Supplementary Material (ESI) for Molecular BioSystems

Supporting Information

Structure-Activity Relationships of derivatives of Exo2, a small molecule inhibitor of secretion and retrograde trafficking pathways in mammalian cells.

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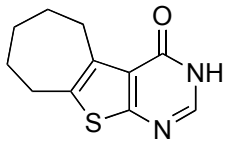
Contents:

1. Experimental Procedures

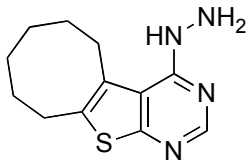
General

All reagents and solvents were purchased from Alfa Aesar, Aldrich and Maybridge and used without further purification. NMR spectra were recorded on a DPX-300 or a DPX-400spectrometer at room temperature (298K). The spectra were recorded in parts per million (ppm) and referenced using residual protio solvents relative to trimethylsilane standard (δ_H = 0ppm). 2D COSY, HMQC and HMBC spectra were used to aid with peak assignments. ESI mass spectra were obtained using a Bruker Esquire 2000 mass spectrometer coupled with an Agilent 1100 HPLC (without a column) as the delivery system. Accurate mass spectra were obtained using a Bruker micro-TOF ESI attached to a time of flight (TOF) analyzer. CHN elemental analyses were carried out by Warwick Analytical Services. Thin Layer Chromatography used in monitoring reaction progress were performed using silica layer (0.25mm) coated alumina plates. Weights were recorded on a balance to 4 decimal places. Exo2[1] and compounds **1**,[1] **2**,[2] **3**,[3] **4**,[4] **5**,[1] **6**,[2] **8**,[5] **9**,[1] **10**,[2] **12**,[6] **16**,[7] **24-29**,[8] **35f**,[9] **35l**,[10] **35m**[10] and **36**[11] were synthesized as described in the literature.



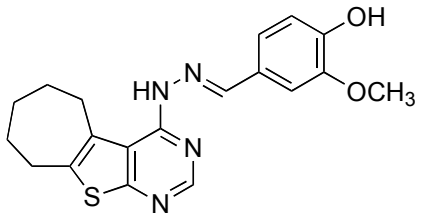
Thienopyrimidone 7: The starting material **3** was heated at 150°C in 5 mL formamide for 5 hours. Upon cooling overnight, the product crystallized as slightly brownish crystals. The resulting crystals were collected and washed with a mixture of cold ethanol/water (1:1) to give the corresponding thienopyrimidone in quantitative yield.

^{1}H NMR (400 MHz, DMSO) δ: 1.17 (m, 2H, CH_2), 1.42 (m, 2H, CH_2), 1.62 (m, 4H, 2 CH_2), 2.87 (m, 2H, CH_2), 3.06 (m, 2H, CH_2), 8.01 (s, 1H, CH), 12.28 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 24.4 (CH_2), 25.3 (CH_2), 25.4 (CH_2), 26.0 (CH_2), 29.9 (CH_2), 31.5 (CH_2), 133.7 (C), 135.0 (C), 144.6 (C), 147.8 (C), 150.0 (CH), 157.7(C). ES-MS *m*/*z* 235.1 (MH^+).



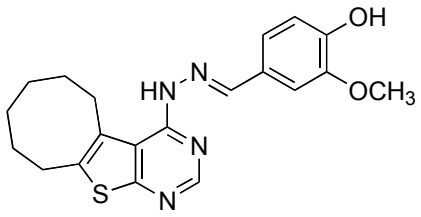
Compound 11: The thienopyrimidone compound **7** was dissolved in hot DMF (10mL), then ice cooled prior to the addition of 2 equiv. of $POCl_3$. The reaction mixture was stirred overnight, diluted with dichloromethane and washed with water and brine. The organic layer was dried with magnesium sulphate, filtered off and concentrated in vacuo to afford the chloride intermediate. Then, the chloride was dissolved in methanol and 10 equiv. of hydrazine monohydrate were added. The mixture was stirred for 2 hours. The resulting precipitate was filtered off and washed with cold methanol to afford the title compound **11** in 37% yield starting from **7** (255mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.27 (m, 2H, CH_2), 1.44 (m, 2H, CH_2), 1.65 (m, 4H, 2 CH_2), 2.50 (brs, 2H, NH_2), 2.83 (m, 4H, 2 CH_2), 6.54 (brs, 1H, NH), 8.41 (s, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.3 (CH_2), 26.0 (CH_2), 26.1 (CH_2), 27.7 (CH_2), 30.1 (CH_2), 31.6 (CH_2), 115.7 (C), 127.7 (C), 137.3 (C), 152.3 (CH), 158.7 (C), 164.8 (C). ES-MS *m*/*z* 249.0 (MH^+), 271.0 (MNa^+).



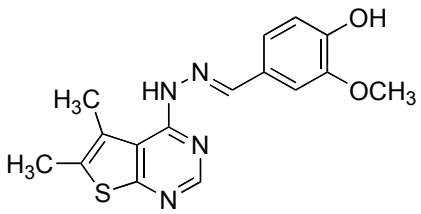
Compound 13: Starting from 80 mg of **10**, and using the procedure for **35a-z**, 72 mg of yellow crystals were obtained (yield: 57%).

^{1}H NMR (400 MHz, DMSO) δ: 1.59 (m, 2H, CH_2), 1.65 (m, 2H, CH_2), 1.86 (m, 2H, CH_2), 2.83 (m, 2H, CH_2), 3.48 (m, 2H, CH_2), 3.88 (s, 3H, CH_3), 6.84 (d, 1H, J = 10.7 Hz, CH), 7.32 (d, 1H, J = 10.7 Hz, CH), 7.61 (s, 1H, CH), 7.78 (s, 1H, CH), 8.33 (s, 1H, CH), 9.47 (brs, 1H, OH), 11.71 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 26.9 (CH_2), 27.4 (CH_2), 27.8 (CH_2), 29.2 (CH_2), 32.1 (CH_2), 55.8 (CH_3), 110.9 (CH), 115.3 (CH), 119.3 (C), 122.4 (CH), 126.9 (C), 136.5 (C), 136.7 (C), 145.8 (CH), 147.8 (C), 148.5 (C), 148.8 (C), 153.5 (CH), 155.3 (C).ES-MS *m*/*z* 369.1 (MH^+). HRMS 369.1380, found 396.1386. Anal. ($C_{19}H_{20}N_4O_2S$.0.3MeOH) C, H, N.



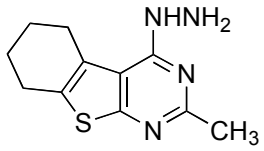
Compound 14: To a solution of 250 mg of **11** in methanol was added 1.2 equivalent of vanillin. The mixture was stirred for 2 hours, diluted with water and extracted with dichloromethane. The organic layer was dried with $MgSO_4$, filtered off and concentrated in vacuo. Crystallisation from diethyl ether gave 100mg of yellow crystals (yield: 26%).

^{1}H NMR (400 MHz, DMSO) δ: 1.27 (m, 2H, CH_2), 1.46 (m, 2H, CH_2), 1.62 (m, 2H, CH_2), 1.68 (m, 2H, CH_2), 2.85 (m, 2H, CH_2), 3.19 (m, 2H, CH_2), 3.88 (s, 3H, CH_3), 6.84 (d, 1H, J = 10.7 Hz, CH), 7.60 (d, 1H, J = 10.7 Hz, CH), 7.79 (s, 1H, CH), 8.30 (s, 1H, CH), 9.45 (brs, 1H, OH) 11.70 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 24.8 (CH_2), 25.8 (CH_2), 26.6 (CH_2), 29.7 (CH_2), 31.7 (CH_2), 51.8 (CH_3), 111.0 (CH), 115.4 (CH), 118.8 (C), 122.2 (CH), 126.8 (C), 126.9 (C), 133.5 (C), 135.1 (C), 143.6 (CH), 144.5 (C), 147.8 (C), 148.7 (C), 153.4 (CH). ES-MS *m*/*z* 383.1 (MH^+). HRMS 393.1536, found 383.1530. Anal. ($C_{20}H_{22}N_4O_2S$.0.2MeOH) C, H, N.



