield). ^{1}H NMR (400 MHz, C_6D_6OS): δ 11.81 p.p.m. (s, 1H), 8.53 (d, *J*=8.4 Hz, 1H), 8.02 (dd, *J1* = 7.9, *J2*=1.5), 7.63 (m, 1H), 7.21 (m, 1H), 4.45 (s, 2H). ^{13}C NMR (125 MHz): δ 169.8, 165.7, 140.4, 134.6, 131.6, 123.9, 120.3, 117.3, 43.9; HRMS (*m/z*): [M+] cald for $C_9H_8ClNO_3$ 213.62, found 213.02

2-(chloromethyl)-3-(2-isopropoxyphenyl)quinazolin-4(3H)-one (general procedure 5)

EDIPA (0.326 mL, 1.87 mmol, 1.0 eq) was added to a solution of 2-(2-chloroethanamido)benzoic acid (0.400 g, 1.87 mmol) at 25^{0}C in dioxane (10 mL) and stirred for 2 minutes before the dropwise addition of phosphorous trichloride (0.309 mL, 2.25 mmol, 1.2 eq). After 5 minutes of stirring, O-isopropoxyaniline (0.311 g, 2.06 mmol, 1.1 eq) was added and the resulting mixture was heated to 70^{0}C and stirred for an additional 6 hr. Upon completion the reaction was carefully quenched with saturated aqueous $NaHCO_3$, diluted with water, and extracted 3 times with EtOAc. The combined organic layers were dried (Na_2SO_4), concentrated, and the crude material was purified by combi flash 0->50% EtOAc in hexanes to provide 2-(chloromethyl)-3-(2-isopropoxyphenyl)quinazolin-4(3H)-one (333 mg, 54% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 p.p.m. (m, 1H), 7.89 (m, 1H), 7.54 (m, 1H), 7.38 (dd, *J1*=7.7, *J2*=1.6, 1H), 7.11 (m, 1H), 4.58 (hept, *J*= 1.5 Hz, 1H), 4.38 (d, *J*=12 Hz, 1H), 4.19 (d, *J*=12 Hz,

1H), 1.26 (d, *J*=6.1, 3H), 1.17 (d, *J*=6.1, 1H), ^{13}C NMR (125 MHz): δ 161.6, 153.0, 152.4, 147.2, 134.5, 131.1, 130.8, 127.6, 127.5, 127.3, 125.3, 121.4, 121.0, 114.3, 71.2, 43.7, 22.2, 21.8; HRMS (*m/z*): [M+] cald for $C_{18}H_{17}ClN_2O_2$ 328.79, found 329.1

3-(2-isopropoxyphenyl)-2-(piperazin-1-ylmethyl)quinazolin-4(3H)-one (general procedure 6)

Piperazine (263 mg, 3.06 mmol, 3.0 eq) was added to a solution of 2-(chloromethyl)-3-(2-isopropoxyphenyl)quinazolin-4(3H)-one (0.335 g, 1.01 mmol) in THF (5 mL) and the resulting mixture was stirred at 25^{0}C for an additional 14 hr. The reaction mixture was then concentrated and purified directly by combiflash 0->20% MeOH in DCM to provide 3-(2-isopropoxyphenyl)-2-(piperazin-1-ylmethyl)quinazolin-4(3H)-one (0.301 g, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 p.p.m. (m, 1H), 7.78 (m, 2H), 7.49 (m, 1H), 7.42 (m, 1H), 7.30 (m, 1H), 7.07 (m, 2H), 4.56 (h, *J*=1.5 Hz, 1H), 3.26 (s, 1H), 2.85 (m, 3 H), 2.65 (s, 3H), 2.52 (m, 2H), 2.37 (m, 1H), 2.23 (m, 2H). ^{13}C NMR (125 MHz): δ 162.1, 153.7, 153.0, 147.2, 134.2, 132.1, 131.1, 130.9, 130.4, 127.4, 127.1, 126.8, 126.4, 121.3, 120.5, 120.4, 114.3, 71.1, 71.0, 61.5, 53.3, 51.3, 45.3, 22.2, 21.8; HRMS (*m/z*): [M+] cald for $C_{22}H_{26}N_4O_2$ 378.47; found, 379.21

2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-3-(2-isopropoxyphenyl)quinazolin-4(3H)-one (general procedure 7)

EDIPA (0.166 mL, 0.954 mmol, 1.2 eq) was added to a solution 3-(2-isopropoxyphenyl)-2-(piperazin-1-ylmethyl)quinazolin-4(3H)-one which was then cooled to 0^{0}C, before the sequential addition of 4-chlorophenoxyacetyl chloride (0.196 g, 0.954 mmol, 1.2 eq) and

4-DMAP (49 mg, 0.390 mmol, 0.5 eq). The mixture was slowly warmed to 25^0C and stirred for an additional 3 hrs. Upon completion, the reaction was quenched with saturated aqueous $NaHCO_3$ and extracted 3 times with dichloromethane. The combined organic layers were dried (Na_2SO_4), concentrated, and the crude material was purified by combi flash 0->5% MeOH in DCM to provide 2-((4-(2-(4 chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-3-(2-isopropoxyphenyl)quinazolin-4(3H)-one (270 mg, 62 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 p.p.m. (dd, *J1* = 8.0 Hz, *J2* = 0.8 Hz, 1H), 7.83-7.75 (m, 2H), 7.54-7.47 (m, 1H), 7.45-7.42 (m, 1H), 7.30-7.23 (m, 3.5 H), 7.10-7.06 (m2H), 6.89-6.86 (m, 2H), 4.64 (s, 1H), 4.57 (h, *J*=1.5, 1H), 3.51-3.44 (m, 4 H), 3.28 (s, 2H), 2.54-2.42 (m, 2H), 2.30-2.26 (m, 2H), 1.21 (d, *J*=6 Hz, 3H), 1.13 (d, *J*=6 Hz, 3 H); ^{13}C NMR (125 MHz): δ 166.1, 162.1, 156.6, 134.5, 130.9, 130.7, 129.7, 127.6, 127.3, 126.8, 126.5, 121.5, 120.7, 116.1, 114.6, 71.4, 68.0, 61.0, 53.1, 52.8, 45.3, 42.1, 22.4, 22.0; HRMS (*m/z*): [M+] cald for $C_{30}H_{31}N_4O_4Cl$ 547.4, found 547.21

2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-3-(4-isopropoxypyridin-3-yl)quinazolin-4(3H)-one (Pyr erastin)

Prepared according to the general procedures described in schemes 1 (using general procedure 2 for the reduction of 4-isopropoxy-3-nitropyridine) and scheme 2, starting from 4-Hydroxy-3-nitropyridine, 5% overall. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (dd, *J1* = 8.0 Hz, *J2*=1.3 Hz, 1H), 7.79-7.76 (m, 1H), 7.71-7.69 (m, 1H), 7.62 (d, *J*=2.4 Hz, 1H), 7.5-7.46 (m, 3H), 7.24-7.21 (m, 4H), 6.87-6.84 (m, 3H), 6.63 (d, *J*=7.8 Hz, 1H), 4.63 (s, 3H), 4.41-4.1 (m, 1H), 3.52-3.42 (m, 9H), 2.60 (m, 1H), 2.39 (m, 4H), 1.54 (d, *J*=6.8 Hz), 10H) ^{13}C NMR (125 MHz): δ 173.4, 166.12, 162.0, 156.4, 138.3, 137.0,

134.6, 129.5, 127.1, 121.1, 120.2, 115.9, 67.8, 61.5, 58.9, 52.9, 45.5, 45.1, 42.0, 29.7, 23.1, 23.0, HRMS (*m/z*): [M+] cald for 548.03; found, 548.21

2-((4-(4-chlorophenylcarbonyl)piperazin-1-yl)methyl)-3-(2-isopropoxyphenyl)quinazolin-4(3H)-one (dMK erastin)

Synthesized from 3-(2-isopropoxyphenyl)-2-(piperazin-1-ylmethyl)quinazolin-4(3H)-one and 4-chlorobenzyoyl chloride using general procedure 7 (82% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 p.p.m. (d, *J*= 8.4 Hz, 1H), 7.76 (m, 2H), 7.49 (m, 1H), 7.41 (m, 1H), 7.37 (m, 2H) 7.34 (m, 3H), 7.07 (m, 1H), 4.55 (h, *J*=1.5 Hz, 1H), 3.65 (s, 2H), 3.30 (s, 3H), 2.48 (s, 2H), 2.25 (s, 2H), 1.23 (d, *J*=6 Hz, 3 H), 1.15 (d, *J*=6 Hz, 3H). ^{13}C NMR (125 MHz): δ 169.2, 162.0, 153.5, 153.1, 147.1, 135.7, 134.3, 134.1, 130.8, 130.5, 129.0, 128.7, 128.6, 127.4, 127.1, 126.9, 126.4, 121.3, 120.5, 114.4, 71.2, 61.0, 53.5, 22.3, 21.8; HRMS (*m/z*): [M+] cald for $C_{29}H_{29}ClN_4O_3$ 517.02; found, 517.21