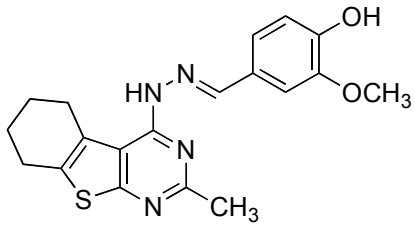
Compound 15: Starting from 70 mg of **12** and using the procedure for **35a-z**, 100 mg of yellow crystals were obtained (yield: 85%).

^{1}H NMR (400 MHz, DMSO) δ: 2.36 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 6.84 (s, 1H, CH), 7.31 (s, 1H, CH), 7.60 (s, 1H, CH), 7.78 (s, 1H, CH), 8.33 (s, 1H, CH), 9.46 (brs, 1H, OH), 11.69 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 12.9 (CH_3), 14.0 (CH_3), 55.8 (CH_3), 105.2 (C), 110.9 (CH), 115.3 (CH), 115.4 (C), 122.3 (CH), 126.9 (C), 129.1 (C), 143.4 (CH), 147.8 (C), 148.8 (C), 153.4 (CH), 153.5 (C). ES-MS *m*/*z* 329.1 (MH^+). HRMS 329.1067, found 329.1069. Anal. ($C_{16}H_{16}N_4O_2S$.0.4MeOH) C, H, N.



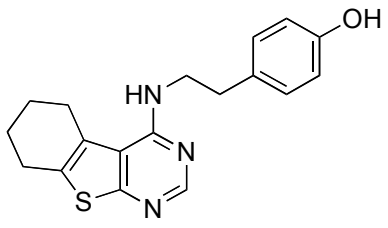
Compound 17: Starting from 190mg of **16** using the procedure described for **11**, 175mg were obtained (49% over 2 steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.82 (m, 4H, 2 CH_2), 2.52 (m, 2H, CH_2), 2.70 (m, 2H, CH_2), 2.76 (s, 3H, CH_3), 4.11 (brs, 2H, NH_2), 6.39 (brs, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.5 (2 CH_2), 25.3 (CH_2), 25.7 (CH_3), 26.2 (CH_2), 112.5 (C), 125.2 (C), 132.7 (C), 158.8 (C), 161.9 (C). ES-MS *m*/*z* 235.0 (MH^+).



Compound 18: Starting from 151 mg of **17**, and using the procedure for **35a-z** below, 150 mg of yellow powder were obtained (yield: 63%).

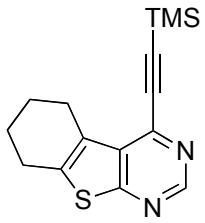
^{1}H NMR (400 MHz, DMSO) δ: 1.79 (m, 4H, 2 CH_2), 2.40 (s, 3H, CH_3), 2.72 (m, 2H, CH_2), 2.97 (m, 2H, CH_2), 3.88 (s, 3H, OCH_3), 6.83 (d, J = 8.2 Hz, 1H, CH), 7.32 (dd, J_1 = 8.2Hz, J_2 = 2.0 Hz, 1H, CH), 7.64 (d, J = 2.0 Hz, 1H, CH), 8.31 (s, 1H, CH), 9.44 (brs, 1H, OH), 11.05 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 21.4 (CH_3), 22.0 (CH_2), 22.4 (CH_2), 24.6 (CH_2), 26.5 (CH_2), 55.7 (CH_3), 111.1 (CH), 115.3 (CH), 116.9 (C), 112.4 (CH), 126.8 (C), 130.6 (C), 130.7 (C), 147.7 (C), 148.4 (C), 148.7 (C), 152.8 (C), 153.5 (CH), 157.2 (C). ES-MS *m*/*z* 369.1 (MH^+). HRMS 369.1380, found 369.1385. Anal. ($C_{19}H_{20}N_4O_2S$.0.15MeOH) C, H, N.



Compound 20: A mixture of chloride **19** (145 mg, 0.64 mmol) and tyramine hydrochloride (112 mg, 0.64 mmol) were heated overnight at 80°C in isopropanol with 2 equivalent of triethylamine (185µL, 1.3 mmol). Upon cooling a white precipitate was collected, dissolved in EtOAc and extracted twice with a diluted HCl

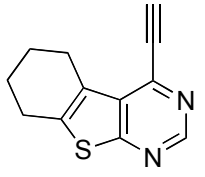
solution and brine. The organic layer was dried with $MgSO_4$, filtered off and concentrated under reduced to afford 90mg of **20** (yield: 43%).

^{1}H NMR (400 MHz, DMSO) δ: 1.85 (m, 4H, 2 CH_2), 2.85 (m, 6H, 3 CH_2), 3.69 (q, *J* = 6.4 Hz, 2H, CH_2), 6.52 (t, 1H, NH), 6.75 (d, *J* = 8.4 Hz, 2H, CH_2), 7.09 (d, *J* = 8.4 Hz, 2H, CH_2), 8.23 (s, 1H, CH), 9.24 (brs, 1H, OH). ^{13}C NMR (100 MHz, DMSO) δ: 21.9 (CH_2), 22.1 (CH_2), 24.8 (CH_2), 25.5 (CH_2), 33.8 (CH_2), 42.2 (CH_2), 115.1 (2 CH), 115.4 (C), 126.4 (C), 129.5 (2 CH), 129.6 (C), 131.2 (C), 152.8 (CH), 155.6 (C), 156.7 (C), 164.4(C). ES-MS *m*/*z* 326.1 (MH^+). HRMS 326.1322, found 326.1311. Anal. ($C_{18}H_{19}N_3OS$) C, H, N,S.



Compound 21: To a mixture of chloride **19** (500mg, 2.23 mmol), copper(I) iodide (44 mg, 0.23 mmol), PPh_3 (120mg, 0.46mmol), $Pd(OAc)_2$ (52mg, 0.23mmol) and DIEA (1.95mL, 11.15mmol) in degassed THF (20mL) was added TMS-acetylene (880μL, 8.92mmol). The resulting black suspension was stirred at room temperature under nitrogen atmosphere for 48hr. The mixture was then diluted with dichloromethane, and washed with an aqueous solution of saturated Na_2CO_3, water and finally brine. The organic layer was dried with $MgSO_4$, filtered off and concentrated under reduced pressure and the residue was purified by chromatography (dichloromethane:ethyl acetate 100:0 to 95:5) to afford **21** in 65% yield (415mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.18 (s, 9H, 3 CH_3), 1.79 (m, 4H, 2 CH_2), 2.75 (m, 2H, CH_2), 2.96 (m, 2H, CH_2), 8.75 (s, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 0.0 (3 CH_3), 22.8 (CH_2), 23.3 (CH_2), 26.1 (CH_2), 26.7 (CH_2), 102.2 (C), 104.2 (C), 128.0 (C), 131.5 (C), 139.9 (C), 142.5 (C), 152.8 (CH), 169.6 (C). ES-MS *m*/*z* 287.1 (MH^+).



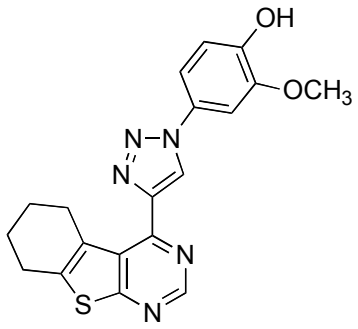
Compound 22: 370mg of **21** (1.29mmol) was stirred overnight at room temperature in 7mL THF with 150μL of a solution of 1M TBAF in THF. The mixture was

quenched with a saturated solution of ammonium chloride and diluted with ethyl acetate. The organic layer was collected, washed with water and brine. The organic layer was dried with $MgSO_4$, filtered off and concentrated under reduced pressure to afford **22** in 87% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.17 (m, 4H, 2 CH_2), 3.14 (m, 2H, CH_2), 3.37 (m, 2H, CH_2), 3.85 (s, 1H, CH), 9.17 (s, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.1 (CH_2), 22.6 (CH_2), 25.2 (CH_2), 26.1 (CH_2), 80.9 (C), 84.3 (CH), 127.2 (C), 132.3 (C), 139.6 (C), 141.2 (C), 152.1 (CH), 169.1 (C). ES-MS *m*/*z* 215.1 (MH^+).

General procedure from the "Click" Chemistry.

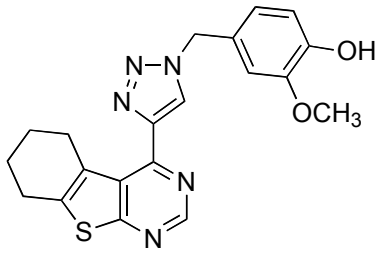
To a solution of azide (60mg, 0.375 mmol) and alkyne **22** (80 mg, 0.375 mmol) in 1:1 EtOH/H_2O (5 mL) was added $CuSO_4$•5 H_2O (10 mg, 0.0375 mmol) and sodium ascorbate (25 mg, 0.113 mmol), each in one portion. The result yellowish cloudy suspension was stirred at room temperature for 36 hours. The resulting mixture was partitioned between water and EtOAc, and the aqueous layer extracted with additional EtOAc (2 x 50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was washed with chloroform to remove soluble impurities to finally afford a white solid.



Compound 30: 80mg (yield: 55%).

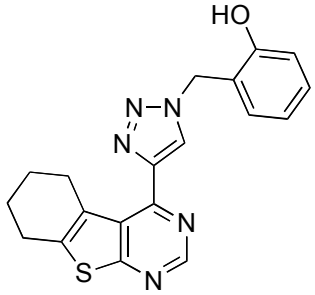
^{1}H NMR (400 MHz, DMSO) δ: 1.74 (m, 2H, CH_2), 1.87 (m, 2H, CH_2), 2.68 (m, 2H, CH_2), 2.95 (m, 2H, CH_2), 3.92 (s, 3H, CH_3), 6.98 (d, J = 8.5 Hz, 1H, CH), 7.44 (dd, J_1 = 8.5 Hz, J_2= 2.4 Hz, 1H, CH), 7.59 (d, J = 2.4 Hz, 1H, CH), 9.06 (s, 1H, CH), 9.33 (s, 1H, CH), 9.60 (brs, 1H, OH). ^{1}H NMR (100 MHz, DMSO) δ: 21.9 (CH_2), 22.0 (CH_2), 25.7 (CH_2), 26.7 (CH_2), 56.0 (CH_3), 105.1 (CH), 112.7 (CH), 115.6 (CH), 124.0 (CH), 127.7 (C), 128.5 (C), 138.4 (C), 145.8 (C), 147.2 (C), 148.2 (C), 150.3

(C), 151.6 (CH), 168.8 (C). ES-MS *m*/*z* 380.1 (MH^+). HRMS 380.1176, found 380.1165. Anal. ($C_{19}H_{17}N_5OS.0.3MeOH$) C, H, N.



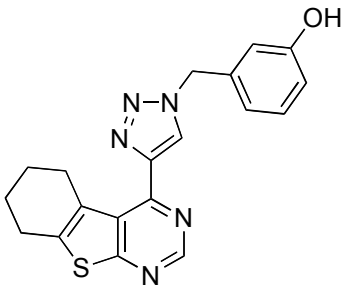
Compound 31: 50mg (yield: 34%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.76 (m, 2H, CH_2), 1.92 (m, 2H, CH_2), 2.74 (m, 2H, CH_2), 2.91 (m, 2H, CH_2), 3.86 (s, 3H, CH_3), 5.53 (s, 2H, CH_2), 5.87 (brs, 1H, OH), 6.89 (s, 1H, CH), 6.95 (s, 2H, 2 CH), 8.01 (s, 1H, CH), 8.95 (s, 1H, CH), 9.59 (brs, 1H, OH). ^{1}H NMR (100 MHz, $CDCl_3$) δ: 22.6 (CH_2), 22.7 (CH_2), 26.4 (CH_2), 27.3 (CH_2), 54.5 (CH_2), 56.0 (CH_3), 110.9 (CH), 114.8 (CH), 114.9 (C), 122.0 (CH), 124.4 (CH), 125.7 (C), 128.1 (2 C), 128.8 (C), 138.9 (C), 146.8 (C), 150.3 (C), 151.4 (CH), 170.1 (C). ES-MS *m*/*z* 394.1 (MH^+). HRMS 394.1332, found 394.1337. Anal. ($C_{20}H_{19}N_5O_2S$) C, H, N, S.

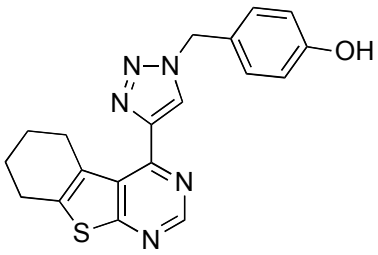


Compound 32: 80mg (yield: 55%).

^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ: 1.74 (m, 2H, CH_2), 1.91 (m, 2H, CH_2), 2.66 (m, 2H, CH_2), 2.90 (m, 2H, CH_2), 5.63 (s, 2H, CH_2), 6.86 (2d, *J* = 7.5 Hz, 2H, 2 CH), 7.20 (t, *J* = 7.5 Hz, 1H, CH), 7.31 (t, *J* = 7.5 Hz, 1H, CH), 8.21 (s, 1H, CH), 8.89 (s, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$+CD_3OD) δ: 22.5 (2 CH_2), 26.3 (CH_2), 27.1 (CH_2), 116.0 (CH), 120.3 (CH), 121.0 125.2 (CH), 128.3 129.1 130.6 (CH), 130.9 (CH), 139.2 (C), 145.5 (C), 150.4 (C), 150.9 (CH), 156.5 (C), 170.0 (C). ES-MS *m*/*z* 364.1 (MH^+). HRMS 364.1227, found 364.1216. Anal. ($C_{19}H_{17}N_5OS$) C, H, N.



Compound 33: 70mg (yield: 47%).^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ: 1.76 (m, 2H, CH_2), 1.91 (m, 2H, CH_2), 2.65 (m, 2H, CH_2), 2.92 (m, 2H, CH_2), 5.56 (s, 2H, CH_2), 6.76 (s, 1H, CH), 6.85 (2d, *J* = 7.9 Hz, 2H, 2 CH), 7.23 (t, *J* = 7.9 Hz, 1H, CH), 8.03 (s, 1H, CH), 8.91 (s, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$+CD_3OD) δ: 22.4 (CH_2), 22.5 (CH_2), 26.3 (CH_2), 27.1 (CH_2), 54.3 (CH_2), 114.9 (CH), 116.2 (CH), 119.4 (CH), 124.8 (CH), 128.0 (C), 129.1 (C), 130.4 (CH), 135.3 (C), 139.4 (C), 146.1 (C), 150.1 (C), 151.0 (CH), 157.7 (C), 170.0 (C). ES-MS *m*/*z* 364.1 (MH^+). HRMS 364.1227, found 364.1221. Anal. ($C_{19}H_{17}N_5OS$.0.3MeOH) C, H, N.

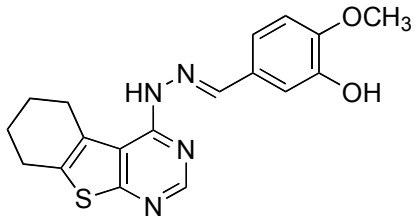


Compound 34: 90mg (yield: 61%).