2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-3-(2-isopropoxy-5-vinylphenyl)quinazolin-4(3H)-one

To a degassed solution of 3-(5-bromo-2-isopropoxyphenyl)-2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)quinazolin-4(3H)-one (Synthesized using the procedures described in scheme 2 using bromo-isopropoxy amine), (6.67 g, 10.8 mmol) in dioxane (100 mmol) PdCl2(PPh3)2 (5%, 0.378 g, 0.539 mmol) was added and the resulting mixture was stirred for 10 min before the addition of tributylvinyl tin (4.73 mL, 16.2 mmol, 1.5 eq). The reaction was heated to 70^0C and stirred for 24 hr, cooled to room temp and a solution of KF (2 M, 16.2 mmol, 8.1 mL, 1.5 eq) was added and then

stirred for an additional 12 hr. Upon completion the reaction was filtered and filtrate was diluted with saturated aqueous $NaHCO_3$ and extracted 3 times with EtOAc. The combined organic layers were dried (Na_2SO_4), concentrated, and the crude material was purified by combi flash 0->5% DCM in methanol to provide 2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-3-(2-isopropoxy-5-vinylphenyl)quinazolin-4(3H)-one (4.47g, 72% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 p.p.m. (d, *J*=2.0 Hz, 1H), 7.86 (dd, *J1* = 8.5 Hz, *J2* = 3.0 Hz, 1H), 7.68 (d, *J*=8.5, 1H), 7.38 (dd, *J1* = 8.4 Hz, *J2* = 2 Hz, 1H), 7.22 (m, 3 H), 7.00 (d, *J*= 8.6 Hz, 1 H), 6.85 (d, *J*=4.8 Hz, 2H), 6.81 (d, *J*=11 Hz, 1H), 5.88 (d, 17.5 Hz, 1H), 5.36 (d, 11 Hz, 1H)4.62 (s, 2H), 4.52 (m, 1H), 3.70 (m, 4.5 H), 3.49 (m, 7H), 2.46 (m, 7 H) 2.32 (m, 1H), 2.22 (m, 1H), 1.21 (d, *J*=6 Hz, 3H), 1.13 (d, *J*=6 Hz, 3 H). ^{13}C NMR (125 MHz): δ 166.0, 161.9, 156.4, 153.1, 152.2, 146.7, 136.4, 135.7, 131.8, 131.0, 130.9, 130.3, 129.5, 127.7, 126.7, 126.2, 124.7, 121.3, 115.9, 115.5, 114.3, 112.4, 71.4, 67.9, 67.0, 62.4, 60.8, 53.6, 52.9, 52.7, 45.2, 42.0, 22.3, 21.8; HRMS (*m/z*): [M+] cald for $C_{32}H_{33}ClN_4O_4$ 573.08; found, 573.27

3-(2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-4-oxoquinazolin-3(4H)-yl)-4-isopropoxybenzaldehyde (AE)

To a solution of 2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-3-(2-isopropoxy-5-vinylphenyl)quinazolin-4(3H)-one (0.625 g, 1.09 mmol) in dioxane:water (3:1, 20 mL) OsO4 (3%, 0.0327 mmol) was added dropwise and the mixture was stirred for 10 min before the addition of NaIO4 (0.332 g, 2.18 mmol, 2.0 eq) in several portions

over 30 min. The reaction was stirred for 24 hr and then diluted with saturated aqueous NaHCO3 and extracted 3 times with EtOAc. The combined organic layers were dried (Na2SO4), concentrated, and the crude material was purified by combi flash 0->5% DCM in methanol to provide 3-(2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-4-oxoquinazolin-3(4H)-yl)-4-isopropoxybenzaldehyde (0.4 g, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.92 p.p.m. (s, 1H), 8.28 (dd, *J1*=7.9, *J2*=1.0 Hz, 1H), 7.96 (dd, *J1*=8.6, *J2*=2.0 Hz, 2H), 7.89 (m, 1H), 7.80 (m, 1H), 7.78 (m, 1H), 7.51 (m, 1H) 7.22 (m, 3H), 7.16 (d, *J*= 8.4, 1H), 6.84 (d, *J*=8.4, 2H), 4.69 (m, 1H), 4.60 (s, 2H), 3.69 (s, 4H), 3.58 (s, 1H), 3.42 (s, 4H), 2.22 (m, 2H), 2.42 (m, 2H), 2.145 (m, 2H), 1.26 (d, *J*=6, 3H), 1.22 (d, *J*=6, 3H), ^{13}C NMR (125 MHz): δ 189.6, 165.9, 161.7, 158.4, 156.4, 152.6, 146.9, 134.6, 133.3, 133.2, 131.9, 129.7, 129.5, 129.4, 127.5, 127.3, 127.1, 127, 126.7, 121.1, 115.9, 121.0, 115.9, 113.5, 72.8, 72.2, 67.8, 67.1, 61.1, 52.9, 52.5, 45.11, 41.9 HRMS (*m/z*): [M+] cald for $C_{31}H_{31}ClN_4O_5$; found, 575.20

2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-3-(2-isopropoxy-5-(piperazin-1-ylmethyl)phenyl)quinazolin-4(3H)-one (PE)

To a solution of 3-(2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-4-oxoquinazolin-3(4H)-yl)-4-isopropoxybenzaldehyde (70 mg, 0.122 mmol) in 1,2-dichloroethane (1 mL) and molecular sieves (50 mg), zinc chloride (0.1 eq, 1.7 mg 0.0122 mmol) and piperazine (63 mg, 0.732 mmol 6.0 eq) were added sequentially. The resulting mixture was stirred at room temp for 3 hr before the addition of a solution of sodium cyanoborohydride (16 mg, 0.244 mmol, 2.0 eq) in methanol (0.5 mL) which was stirred for an additional 1 hr at 25^{0}C before being heated to 40^{0}C for 3 hr. Upon

completion the reaction was filtered, concentrated and purified directly by combiflash 0->20% MeOH in DCM to provide 2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-3-(2-isopropoxy-5-(piperazin-1-ylmethyl)phenyl)quinazolin-4(3H)-one (36 mg, 46% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 p.p.m. (d, *J*= 8.0 Hz, 1H), 7.76 (m, 2H), 7.49 (m 1H), 7.35 (m, 1H), 7.22 (m, 3H), 7.03 (d, *J*=8.4 Hz, 2H), 4.68 (s, 2H), 4.54 (m, 1H), 3.59 (m, 3H), 3.44 (m, 3H), 3.27 (s, 2 H), 3.12 (m, 5 H), 2.62 (m, 6 H) 2.36 (s, 3H), 2.25 (s, 1H), 1.21 (d, *J*=6 Hz, 3H), 1.13 (d, *J*=6 Hz, 3 H), ^{13}C NMR (125 MHz): δ 162.2, 156.4, 153.1, 152.3, 147.1, 134.5, 131.2, 131.1, 129.6, 128.9, 127.5, 127.1, 127.0, 126.0, 121.1, 116.3, 114.4, 77.2, 71.3, 67.4, 61.3, 60.8, 52.7, 49.5, 44.9, 44.2, 42.1, 22.2, 21.8; HRMS (*m/z*): [M+] cald for $C_{35}H_{41}ClN_6O_4$ 645.19; found, 645.29

2-((4-(2-(4-chlorophenoxy)ethanoyl)piperazin-1-yl)methyl)-3-(2-isopropoxy-5-(morpholinomethyl)phenyl)quinazolin-4(3H)-one (MEII)

Prepared from AE according to the procedure described for PE, 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 p.p.m. (dd, *J1*= 8 Hz, *J2*= 1 Hz, 1H), 7.77 (m, 2H) 7.49 (m, 1H), 7.38 (dd, *J1*= 8.4 Hz, *J2*= 2 Hz, 1H), 7.22 (m, 3 H), 7.00 (d, *J*= 8.6 Hz, 1 H), 6.85 (d, *J*=4.8 Hz, 2H), 4.60 (s, 2H), 4.51 (m, 1H), 3.69 (m, 4H), 3.52 (m, 6.5 H), 3.27 (s, 2H), 2.52 (s, 1H), 2.50 (s, 4H), 2.38 (s, 1H), 2.34 (s, 1H), 2.30 (s, 1H), 1.21 (d, *J*= 6.1 Hz, 3H), 1.13 (d, *J*=6.1, 3H), ^{13}C NMR (125 MHz): δ 166.0, 156.4, 153.3, 152.3, 147.1, 134.3, 130.9, 130.3, 129.5, 127.4, 127.1, 126.9, 126.7, 126.2, 121.26, 121.2, 115.9, 114.2, 7.3, 67.9, 67.0, 62.4, 60.8, 53.6, 52.9, 52.7, 45.2, 42.0, 22.3, 21.8; HRMS (*m/z*): [M+] cald for $C_{35}H_{50}ClN_5O_5$ 646.18; found, 646.28

Synthesis of RSL3 analogs

Materials

Chemicals: Solvents, inorganic salts, and organic reagents were purchased from commercial sources (i.e. Sigma-Aldrich) and used without further purification unless otherwise mentioned.