^{1}H NMR (400 MHz, DMSO) δ: 1.68 (m, 2H, CH_2), 1.84 (m, 2H, CH_2), 2.91 (m, 2H, CH_2), 3.33 (m, 2H, CH_2), 5.60 (s, 2H, CH_2), 6.79 (d, *J* = 8.4Hz,2H, 2 CH), 7.27 (d, *J* = 8.4Hz,2H, 2 CH), 8.68 (s, 1H, CH), 8.99 (s, 1H, CH), 9.59 (brs, 1H, OH). ^{1}H NMR (100 MHz, DMSO) δ: 21.9 (CH_2), 22.1 (CH_2), 25.6 (CH_2), 26.6 (CH_2), 52.8 (CH_2), 115.5 (2 CH), 125.9 (CH), 127.6 (C), 127.9 (C), 129.7 (2 CH), 138.2 (C), 145.2 (C), 150.7 (C), 151.6 (CH), 157.5 (C), 168.6 (C). ES-MS *m*/*z* 364.1 (MH^+). HRMS 364.1227, found 364.1222. Anal. ($C_{19}H_{17}N_5OS$. MeOH) C, H, N.

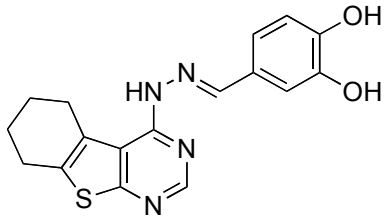
General procedure for the synthesis of hydrazones 35a-z.

To a solution of 1 equivalent of hydrazine compound **9** in methanol was added 1.2 equivalent of aldehyde. The mixture was stirred for 2 hours, and the resulting precipitate was collected and recrystallised from methanol to afford the Exo2 analogues.



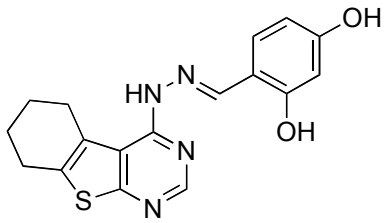
Compound 35a: Starting from 100 mg of **9**, 90 mg of yellow crystals were obtained (yield: 56%).

^{1}H NMR (400 MHz, DMSO) δ: 1.79 (m, 4H, 2 CH_2), 2.75 (m, 2H, CH_2), 2.98 (m, 2H, CH_2), 3.84 (s, 3H, CH_3), 7.00 (d, 1H, *J* = 10.7 Hz, CH), 7.28 (d, 1H, *J* = 10.7 Hz, CH), 7.47 (s, 1H, CH), 7.76 (s, 1H, CH), 8.26 (s, 1H, CH), 9.00 (brs, 1H, OH), 11.72 (brs, 1H, NH). ^{13}C NMR (75 MHz, DMSO) δ: 22.0 (CH_2), 22.4 (CH_2), 24.6 (CH_2), 26.5 (CH_2), 55.6 (CH_3), 111.5 (CH), 113.9 (CH), 118.8 (C), 120.6 (CH), 128.4 (C), 130.8 (C), 132.0 (C), 143.9 (CH), 146.4 (C), 148.2 (C), 149.4 (C), 153.1 (CH), 156.8 (C). ES-MS *m/z* 355.1 (MH^+). HRMS 355.1223, found 355.1233. Anal. ($C_{18}H_{18}N_4O_2S$.0.2MeOH) C, H, N.



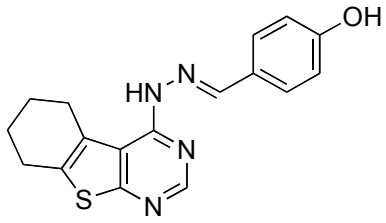
Compound 35b: Starting from 100 mg of **9**, 150 mg of pale yellow crystals were obtained (yield: 97%).

^{1}H NMR (400 MHz, DMSO) δ: 1.79 (m, 4H, 2 CH_2), 2.75 (m, 2H, CH_2), 2.98 (m, 2H, CH_2), 6.80 (d, J= 10.7 Hz, 2 CH), 7.20 (d, J= 10.7 Hz, 2 CH), 7.38 (s, 1H, CH), 7.74 (s, 1H, CH), 8.22 (s, 1H, CH), 8.95 (brs, 1H, OH), 9.32 (brs, 1H, OH), 11.67 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 22.0 (CH_2), 22.4 (CH_2), 24.7 (CH_2), 26.5 (CH_2), 114.7 (CH), 115.3 (CH), 118.9 (C), 120.5 (CH), 127.0 (C), 130.9 (C), 131.9 (C), 145.4 (C), 147.6 (C), 148.0 (C), 148.6 (CH), 153.4 (CH), 156.6(C). ES-MS *m/z* 341.1 (MH^+). HRMS 341.1067, found 341.1063. Anal. ($C_{17}H_{16}N_4O_2S$.0.5MeOH) C, H, N.



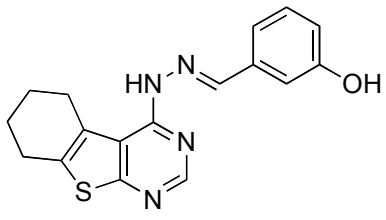
Compound 35c: Starting from 100 mg of **9**, 120 mg of yellow crystals were obtained (yield: 77%).

^{1}H NMR (500 MHz, DMSO) δ: 1.79 (m, 4H, 2 CH_2), 2.77 (m, 2H, CH_2), 2.98 (m, 2H, CH_2), 6.26 (s, 1H, CH), 6.35 (d, 1H, *J* = 7.5 Hz, CH), 7.53 35 (d, 1H, *J* = 7.5 Hz, CH), 7.73 (s, 1H, CH), 8.50 (s, 1H, CH), 9.89 (brs, 1H, OH), 10.39 (brs, 1H, OH), 11.57 (brs, 1H, NH). ^{13}C NMR (125 MHz, DMSO) δ: 22.5 (CH_2), 22.9 (CH_2), 25.5 (CH_2), 27.1 (CH_2), 102.8 (CH), 108.1 (CH), 112.4 (C), 119.3 (C), 131.3 (C), 131.8 (CH), 132.5 (C), 144.5 (CH), 147.0 (C), 155.1 (CH), 156.9 (C), 159.3 (C), 161.0 (C). ES-MS *m*/*z* 363.1 (MNa^+). HRMS 363.0886, found 363.0896.



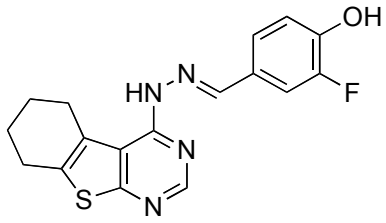
Compound 35d: Starting from 45 mg of **9**, 60 mg of yellow crystals were obtained (yield: 90%).

^{1}H NMR (300 MHz, DMSO) δ: 1.77 (m, 4H, 2 CH_2), 2.73 (m, 2H, CH_2), 2.97 (m, 2H, CH_2), 6.82 (m, 2H, 2 CH), 7.76 (m, 3H, 3 CH), 8.28 (s, 1H, CH), 9.88 (brs, 1H, OH), 11.70 (brs, 1H, NH). ^{13}C NMR (75 MHz, DMSO) δ: 22.3 (CH_2), 22.4 (CH_2), 25.0 (CH_2), 27.2 (CH_2), 115.4 (CH), 118.4 (C), 126.5 (C), 129.5 (CH), 130.8 (C), 131.9 (C), 143.9 (CH), 148.1 (C), 152.9 (CH), 156.7 (C), 159.1 (C). ES-MS *m*/*z* 325.1 (MH^+). HRMS 325.1118, found 325.1123. Anal. ($C_{17}H_{16}N_4OS.0.4H_2O$) C, H, N.



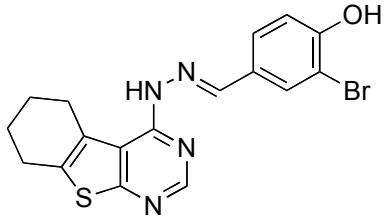
Compound 35e: Starting from 105 mg of **9**, 120 mg of yellow crystals were obtained (yield: 78%).

^{1}H NMR (400 MHz, DMSO) δ: 1.80 (m, 4H, 2 CH_2), 2.76 (m, 2H, CH_2), 3.00 (m, 2H, CH_2), 6.84 (d, 1H, J = 10.7 Hz, CH), 7.24 (t, 1H, J = 10.7 Hz, CH), 7.35 (s+d, 2H, J = 10.7 Hz, 2 CH), 7.79 (s, 1H, CH), 8.31 (s, 1H, CH), 9.52 (brs, 1H, OH), 11.8 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 21.8 (CH_2), 22.6 (CH_2), 24.0 (CH_2), 25.9 (CH_2), 114.2 (CH), 116.8 (CH), 118.8 (C), 118.9 (CH), 129.4 (C), 130.8 (C), 132.2 (C), 136.7 (C), 143.9 (CH), 148.9 (C), 153.1 (CH), 157.2 (C), 157.5 (C). ES-MS *m*/*z* 325.0 (MH^+). HRMS 325.1118, found 325.1113. Anal. ($C_{17}H_{16}N_4OS$) C, H, N.



Compound 35g: Starting from 97 mg of **9**, 75 mg of orange crystals were obtained (yield: 85%).

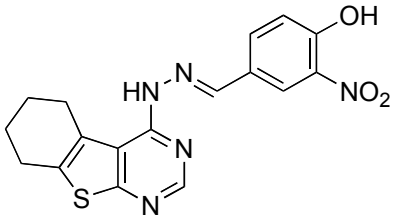
^{1}H NMR (300 MHz, DMSO) δ: 1.76 (m, 4H, 2 CH_2), 2.73 (m, 2H, CH_2), 2.96 (m, 2H, CH_2), 6.98 (t, $^3J_{HH}$ = $^4J_{HF}$ = 8.3 Hz, 1H, CH),), 7.44 (dd, $^3J_{HH}$ = 8.3Hz, $^4J_{HH}$ = 1.6 Hz, 1H, CH), 7.76 (s, 1H, CH), 7.92 (dd, $^3J_{HF}$ = 12.6 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, CH), 8.27 (s, 1H, CH), 10.30 (brs, 1H, OH), 11.80 (brs, 1H, NH). ^{13}C NMR (75 MHz, DMSO) δ: 21.9 (CH_2), 22.3 (CH_2), 24.6 (CH_2), 26.5 (CH_2), 114.1 (CH, $^2J_{CF}$ = 19 Hz), 117.3 (CH, $^3J_{CF}$ = 2.4 Hz), 118.8 (C), 125.4 (CH), 127.4 (CH, $^3J_{CF}$ = 5 Hz), 130.8 (C), 132.1 (C), 143.9 (CH), 146.6 (CH, $^2J_{CF}$ = 13 Hz), 148.7 (C), 148.6 (C), 151.2 (C, $^1J_{CF}$ = 240 Hz), 151.8 (CH), 157.0 (C). ^{19}F NMR (282 MHz, DMSO) δ: -136.1. ES-MS *m*/*z* 343.1 (MH^+). HRMS 343.1023, found 343.1035. Anal. ($C_{17}H_{15}N_4OFS.1H_2O$) C, H, N.



Compound 35h: Starting from 90 mg of **9**, 75 mg of orange crystals were obtained (yield: 45%).

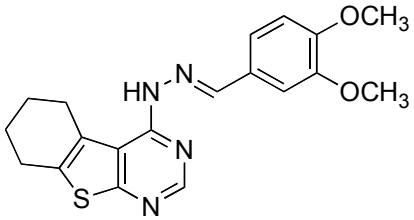
^{1}H NMR (300 MHz, DMSO) δ: 1.77 (m, 4H, 2 CH_2), 2.74 (m, 2H, CH_2), 2.96 (m, 2H, CH_2), 6.99 (s, 1H, CH), 7.66 (s, 1H, CH), 7.76 (s, 1H, CH), 8.21 (s, 1H, CH), 8.27 (s,

1H, CH), 10.70 (brs, 1H, OH), 11.80 (brs, 1H, NH). ^{13}C NMR (75 MHz, DMSO) δ: 22.0 (CH_2), 22.4 (CH_2), 24.6 (CH_2), 26.5 (CH_2), 110.0 (C), 116.0 (CH), 126.0 (C), 128.2 (C), 129.0 (CH), 130.8 (C), 131.4 (CH), 132.1 (C), 143.2 (C), 143.9 (CH), 148.6 (C), 151.5 (CH), 155.4(C). ES-MS *m/z* 403.0 (MH^+, ^{79}Br), 405.0 (MH^+, ^{81}Br). HRMS 403.0223 (^{79}Br), 405.0203 (^{81}Br), found 403.0232 (^{79}Br), 405.0192 (^{81}Br). Anal. ($C_{17}H_{15}N_4OSBr.0.3MeOH$) C, H, N.



Compound 35i: Starting from 100 mg of **9**, 140 mg of bright orange crystals were obtained (yield: 85%).

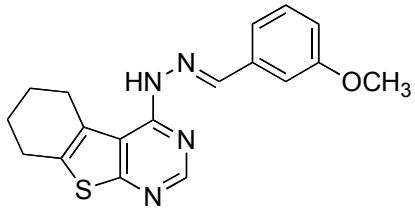
^{1}H NMR (400 MHz, DMSO) δ: 1.78 (m, 4H, 2 CH_2), 2.75 (m, 2H, CH_2), 2.99 (m, 2H, CH_2), 7.19 (d, J = 8.4 Hz, 1H, CH), 7.80 (s, 1H, CH), 8.11 (d, J = 8.4 Hz, 1H, CH), 8.37 (s, 1H, CH), 8.44 (s, 1H, CH), 11.88 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 22.0 (CH_2), 22.3 (CH_2), 24.6 (CH_2), 26.5 (CH_2), 118.8 (C), 119.0 (CH), 122.1 (C), 123.7 (CH), 127.2 (C), 130.8 (2 C), 132.3 (C), 133.8 (CH), 137.5 (C), 143.8 (CH), 150.7 (CH), 152.7 (C). ES-MS *m/z* 370.1 (MH^+). HRMS 370.0968, found 370.0972. Anal. ($C_{17}H_{15}N_5OS$) C, H, N, S.



Compound 35j: Starting from 105 mg of **9**, 62 mg of yellow crystals were obtained (yield: 35%).

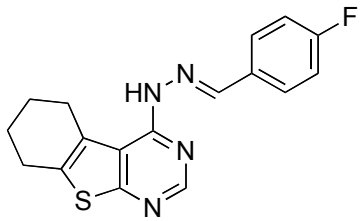
^{1}H NMR (400 MHz, DMSO) δ: 1.80 (m, 4H, 2 CH_2), 2.74 (m, 2H, CH_2), 3.00 (m, 2H, CH_2), 3.82 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 7.02 (d, J = 8.2 Hz, 1H, CH), 7.38 (dd, J_1 = 8.2Hz, J_2 = 2.0 Hz, 1H, CH), 7.65 (d, J = 2.0 Hz, 1H, CH), 7.79 (s, 1H, CH), 8.34 (s, 1H, CH), 11.72 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 22.0 (CH_2), 22.4 (CH_2), 24.6 (CH_2), 26.6 (CH_2), 55.5 (CH_3), 55.7 (CH_3), 110.0 (CH), 111.4 (CH), 122.2 (C), 128.2 (CH), 130.9 (C), 132.1 (C), 143.9 (CH), 148.3 (C), 148.9 (C), 150.6

(C), 153.1 (CH), 156.8 (C). ES-MS *m*/*z* 369.1 (MH^+). HRMS 369.1380, found 369.1371. Anal. ($C_{19}H_{20}N_4O_2S$) C, H, N.