Chromatography: Merck pre-coated 0.25 mm silica plates containing a 254 nm fluorescence indicator were used for analytical thin-layer chromatography. Flash chromatography was performed on 230-400 mesh silica (SiliaFlashÒ P60) from Silicycle.

Spectroscopy: NMR spectra were obtained on a Bruker DPX 300, 400 MHz, Avance III 400SL or Avance III 500 spectrometer. CI-MS spectra were taken on a Nermag R-10-10 instrument and high resolution MS were taken on a double focusing sector type mass spectrometer HX-110A (JEOL Ltd. Tokyo Japan).

Synthesis and characterization of the four diastereomers of RSL3

A general protocol for the Pictet-Spengler and chloroacetylation reactions is described: To a suspension of 1.2 equivalents of the corresponding S or R tryptophan methyl ester hydrochloride in CH_2Cl_2 was added 1.3 equivalents of NEt_3 at room temperature.

The mixture was stirred for 1 hour and then filtered. The filtrate was concentrated to give the required product, tryptophan methyl ester as clear oil, which was dried under vacuum for 10 minutes. Tryptophan methyl ester, along with activated molecular sieves, was

dissolved in anhydrous dichloromethane. Methyl 4-formylbenzoate (1 equivalent) and 0.1 equivalent TFA were added to the reaction mixture and the solution was refluxed for one hour. Then 3 equivalents of TFA were added to the solution, and the reaction was allowed to stir under reflux overnight. The reaction mixture was cooled to room temperature and quenched with 30% NaOH. The organic phase was separated, washed with brine and dried with Na_2SO_4 and then concentrated to give the crude product. The compound was purified by dry-loaded silica gel chromatography in a gradient elution from 100% hexane with 1% NEt_3 to 20:80 EtOAc:hexane with 1% NEt_3 to give two separate diastereomers. Mixed fractions were re-purified with the same protocol. The four diastereomers were obtained in the following yields: (1*R*, 3*S*)-RSL3 intermediate 18%, (1*S*, 3*S*)-RSL3 intermediate 34%, (1*R*, 3*R*)-RSL3 intermediate 41% and (1*S*, 3*R*)-RSL3 intermediate 16%.

The corresponding RSL3 intermediate (one of the stereoisomers) was dissolved in anhydrous CH_2Cl_2 and 1.1 equivalents of sodium bicarbonate was added. To this were added 0.5 equivalents of chloroacetyl chloride dropwise at 0°C. Consecutive half equivalents of chloroacetyl chloride were added and the reaction was followed by MS and TLC until all the starting material was completely consumed. The reaction was filtered and the filtrate was extracted, washed with brine, dried with Na_2SO_4 and concentrated. The compound was purified by silica gel chromatography in 20:80 EtOAc:hexane. A similar protocol was used to obtain the chloroacetamide derivative for the other three diastereomers. The four diastereomers were obtained in the following

3 yields: (1*R*, 3*S*)-RSL3 (49%), (1*S*, 3*S*)-RSL3 (55%), and (1*R*, 3*R*)-RSL3 (42%), (1*S*, 3*R*)-RSL3 (79%).

(1*S*, 3*R*)-RSL3

^{1}H NMR (500 MHz, DMF) δ 8.19 (s, 1H), 8.10 (s, 2H), 7.87 (s, 2H), 7.69 (d, J = 6.9 Hz, 1H), 7.48 (d, J = 6.9 Hz, 1H), 7.22 (dd, J = 19.1, 7.2 Hz, 2H), 6.52 (s, 1H), 5.56 (s, 1H), 4.83 (d, J = 12.8 Hz, 1H), 4.51 (d, J = 12.3 Hz, 1H), 4.04 (s, 3H), 3.90 – 3.71 (m, 4H), 3.65 (d, J = 13.4 Hz, 1H). ^{13}C NMR (75 MHz, DMF) δ 171.93, 171.27, 168.63, 167.63, 166.71, 166.34, 137.86, 137.30, 134.12, 133.31, 129.91, 129.63, 127.19, 126.82, 125.25, 122.03, 119.54, 118.37, 111.85, 110.35, 57.58, 57.00, 52.42, 51.93, 43.23, 23.75.

HRMS (m/z): [MH]+ calculated for $C_{23}H_{21}ClN_2O_5$, 440.1139; found 440.1143.

(1*R*, 3*R*)-RSL3

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.93 (s, 1H), 7.98 (d, J = 8.2 Hz, 2H), 7.65 (d, J = 7.7 Hz, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 8.0 Hz, 2H), 7.25 – 7.16 (m, 1H), 7.11 (d, J = 7.1 Hz, 1H), 7.02 (s, 1H), 5.41 (d, J = 6.5 Hz, 1H), 5.00 (d, J = 13.8 Hz, 1H), 4.61 (d, J = 13.7 Hz, 1H), 3.67 (d, J = 15.9 Hz, 1H), 3.27 (dd, J = 15.5, 6.5 Hz, 1H), 3.02 (s, 3H). ^{13}C NMR (75 MHz, CDCl3) δ 170.74, 167.25, 144.89, 136.95, 136.00, 135.98, 132.86, 132.60, 130.49, 130.47, 129.86, 129.66, 129.47, 128.32, 128.27, 126.84, 122.91, 120.10, 118.99, 111.63, 108.19, 65.53, 27.21, 22.12, 19.68.

HRMS (m/z): [MH]+ calculated for $C_{23}H_{21}ClN_2O_5$, 440.1139; found 440.1146.

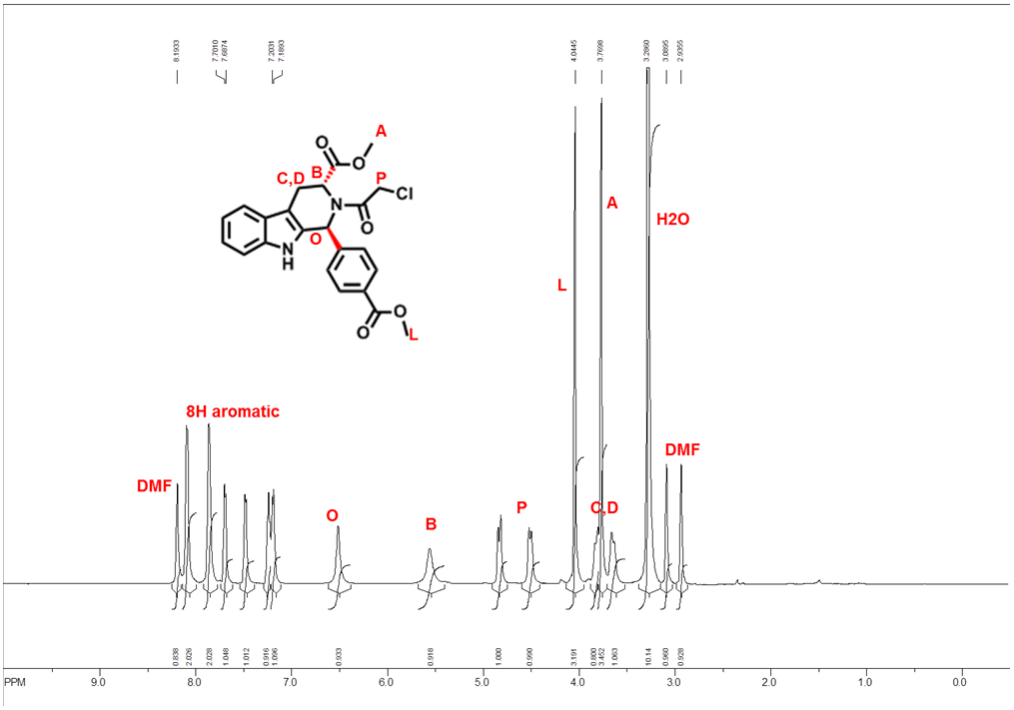
(1*S*, 3*S*)-RSL3

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.94 (s, 1H), 8.05 (s, 1H), 7.98 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 7.7 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.36 (d, J = 8.0 Hz, 2H), 7.24 – 7.16 (m, 1H), 7.13 (dd, J = 10.9, 3.9 Hz, 2H), 7.03 (s, 1H), 5.42 (d, J = 6.5 Hz, 1H), 5.00 (d, J = 13.8 Hz, 1H), 4.61 (d, J = 13.8 Hz, 1H), 3.92 (s, 3H), 3.68 (d, J = 15.9 Hz, 2H), 3.28 (dd, J = 15.5, 6.4 Hz, 1H), 3.03 (s, 3H). ^{13}C NMR (75 MHz, DMF) δ 171.11, 168.52, 167.84, 167.58, 166.84, 165.61, 163.05, 162.66, 162.27, 157.85, 145.68, 144.09, 137.74, 136.34, 130.11, 129.84, 129.53, 127.46, 127.01, 123.73, 118.74, 111.01, 107.66, 79.56, 56.18, 55.36, 55.07, 53.79.

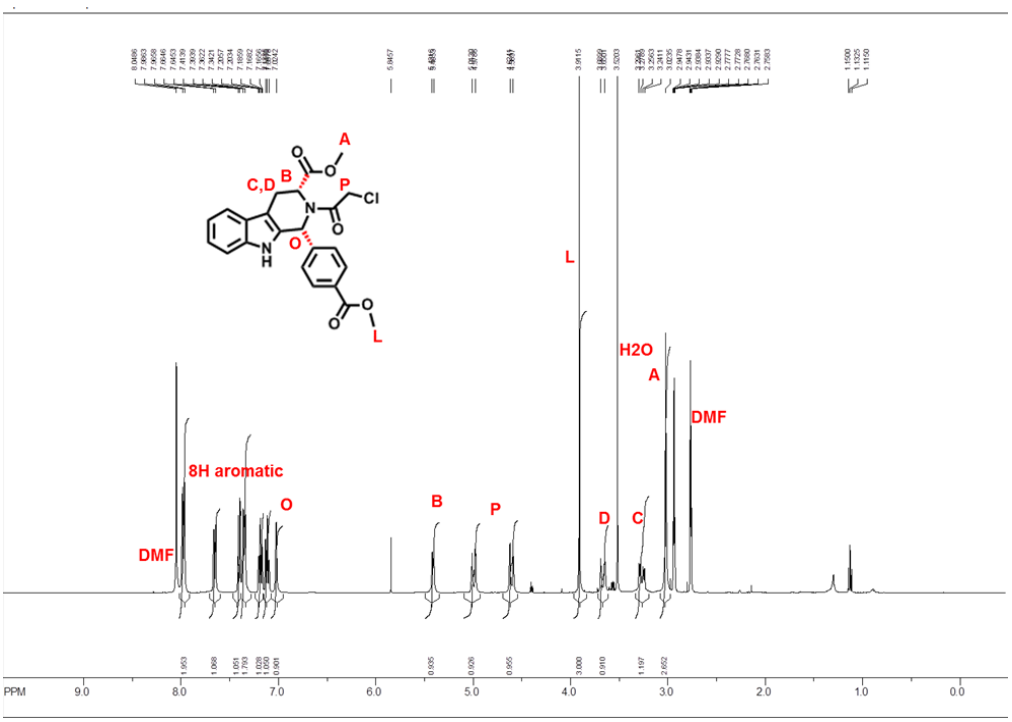
(1*R*, 3*S*)-RSL3

^{1}H NMR (300 MHz, DMF) δ 10.99 (s, 1H), 8.10 (d, J = 8.2 Hz, 2H), 7.86 (d, J = 8.2 Hz, 2H), 7.70 (d, J = 7.4 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.34 – 7.04 (m, 2H), 6.52 (s, 1H), 5.56 (s, 1H), 4.84 (d, J = 13.6 Hz, 1H), 4.51 (d, J = 13.6 Hz, 1H), 4.04 (s, 3H), 3.83 (d, J = 15.6 Hz, 1H), 3.65 (dd, J = 15.6, 4.6 Hz, 1H). ^{13}C NMR (75 MHz, DMF) δ 172.08, 168.79, 166.86, 138.03, 134.27, 130.08, 129.81, 127.36, 126.99, 122.21, 119.72, 118.54, 112.02, 105.65, 57.75, 57.17, 52.59, 52.09, 43.39, 23.92.