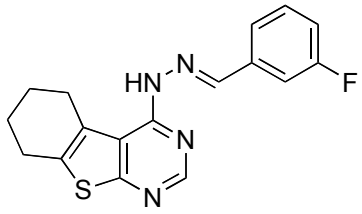
Compound 35k: Starting from 115 mg of **9**, 98 mg of yellow crystals were obtained (yield: 56%).

^{1}H NMR (400 MHz, DMSO) δ: 1.80 (m, 4H, 2 CH_2), 2.76 (m, 2H, CH_2), 3.00 (m, 2H, CH_2), 3.84 (s, 3H, CH_3), 7.00 (d, *J* = 7 Hz, 1H, CH), 7.37 (t, *J* = 7 Hz, H, CH), 7.50 (d, *J* = 7 Hz, 1H, CH), 7.58 (s, 1H, CH), 7.81 (s, 1H, CH), 8.39 (s, 1H, CH), 11.84 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 22.0 (CH_2), 22.4 (CH_2), 24.7 (CH_2), 26.6 (CH_2), 55.2 (CH_3), 112.7 (CH), 115.3 (CH), 118.8 (C), 120.6 (CH), 129.5 (C), 130.9 (CH), 132.3 (C), 136.8 (C), 149.1 (C), 152.8 (CH), 159.5 (C). ES-MS *m*/*z* 361.1 (MNa^+). HRMS 361.1094, found 361.1078. Anal. ($C_{18}H_{18}N_4OS$) C, H, N, S.



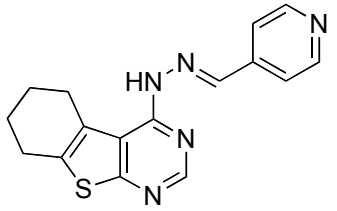
Compound 35n: Starting from 100 mg of **9**, 110 mg of yellow crystals were obtained (yield: 74%).

^{1}H NMR (400 MHz, DMSO) δ: 1.78 (m, 4H, 2 CH_2), 2.75 (m, 2H, CH_2), 2.99 (m, 2H, CH_2), 7.29 (t, 2H, $J_{HH} = {}^3J_{HF}$ = 8.5Hz, 2 CH), 7.80 (s, 1H, CH), 8.02 (t, 2H, $J_{HH} = {}^3J_{HF}$ = 8.5Hz, 2 CH), 8.4 (s, 1H, CH), 11.86 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 22.0 (CH_2), 22.4 (CH_2), 25.0 (CH_2), 26.5 (CH_2), 115.5 (CH, $^2J_{CF}$ = 21.5Hz), 118.8 (C), 129.7 (CH, $^3J_{CF}$ = 8.2Hz), 130.8 (C), 132.1 (C), 132.2 (C), 143.8 (CH), 149.1 (C), 151.6 (CH), 157.3 (C), 163.0 (C, $^1J_{CF}$ = 245Hz). ^{19}F NMR (282 MHz, DMSO) δ: -111.1. ES-MS *m*/*z* 327.1 (MH^+). HRMS 327.1074, found 327.1083. ($C_{17}H_{15}N_4FS.0.4MeOH$) C, H, N.



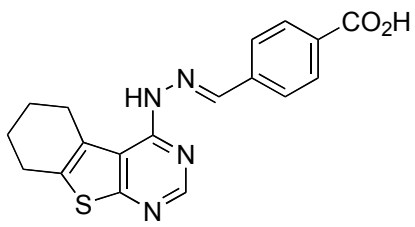
Compound 35o: Starting from 95 mg of **9**, 80 mg of yellow crystals were obtained (yield: 57%).

^{1}H NMR (400 MHz, DMSO) δ: 1.80 (m, 4H, 2 CH_2), 2.76 (m, 2H, CH_2), 3.00 (m, 2H, CH_2), 7.24 (t, 1H, $J_{HH} = {}^3J_{HF}$ = 8Hz, CH), 7.48 (q, 1H, $J_{HH} = {}^4J_{HF}$ = 8 Hz, CH), 7.69 (d, 1H, J = 8 Hz, CH), 7.83 (s, 1H, CH), 7.94 (t, 1H, $^3J_{HF}$ = 8 Hz, CH), 8.41 (s, 1H, CH), 11.94 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 22.5 (CH_2), 22.8 (CH_2), 25.0 (CH_2), 31.6 (CH_2), 113.0 (CH, $^2J_{CF}$ = 22 Hz), 116.2 (CH, $^2J_{CF}$ = 22 Hz), 118.8 (C), 124.5 (CH), 130.4 (CH), 130.8 (C), 132.8 (C), 138.1 (C), 143.8 (CH), 149.6 (C), 151.4 (CH), 157.7 (C), 162.5 (C, $^1J_{CF}$ = 230 Hz). ^{19}F NMR (282 MHz, DMSO) δ: -113.3. ES-MS *m/z* 327.1 (MH^+). HRMS 327.1074, found 327.1087. Anal. ($C_{17}H_{15}N_4FS.0.5H_2O$) C, H, N.



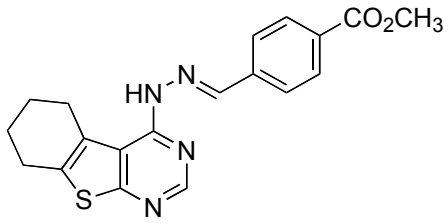
Compound 35p: Starting from 180 mg of **9**, 210 mg of yellow crystals were obtained (yield: 83%).

^{1}H NMR (400 MHz, DMSO) δ: 1.80 (m, 4H, 2 CH_2), 2.77 (m, 2H, CH_2), 3.01 (m, 2H, CH_2), 7.89 (m, 3H, 3 CH), 8.40 (s, 1H, CH), 8.65 (d, J = 7 Hz, 2H, 2CH), 12.06 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 21.5 (CH_2), 21.9 (CH_2), 24.4 (CH_2), 26.0 (CH_2), 121.4 (2 CH), 125.5 (C), 131.0 (C), 132.8 (C), 142.4 (CH), 149.9 (3 CH), 151.1 (C), 155.9 (C). ES-MS *m/z* 310.1 (MH^+). HRMS 332.094, found 332.0927. Anal. ($C_{16}H_{15}N_5S.0.1MeOH$) C, H, N.



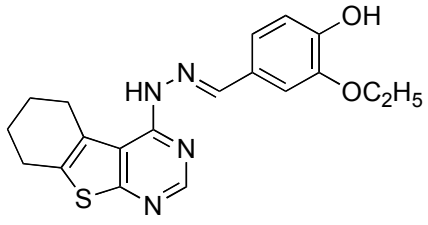
Compound 35q: Starting from 100 mg of **9**, 130 mg of pale yellow precipitate were obtained (yield: 81%).

^{1}H NMR (300 MHz, DMSO) δ: 1.78 (m, 4H, 2 CH_2), 2.75 (m, 2H, CH_2), 2.99 (m, 2H, CH_2), 3.39 (brs, 1H, OH), 7.85 (s, 1H, CH), 7.98 (d, J= 10.7 Hz, 2 CH), 8.04 (d, J= 10.7 Hz, 2 CH), 8.45 (s, 1H, CH), 12.0 (brs, 1H, NH). ^{13}C NMR (75 MHz, DMSO) δ: 22.0 (CH_2), 22.3 (CH_2), 24.7 (CH_2), 26.5 (CH_2), 118.8 (C), 127.6 (2 CH), 129.4 (2 CH), 130.8 (C), 131.2 (C), 132.5 (C), 139.5 (C), 143.9 (CH), 149.7 (C), 151.6 (CH), 167.0 (COOH). ES-MS *m/z* 353.0 (MH^+). HRMS 353.1067, found 353.1069. Anal. ($C_{18}H_{16}N_4O_2S$.0.4MeOH) C, H, N.