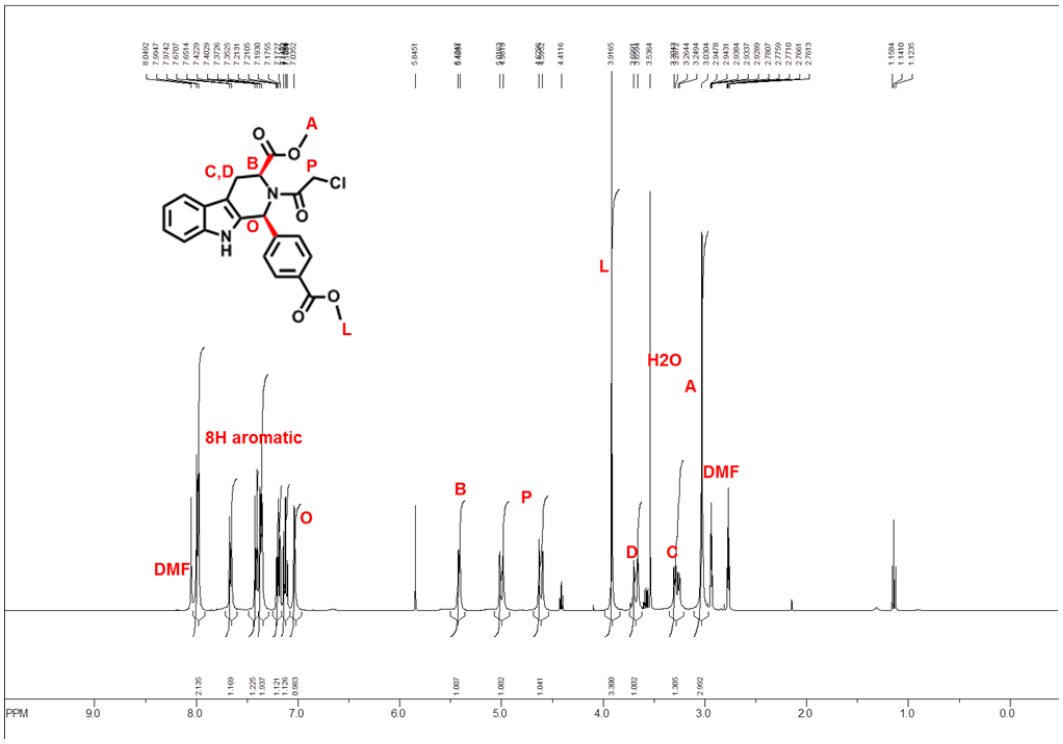
(1*S*, 3*R*)-RSL3



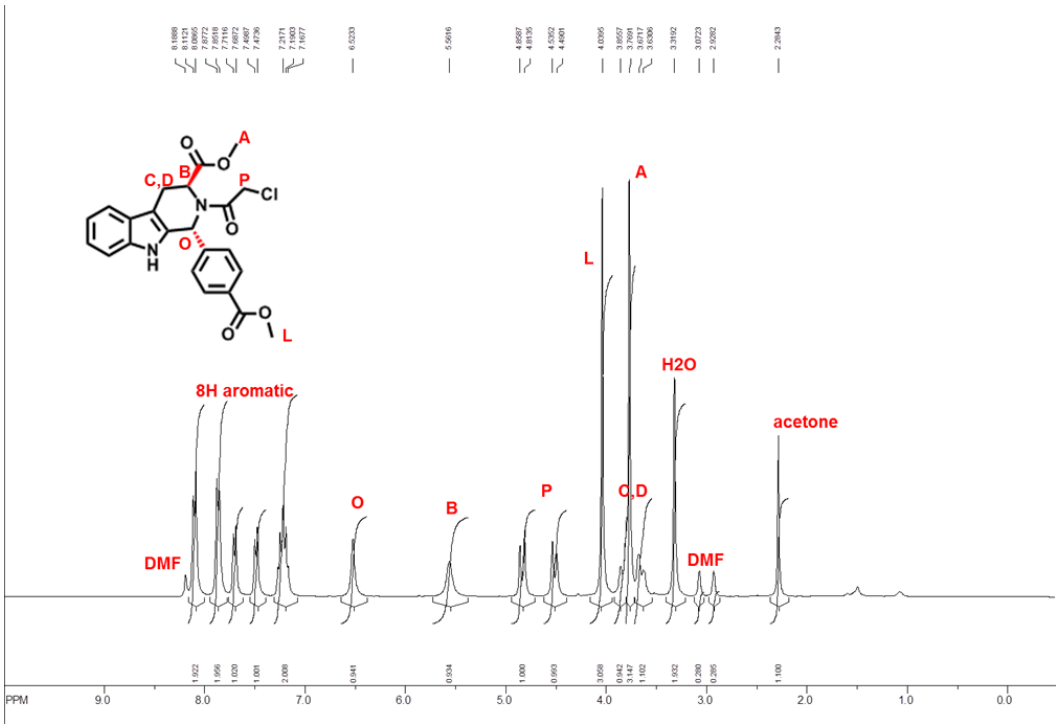
(1*R*, 3*R*)-RSL3



(1*S*, 3*S*)-RSL3



(1*R*, 3*S*)-RSL3



Synthesis and characterization of RSL3-fluorescein probes

Fluorescein-azide intermediate

Based on a published protocol (Gallagher et al., 2009), 11-azido-3,6,9-trioxaundecan-1-amine (63 μL, 0.3 mmol) was added to a solution of 5(6)-carboxyfluorescein-N-succinimidyl ester (50 mg, 0.1 mmol) dissolved in anhydrous DMF (0.5 mL). The reaction was stirred under N_2 overnight and then concentrated in vacuo. The crude product was dissolved in dry pyridine (0.5 mL); isobutyric anhydride (~1 mL, 1 mmol) was added and allowed to stir at room temperature for 2 h. Methanol (0.25 mL) was added to the mixture and incubated for 5 minutes to quench unreacted isobutyric anhydride. The solvent was removed in vacuo and the crude product was purified by silica gel chromatography in 5:95 MeOH: CH_2Cl_2 (Rf = 0.28) to give the protected fluorescein azide with 73% yield over two steps.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (s, 1H), 8.22 (d, J = 7.7 Hz, 1H), 8.11 (d, J = 6.0 Hz, 1H), 7.62 (s, 1H), 7.28 (d, J = 7.2 Hz, 1H), 7.10 (d, J = 6.5 Hz, 2H), 6.82 (d, J = 5.0 Hz, 4H), 3.72 (t, J = 5.9 Hz, 7H), 3.68 – 3.64 (m, 2H), 3.61 (dd, J = 13.2, 5.1 Hz, 2H), 3.56 – 3.47 (m, 2H), 3.41 – 3.33 (m, 1H), 3.32 – 3.24 (m, 1H), 2.82 (dt, J = 13.9, 7.0 Hz, 2H), 1.33 (d, J = 7.0 Hz, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.95, 168.29, 165.55, 155.19, 153.38, 152.54, 151.58, 151.48, 141.45, 137.03, 134.79, 129.44, 128.95, 128.78, 128.14, 126.53, 125.47, 124.49, 123.51, 122.66, 117.89, 117.84, 115.72, 115.67, 110.47, 110.42, 81.95, 81.85, 50.65, 50.59, 40.21, 40.13, 34.19, 18.83.

(1*S*, 3*R*) & (1*R*,3*R*) RSL3-alkyne intermediate

Propargyl 4-formyl benzoate was prepared by stirring 4-formyl benzoic acid (500 mg, 3.33 mmol) and K_2CO_3 (507 mg, 3.67 mmol) in anhydrous DMF for 30 min at 70°C. The reaction mixture was then cooled to 0°C and propargyl bromide (1.15 mL, 3.67 mmol) was added dropwise. The mixture was again heated overnight until it became a clear yellow color. The reaction was filtered, concentrated and purified by silica gel column chromatography in 10:90 EtOAc:hexane to give propargyl 4-formyl benzoate in 80% yield.

The Pictet-Spengler reaction was performed with (D)-tryptophan methyl ester hydrochloride (638 mg, 2.92 mmol) and propargyl 4-formyl benzoate (500 mg, 2.66 mmol) as described above. The 1-propargyl ester RSL3 intermediate was purified by silica gel chromatography by gradient elution from 0:100 to 50:50 EtOAc:hexane with 1% NEt_3 (R_f = 0.53 (1*R*, 3*R*), 0.35 (1*S*, 3*R*)) The 1-propargyl ester intermediates were chloroacetylated as described above and purified by gradient elution from 0:100 to 50:50 EtOAc:hexane to give (1*S*, 3*R*) and (1*R*, 3*R*)-RSL3-alkyne probe in near quantitative yield.

(1*R*, 3*R*)-RSL3-alkyne: ^{1}H NMR (400 MHz, DMF) δ 10.90 (s, 1H), 8.02 (d, J = 7.9 Hz, 2H), 7.66 (d, J = 7.6 Hz, 1H), 7.43 (d, J = 7.5 Hz, 3H), 7.21 (d, J = 7.1 Hz, 1H), 7.13 (t, J = 7.3 Hz, 1H), 7.04 (s, 1H), 5.42 (s, 1H), 5.06 (s, 2H), 4.97 (d, J = 13.0 Hz, 1H), 4.61 (d, J = 13.0 Hz, 1H), 3.79 – 3.65 (m, 1H), 3.55 (s, 1H), 3.29 (dd, J = 15.8, 6.5 Hz, 1H). ^{13}C NMR (101 MHz, DMF) δ 171.13, 167.90, 165.69, 146.12, 137.78, 130.30,

129.71, 129.53, 127.01, 122.51, 119.62, 118.82, 112.00, 107.71, 78.90, 77.46, 53.89, 53.01, 52.67, 52.02, 43.76, 42.03, 22.09.

(1*S*, 3*R*)-RSL3-alkyne: ^{1}H NMR (400 MHz, DMF) δ 10.83 (s, 1H), 7.99 (d, J = 7.9 Hz, 2H), 7.75 (d, J = 7.9 Hz, 2H), 7.57 (d, J = 7.6 Hz, 1H), 7.35 (d, J = 7.9 Hz, 1H), 77.09 (dt, J = 21.2, 7.2 Hz, 3H), 6.40 (s, 1H), 5.43 (s, 1H), 5.01 (d, J = 1.9 Hz, 2H), 4.69 (d, J = 13.6 Hz, 1H), 4.38 (d, J = 13.5 Hz, 1H), 3.70 (d, J = 15.3 Hz, 1H), 3.52 (d, J = 11.8 Hz, 1H). ^{13}C NMR (101 MHz, DMF) δ 178.32, 171.54, 168.29, 165.14, 137.54, 133.66, 129.72, 128.65, 126.97, 126.47, 121.71, 119.21, 118.03, 111.51, 78.35, 76.36, 57.25, 52.28, 52.07, 42.83, 23.41.

(1*S*, 3*R*) & (1*R*, 3*R*)- RSL3-fluorescein probe

Protected fluorescein azide (20 mg, 28 μmol) and either (1*S*, 3*R*) or (1*R*, 3*R*) RSL3-alkyne (15 mg, 34 μmol) as described above, were dissolved in THF and sodium ascorbate (2.8 mg, 14 μmol) and copper sulphate pentahydrate (0.4 mg, 1.4 μmol) dissolved in water was added. The reaction was stirred overnight at 40°C. The final product was purified using TLC preparatory plates run in 5:95 MeOH: CH_2Cl_2 in 83% yield.

(1*S*, 3*R*)-fluorescein-RSL3 (active probe, A): ^{1}H NMR (400 MHz, DMF) δ 10.81 (s, 1H), 8.63 (s, 1H), 8.59 (s, 1H), 8.51 (s, 1H), 8.38 (d, J = 6.6 Hz, 1H), 8.32 (d, J = 8.0 Hz, 1H), 8.17 (d, J = 9.5 Hz, 1H), 7.92 (d, J = 5.6 Hz, 3H), 7.68 (d, J = 7.5 Hz, 2H), 7.53 (dd, J = 7.9, 4.1 Hz, 2H), 7.30 (d, J = 2.0 Hz, 3H), 7.12 – 6.94 (m, 6H), 6.33 (s, 1H), 5.44 (d, J

= 5.1 Hz, 3H), 4.67 (d, J = 13.5 Hz, 1H), 4.59 (dt, J = 10.9, 5.4 Hz, 2H), 4.35 (d, J = 13.3 Hz, 1H), 3.91 (dt, J = 14.9, 5.4 Hz, 2H), 3.71 (t, J = 5.6 Hz, 2H), 3.68 – 3.53 (m, 12H), 3.52 – 3.39 (m, 4H), 2.89 (dd, J = 14.0, 7.0 Hz, 2H), 1.32 (d, J = 7.0 Hz, 12H). ^{13}C NMR (101 MHz, DMF) δ 174.56, 171.70, 168.12, 154.4, 129.95, 128.28, 124.64, 123.78, 120.02, 114.84, 110.42, 72.18, 69.94, 68.68, 52.17, 50.82, 46.81, 43.35, 41.34, 32.59, 22.35,18.51. HRMS (m/z): [MH]+ calculated for $C_{62}H_{61}ClN_6O_{16}$, 1181.3911; found 1182.4039.

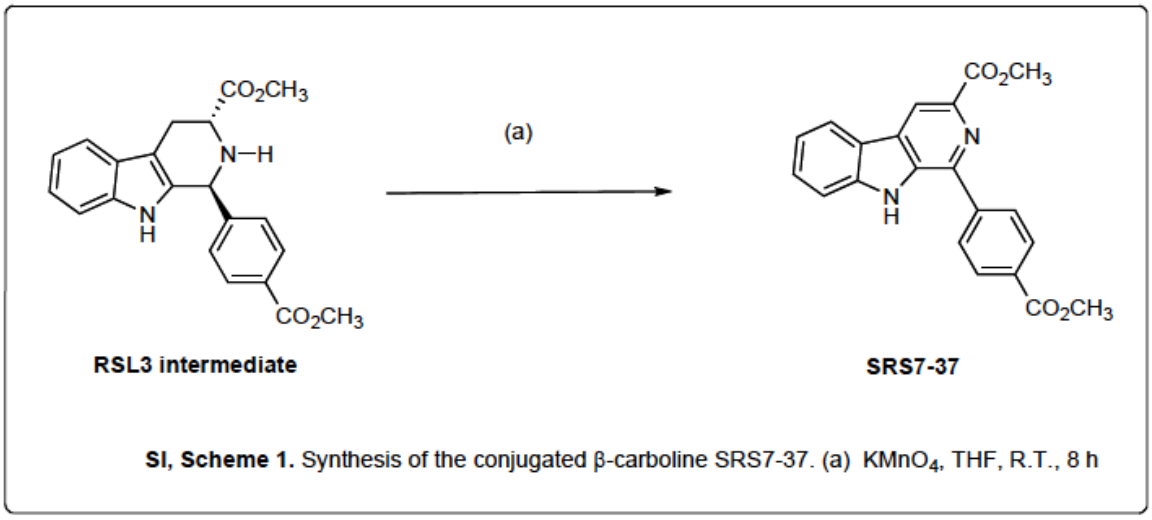
(1*R*, 3*R*)-fluorescein-RSL3 (inactive probe, I): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (d, J = 10.0 Hz, 1H), 8.36 (s, 3H), 8.17 (dd, J = 8.0, 1.5 Hz, 3H), 8.10 (dd, J = 8.0, 1.3 Hz, 2H), 7.96 (d, J = 8.0 Hz, 2H), 7.89 – 7.80 (m, 2H), 7.77 (s, 2H), 7.68 (s, 2H), 7.66 – 7.55 (m, 1H), 7.41 – 7.29 (m, 2H), 7.23 – 7.14 (m, 2H), 7.12 (d, J = 5.8 Hz, 1H), 7.07 – 7.03 (m, 1H), 6.81 (t, J = 2.9 Hz, 1H), 6.79 (d, J = 1.2 Hz, 1H), 6.77 (dd, J = 7.5, 2.3 Hz, 1H), 5.40 (d, J = 20.6 Hz, 2H), 4.98 (s, 1H), 4.61 – 4.50 (m, 1H), 4.48 – 4.30 (m, 1H), 4.22 (dd, J = 12.6, 3.6 Hz, 1H), 3.94 – 3.85 (m, 1H), 3.84 – 3.75 (m, 1H), 3.70 (d, J = 15.7 Hz, 1H), 3.65 – 3.53 (m, 7H), 3.53 – 3.36 (m, 4H), 3.24 (dd, J = 15.9, 6.8 Hz, 1H), 3.04 (s, 2H), 2.92 – 2.71 (m, 2H), 1.32 (t, J = 10.6 Hz, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.41, 168.73, 167.55, 165.97, 155.66, 152.94, 151.96, 144.79, 142.97, 137.08, 135.33, 129.87, 129.18, 126.80, 126.61, 125.81, 125.41, 125.29, 124.86, 123.69, 123.21, 123.03, 120.16, 118.92, 118.26, 116.11, 115.99, 111.63, 110.85, 110.73, 107.96, 82.45, 77.82, 77.60, 77.40, 76.97, 70.90, 70.83, 70.77, 70.59, 70.40, 69.78, 69.64, 58.72, 58.48, 54.13, 52.57, 50.70, 50.59, 42.35, 40.48, 36.29, 34.59, 30.07, 29.70, 27.60, 26.02, 25.91, 22.12,

19.23, 14.48, 0.37. HRMS (m/z): [MH]+ calculated for $C_{62}H_{61}ClN_6O_{16}$, 1181.3911; found 1181.3926.

Synthesis and characterization of (1*S*, 3*R*)-RSL3 electrophile analogs

We developed a structure-activity relationship (SAR) of the parent RSL3 compound to confirm the importance of the chloroacetamide moiety for potency. The syntheses of analogs in Figure S3 are shown below.

SRS7-37



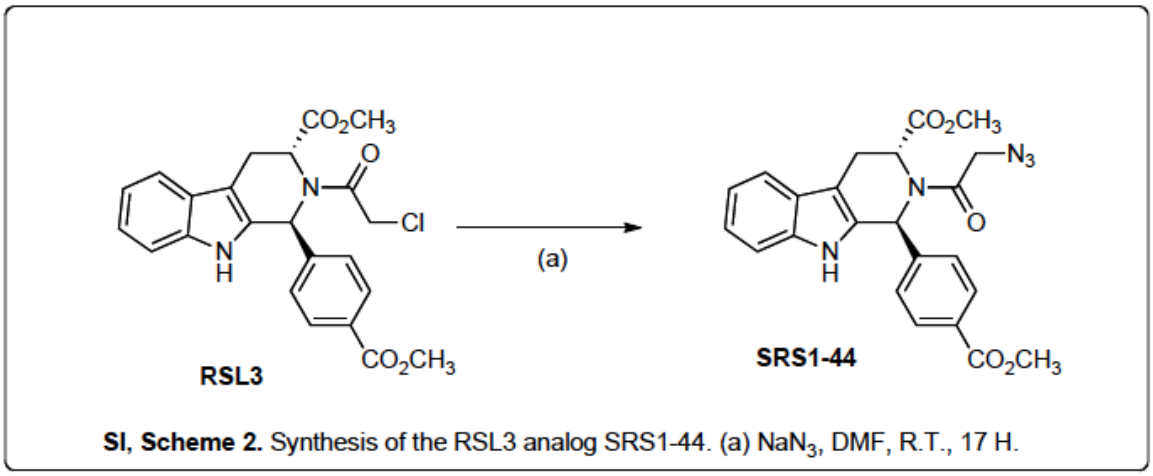
SI, Scheme 1. Synthesis of the conjugated β-carboline SRS7-37. (a) $KMnO_4$, THF, R.T., 8 h

To the **RSL3 intermediate** compound (30 mg, 0.082 mmol) in dry THF was added potassium permanganate (39 mg, 0.247 mmol). The mixture was stirred at room temperature for 8 h. The solid was filtered through celite and the solvent removed under vacuum. The crude product was purified using preparative TLC (DCM/MeOH) to provide compounds **SRS7-37** (20 mg, 0.055 mmol, 68%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (m, 1H), 7.90 (d, J = 8.2 Hz, 1H), 8.26-8.22 (m, 3H), 8.10 (d, J = 8.0 Hz, 2H), 7.65-7.61 (m, 2H), 7.41 (t, J = 7.8 Hz, 1H), 4.08 (s, 3H),

3.98 (s, 3H); MS (APCI+) 360.79.