Compound 35r: Starting from 85 mg of **9**, 96 mg of yellow crystals were obtained (yield: 68%).

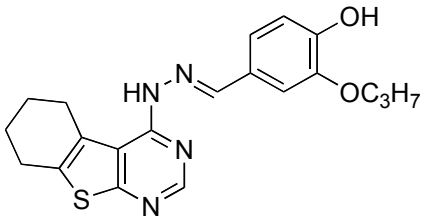
^{1}H NMR (300 MHz, DMSO) δ: 1.78 (m, 4H, 2 CH_2), 2.50 (m, 2H, CH_2), 2.75 (m, 2H, CH_2), 3.87 (s, 3H, CH_3), 7.85 (s, 1H, CH), 7.99 (d, *J* = 7 Hz, 2H, 2 CH), 8.06 (d, *J* = 7 Hz, 2H, 2 CH), 8.44 (s, 1H, CH), 11.99 (brs, 1H, NH). ^{13}C NMR (75 MHz, DMSO) δ: 22.0 (CH_2), 22.3 (CH_2), 24.7 (CH_2), 26.6 (CH_2), 52.2 (CH_3), 118.6 (C), 127.6 (CH), 129.2 (CH), 130.8 (C), 132.6 (C), 139.9 (C), 143.8 (C), 151.4 (CH), 163.7 (C), 165.9 (C). ES-MS *m/z* 367.1 (MH^+). HRMS 367.1223, found 367.1237. Anal. ($C_{19}H_{18}N_4O_2S$) C, H, N, S.



Compound 35s: Starting from 145 mg of **9**, 180 mg of yellow crystals were obtained (yield: 74%).

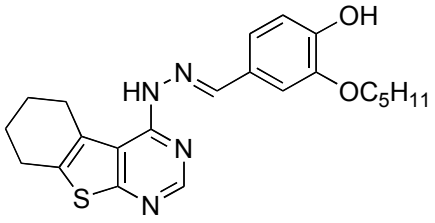
^{1}H NMR (400 MHz, DMSO) δ: 1.39 (t, *J* = 7.5 Hz, 3H, CH_3), 1.79 (m, 4H, 2 CH_2), 2.75 (m, 2H, CH_2), 2.99 (m, 2H, CH_2), 4.12 (q, *J* = 7.5 Hz, 2H, CH_2), 6.85 (d, *J* = 7 Hz, 1H, CH), 7.29 (d, *J* = 7 Hz, 1H, CH), 7.59 (s, 1H, CH), 7.78 (s, 1H, CH), 8.28 (s,

1H, CH), 9.38 (brs, 1H, OH), 11.67 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 14.8 (CH_3), 22.0 (CH_2), 22.4 (CH_2), 24.7 (CH_2), 28.6 (CH_2), 64.9 (CH_2), 112.2 (CH), 115.4 (CH), 118.9 122.4 (CH), 126.9 (C), 130.8 (C), 132.0 (C), 143.9 (CH), 147.0 (C), 148.0 (C), 149.0 (C), 153.4 (CH), 156.6 (C). ES-MS *m*/*z* 369.1 (MH^+). HRMS 369.1380, found 369.1390. Anal. ($C_{19}H_{20}N_4O_2S$.0.5MeOH) C, H, N.



Compound 35t: Starting from 90 mg of **9**, 103 mg of yellow crystals were obtained (yield: 66%).

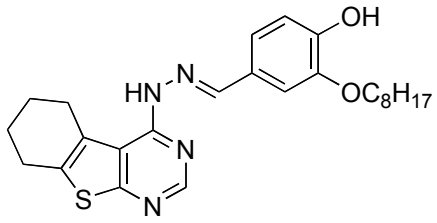
^{1}H NMR (400 MHz, DMSO) δ: 1.02 (t, *J* = 9.7 Hz, 3H, CH_3), 1.79 (q, *J* = 9.7 Hz, 2H, CH_2), 1.79 (m, 4H, 2 CH_2), 2.73 (m, 2H, CH_2), 2.97 (m, 2H, CH_2), 4.01 (t, *J* = 9.7 Hz, 1H, CH), 4.12 (t, *J* = 9.7 Hz, 1H, CH), 6.83 (d, *J* = 10.7 Hz, 1H, CH), 7.27 (d, *J* = 10.7 Hz, 1H, CH), 7.56 (s, 1H, CH), 7.76 (s, 1H, CH), 9.34 (brs, 1H, OH), 11.66 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 10.5 (CH_3), 22.0 (CH_2), 22.1 (2 CH_2), 22.4 (CH_2), 24.6 (CH_2), 26.5 (CH_2), 69.9 (CH_2), 112.0 (CH), 115.3 (CH), 118.9 122.3 (CH), 126.9 (C), 130.8 (C), 131.9 (C), 143.9 (CH), 147.1 (C), 148.0 (C), 148.9 (C), 153.4 (CH), 156.6 (C). ES-MS *m*/*z* 383.1 (MH^+). HRMS 383.1536, found 383.1527. Anal. ($C_{20}H_{22}N_4O_2S$.0.5MeOH) C, H, N.



Compound 35u: Starting from 100 mg of **9**, 130 mg of yellow crystals were obtained (yield: 70%).

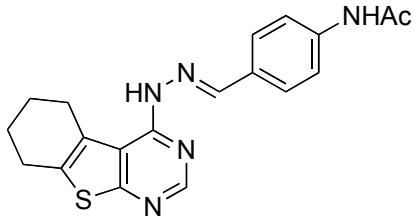
^{1}H NMR (400 MHz, DMSO) δ: 0.93 (t, *J* = 7.3 Hz, 3H, CH_3), 1.39 (m, 2H, CH_2), 1.43 (m, 2H, CH_2), 1.78 (m, 6H, 3 CH_2), 2.75 (m, 2H, CH_2), 2.98 (m, 2H, CH_2), 4.05 (t, *J* = 7.3 Hz, 1H, CH), 4.10 (t, *J* = 7.3 Hz, 1H, CH), 6.84 (d, *J* = 10.7 Hz, 1H, CH), 7.28 (d, *J* = 10.7 Hz, 1H, CH), 7.57 (s, 1H, CH), 7.78 (s, 1H, CH), 8.28 (s, 1H, CH), 9.33 (brs, 1H, OH), 11.67 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 13.9

(CH_3), 21.9 (CH_2), 22.0 (CH_2), 22.4 (CH_2), 24.6 (CH_2), 26.5 (CH_2), 27.7 (CH_2), 28.5 (CH_2), 68.4 (CH_2), 112.1 (CH), 115.3 (CH), 118.9 (C), 122.3 (CH), 126.9 (C), 130.8 (C), 132.0 (C), 143.4 (CH), 147.1 (C), 148.0 (C), 149.0 (C), 153.4 (CH), 156.6 (C). ES-MS *m/z* 411.1 (MH^+). HRMS 411.1849, found 411.1839. Anal. ($C_{22}H_{26}N_4O_2S.0.6MeOH$) C, H, N, S.



Compound 35v: Starting from 66 mg of **5**, 60 mg of yellow crystals were obtained (yield: 44%).