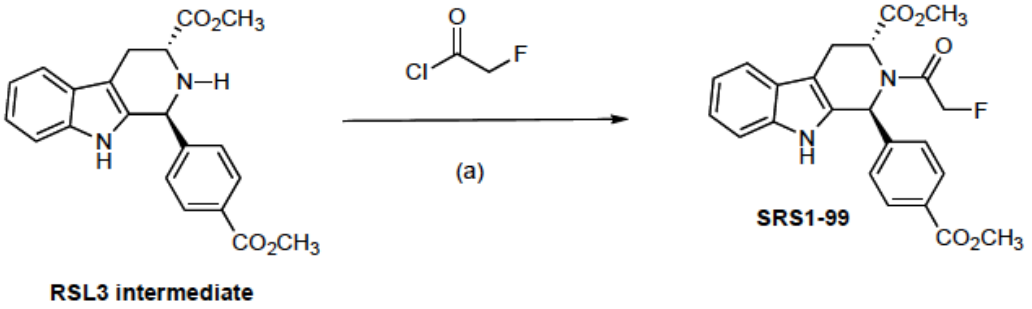
SRS1-44



SI, Scheme 2. Synthesis of the RSL3 analog SRS1-44. (a) NaN_3, DMF, R.T., 17 H.

To **(1*S*, 3*R*)-RSL3** (34 mg, 0.077 mmol) in dry DMF, at 0 ^{O}C, was added sodium azide (NaN_3) (15 mg, 0.231 mmol). The mixture was stirred at room temperature for 17 hours, then poured in water. The organic layer was extracted with ethyl acetate and dried over $MgSO_4$. The organic solvent was removed under vacuum. The crude product was purified via flash column chromatography (CH_2Cl_2:methanol, 20:1) to give the desired RSL3 analog **SRS1-44** (33 mg, 0.073 mmol, 96%). ^{1}H NMR (400 MHz, DMF-d_7) δ 10.87 (m, 1H), 7.94 (d, J = 7.6 Hz, 2H), 7.72 (d, J = 7.6 Hz, 1H), 7.53 (d, J = 7.2 Hz, 1H), 7.33 (d, J = 7.2 Hz, 1H), 7.10-7.02 (m, 2H), 6.35 (s, 1H), 5.37 (m, 1H), 4.45 (s, 1H), 4.11 (m, 1H), 3.89 (s, 3H), 3.62 (s, 3H), 3.33 (m, 1H), 3.14 (s, 2H); MS (APCI+) 448.00.

SRS1-99

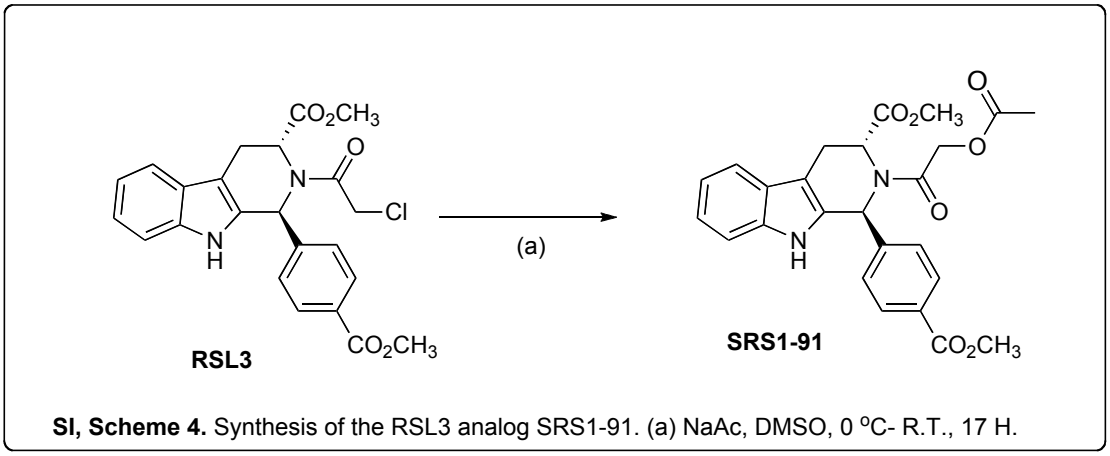


SI, Scheme 3. Synthesis of the RSL3 analog SRS1-99. (a) DIPEA, CH_2Cl_2, 0 °C- R.T., 17 H.

To the **RSL3 intermediate** compound (50 mg, 0.137 mmol) in dry CH_2Cl_2, at 0 °C, was added N,N-diisopropylethylamine (DIPEA) (71.8 µL, 0.412 mmol) and fluoroacetyl chloride (26.5 mg, 0.274 mmol). The mixture was stirred at room temperature for 17 h. The organic solvent was removed under vacuum. The crude product was purified via flash column chromatography (CH_2Cl_2:methanol, 40:1) to give the desired RSL3 analog **SRS1-99** (30 mg, 0.071 mmol, 52 %). ^{1}H NMR (400 MHz, DMF-d_7) δ 11.22 (m, 1H), 7.91 (m, 2H), 7.74 (m, 2H), 7.54 (d, J = 7.6 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 7.08-7.01 (m, 2H), 6.26 (s, 1H), 5.52 (m, 1H), 5.35 (s, 2H), 3.88 (s, 3H), 3.66 (m, 2H), 3.61 (s, 3H); MS (APCI+) 424.42.

SRS1-91

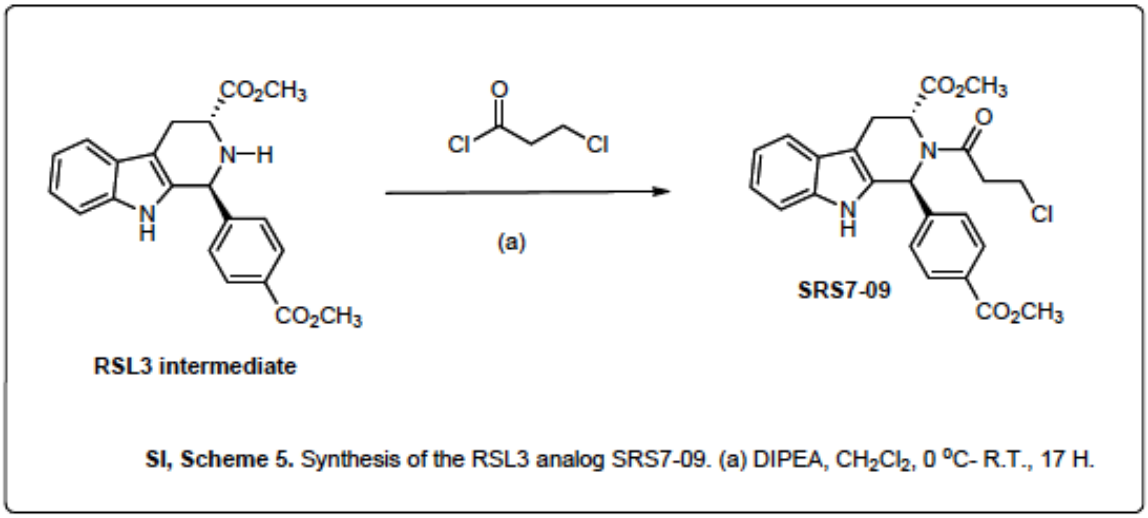
To the **(1*S*, 3*R*)-RSL3** compound (26 mg, 0.059 mmol) in dry DMSO-d6 was added sodium acetate (19.3 mg, 0.236 mmol). The reaction was followed by ^{1}H NMR spectroscopy until the RSL3 compound was consumed. The mixture was stirred at room temperature for 17 h, and then poured into water. The organic layer was extracted with ethyl acetate and dried over MgSO4. The organic solvent was removed under vacuum



SI, Scheme 4. Synthesis of the RSL3 analog SRS1-91. (a) NaAc, DMSO, 0 ^{o}C- R.T., 17 H.

and the crude product was purified via flash column chromatography (CH_2Cl_2:methanol, 40:1) to give the desired RSL3 analog **SRS1-91** (12 mg, 0.026 mmol, 44 %). ^{1}H NMR (400 MHz, DMF-d_7) δ 10.89 (m, 1H), 7.90 (m, 2H), 7.76 (m, 2H), 7.51 (d, J = 7.6 Hz, 1H), 7.33 (d, J = 7.6 Hz, 1H), 7.09-7.01 (m, 2H), 6.24 (s, 1H), 5.50 (m, 1H), 5.33 (s, 2H), 3.86 (s, 3H), 3.62 (m, 2H), 2.31 (s, 2H); MS (APCI+) 464.47.

SRS7-09

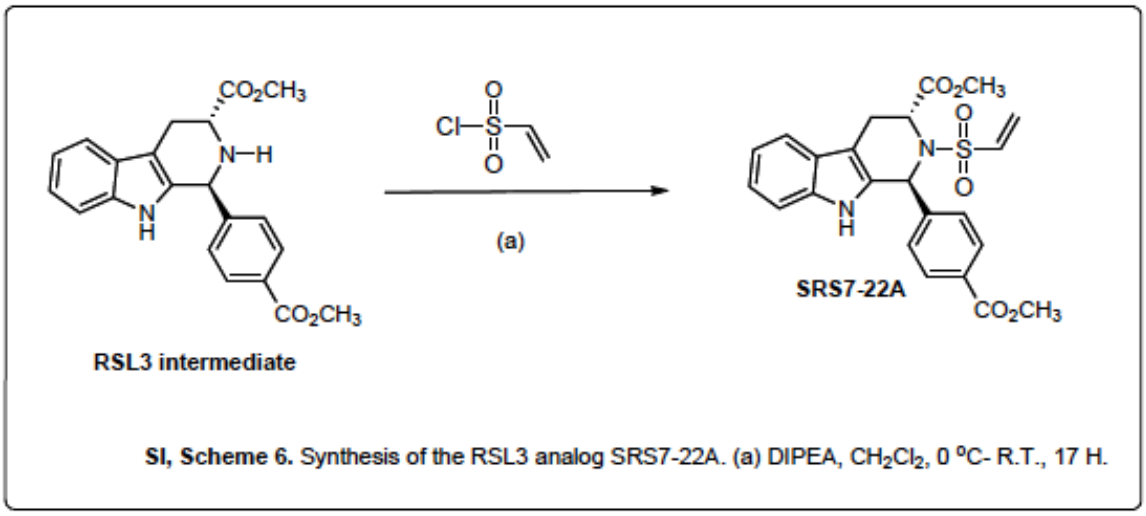


SI, Scheme 5. Synthesis of the RSL3 analog SRS7-09. (a) DIPEA, CH_2Cl_2, 0 ^{o}C- R.T., 17 H.

To the **RSL3 intermediate** compound (50 mg, 0.137 mmol) in dry CH_2Cl_2, at 0 ^{O}C, was added N,N-diisopropylethylamine (DIPEA) (20 µL, 0.274 mmol) and 3-chloropropionyl

chloride (26.2 µL, 0.274 mmol). The mixture was stirred at room temperature for 17 h. The organic solvent was removed under vacuum. The crude product was purified via flash column chromatography (CH_2Cl_2:methanol, 40:1) to give the desired RSL3 analog **SRS7-09** (49 mg, 0.108 mmol, 79%). ^{1}H NMR (400 MHz, DMF-d_7) δ 10.39 (m, 1H), 7.52 (d, J = 7.2 Hz, 2H), 7.30 (d, J = 7.2 Hz, 2H), 7.11 (d, J = 7.6 Hz, 1H), 6.92 (d, J = 7.6 Hz, 1H), 6.68-6.59 (m, 2H), 5.97 (s, 1H), 5.01 (m, 1H), 3.47 (s, 3H), 3.40 (t, J = 6.4 Hz, 2H), 3.06 (s, 3H), 2.89 (m, 1H), 2.83 (m, 1H), 2.75 (m, 2H); MS (APCI+) 454.77.

SRS7-22A

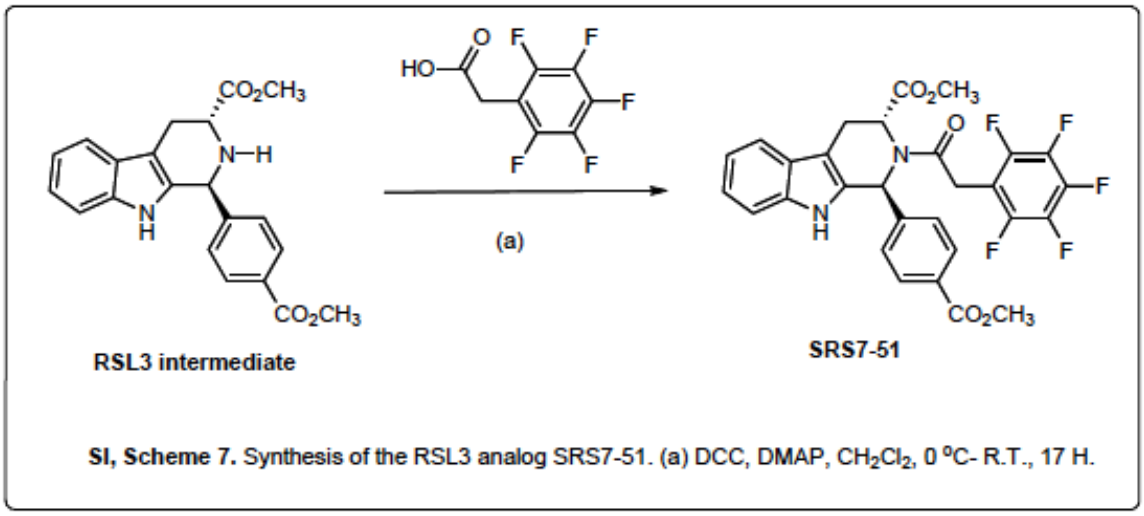


SI, Scheme 6. Synthesis of the RSL3 analog SRS7-22A. (a) DIPEA, CH_2Cl_2, 0 ^{O}C- R.T., 17 H.

To the **RSL3 intermediate** compound (50 mg, 0.137 mmol) in dry CH_2Cl_2, at 0 ^{O}C, was added N,N-diisopropylethylamine (DIPEA) (24 µL, 0.137 mmol) and vinylsulfonyl chloride (35mg, 0.274 mmol). The mixture was stirred at room temperature for 17 h. The organic solvent was removed under vacuum. The crude product was purified via flash column chromatography (CH_2Cl_2:methanol, 40:1) to give the desired RSL3 analog **SRS7-22A** (35 mg, 0.077 mmol, 56%). ^{1}H NMR (400 MHz, DMF-d_7) δ 10.65 (m, 1H), 7.96 (d, J = 8.0 Hz, 2H), 7.58-7.54 (m, 3H), 7.32 (d, J = 8.0 Hz, 1H), 7.13-7.03 (m, 2H),

6.57 (dd, J = 10.0, 16.0 Hz, 1H), 6.26 (s, 1H), 5.95 (d, J = 16.0 Hz, 1H), 5.83 (d, J = 10.0 Hz, 1H), 4.85 (t, J = 5.2 Hz, 1H), 3.90 (s, 3H), 3.67 (s, 3H), 3.46 (m, 2H); MS (APCI+) 455.16.

SRS7-51



SI, Scheme 7. Synthesis of the RSL3 analog SRS7-51. (a) DCC, DMAP, CH_2Cl_2, 0 ^{0}C- R.T., 17 H.

To the **RSL3 intermediate** compound (50 mg, 0.137 mmol) in dry CH_2Cl_2, at 0 ^{0}C, was added 4-(dimethylamino)pyridine (DMAP) (3.5 mg, 0.027 mmol), N,N'-dicyclohexylcarbodiimide (DCC) (34.0 mg, 0.165 mmol) and pentafluorophenylacetic acid (37.3 mg, 0.165 mmol). The mixture was stirred at room temperature for 17 hours. The organic solvent was removed under vacuum. The crude product was purified via flash column chromatography (CH_2Cl_2:methanol, 40:1) to give the desired RSL3 analog SRS7-51 (60 mg, 0.105 mmol, 77%). ^{1}H NMR (400 MHz, DMF-d_7) δ 10.84 (m, 1H), 7.93 (m, 2H), 7.75 (m, 2H), 7.54 (d, J = 7.6 Hz, 1H), 7.33 (d, J = 7.6 Hz, 1H), 7.10-7.05 (m, 2H), 6.44 (s, 1H), 4.35 (m, 1H), 3.89 (s, 3H), 3.63 (s, 3H), 3.34 (m, 2H), 3.07 (s, 2H); MS (APCI+) 572.52.