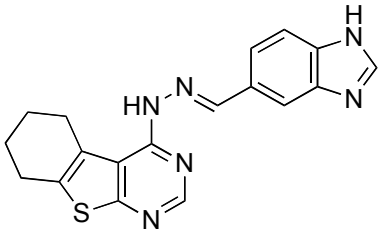
^{1}H NMR (400 MHz, DMSO) δ: 1.28 (m, 9H, CH_3 + 3 CH_2) 1.46 (m, 2H, CH_2), 1.77 (m, 8H, 4 CH_2), 2.75 (m, 2H, CH_2), 2.98 (m, 2H, CH_2), 4.05 (t, *J* = 7 Hz, 1H, CH), 4.10 (t, *J* = 7 Hz, 1H, CH), 6.84 (d, *J* = 8 Hz, 1H, CH), 7.28 (d, *J* = 8 Hz, 1H, CH), 7.56 (s, 1H, CH), 7.79 (s, 1H, CH), 8.28 (s, 1H, CH), 9.33 (brs, 1H, OH), 11.67 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 13.9 (CH_3), 22.0 (2 CH_2), 22.1 (CH_2), 22.4 (CH_2), 24.6 (CH_2), 25.6 (CH_2), 26.5 (CH_2), 28.7 (CH_2), 28.8 (CH_2), 31.3 (CH_2), 68.4 (CH_2), 112.1 (CH), 115.4 (CH), 118.9 (C), 122.5 (CH), 126.9 (C), 130.8 (C), 131.9 (C), 143.4 (CH), 147.1 (C), 148.0 (C), 149.0 (C), 153.4 (CH), 156.6 (C). ES-MS *m/z* 453.2 (MH^+). HRMS 453.2319, found 453.2307. Anal. ($C_{25}H_{32}N_4O_2S.0.5MeOH$) C, H, N.



Compound 35w: Starting from 115 mg of **9**, 180 mg of yellow crystals were obtained (yield: 94%).

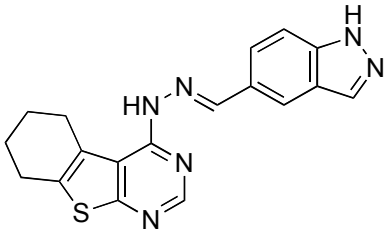
^{1}H NMR (400 MHz, DMSO) δ: 1.79 (m, 4H, 2 CH_2), 2.08 (s, 3H, CH_3), 2.76 (m, 2H, CH_2), 3.00 (m, 2H, CH_2), 7.67 (d, *J* = 7 Hz, 2H, 2 CH), 7.79 (s, 1H, CH), 7.88 (d, *J* = 7 Hz, 2H, 2 CH), 8.33 (s, 1H, CH), 10.11 (brs, 1H, NH),11.77 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 22.0 (CH_2), 22.4 (CH_2), 24.1 (CH_3), 24.7 (CH_2), 26.6

(CH_2), 118.6 (2 CH), 118.9 (C), 128.3 (2 CH), 130.1 (C), 130.8 (C), 132.1 (C), 140.7 (C), 143.9 (CH), 148.6 (C), 152.5 (CH), 157.0 (C), 168.4 (C). ES-MS *m*/*z* 336.1 (MH^+). HRMS 366.1383, found 366.1384. Anal. ($C_{19}H_{19}N_5OS$) C, H, N, S.



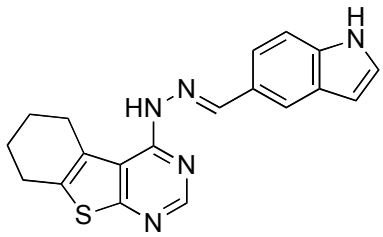
Compound 35x: Starting from 144 mg of **9**, 198 mg of yellow crystals were obtained (yield: 87%).

^{1}H NMR (400 MHz, DMSO) δ: 1.80 (m, 4H, 2 CH_2), 2.76 (m, 2H, CH_2), 3.03 (m, 2H, CH_2), 4.12 (brs, 1H, NH), 7.63 (d, *J* = 8.7 Hz, 1H, CH), 7.80 (s, 1H, CH), 7.92 (s, 1H, CH), 8.15 (s, 1H, CH), 8.30 (s, 1H, CH), 8.52 (s, 1H, CH), 11.84 (brs, 1H, NH), 13.26 (brs, 1H, NH). ^{13}C NMR (125 MHz, DMSO) δ: 22.5 (CH_2), 22.9 (CH_2), 25.2 (CH_2), 27.0 (CH_2), 118.5 (C), 119.4 (C), 122.3 (CH), 130.0 (C), 131.4 (C), 132.6 (C), 143.7 (3 CH), 144.5 (CH), 149.0 (C), 154.5 (CH), 157.4 (C). ES-MS *m*/*z* 349.1 (MH^+). HRMS 349.1230, found 349.1234. Anal. ($C_{18}H_{16}N_6S$.1MeOH) C, H, N.



Compound 35y: Starting from 125 mg of **9**, 179 mg of yellow crystals were obtained (yield: 90%).

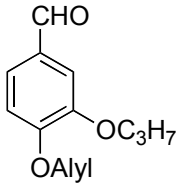
^{1}H NMR (400 MHz, DMSO) δ: 1.80 (m, 4H, 2 CH_2), 2.76 (m, 2H, CH_2), 3.02 (m, 2H, CH_2), 4.12 (brs, 1H, NH), 7.59 (d, *J* = 8.7 Hz, 1H, CH), 7.81 (s, 1H, CH), 8.16 (m, 3H, 3 CH), 8.51 (s, 1H, CH), 11.85 (brs, 1H, NH), 13.25 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ: 22.0 (CH_2), 22.4 (CH_2), 24.6 (CH_2), 26.6 (CH_2), 110.3 (CH), 118.9 (C), 121.8 (CH), 122.9 (C), 125.0 (CH), 128.3 (C), 130.9 (C), 132.1 (C), 134.3 (CH), 140.5 (C), 143.8 (CH), 148.5 (C), 153.7 (CH), 156.9 (C). ES-MS *m*/*z* 349.1 (MH^+). HRMS 349.1230, found 349.1232. Anal. ($C_{18}H_{16}N_6S$.1MeOH) C, H, N.



Compound 35z: Starting from 100 mg of **9**, 90 mg of purple crystals were obtained (yield: 57%).

^{1}H NMR (300 MHz, DMSO) δ: 1.79 (m, 4H, 2 CH_2), 2.74 (m, 2H, CH_2), 3.00 (m, 2H, CH_2), 6.49 (m, 1H, CH), 7.39 (m, 1H, CH), 7.42 (d, *J* = 8.6 Hz, 1H, CH), 7.76 (s, 1H, CH), 7.84 (d, *J* = 8.6 Hz, 1H, CH), 8.02 (s, 1H, CH), 8.45 (s, 1H, CH), 11.29 (brs, 1H, NH), 11.73 (brs, 1H, NH). ^{13}C NMR (75 MHz, DMSO) δ: 22.0 (CH_2), 22.4 (CH_2), 24.6 (CH_2), 26.5 (CH_2), 101.8 (CH), 111.5 (CH), 118.9 (C), 120.6 (CH), 121.3 (CH), 126.2 (CH), 126.6 (C), 127.6 (C), 130.8 (C), 131.8 (C), 136.9 (C), 144.0 (CH), 148.0 (C), 154.8 (CH), 156.6 (C). ES-MS *m*/*z* 348.1 (MH^+). HRMS 348.1277, found 348.1290. Anal. ($C_{19}H_{17}N_5S$) C, H, N, S.

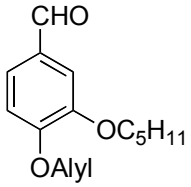
Aldehydes syntheses.



4-allyloxy-3-propyloxybenzaldehyde 37a: A solution of 2.98mmol of 4-allyloxy-3-hydroxybenzaldehyde **36**, 1.5 equivalent of K_2CO_3 (618mg) and 0.95 equivalent of 1-bromopropane (130µL) in acetone was stirred overnight at 40°C. The mixture was diluted with dichloromethane and washed twice with an aqueous solution of NaOH 2M and brine. The organic layer was dried with $MgSO_4$, filtered off and concentrated under reduced pressure to afford 400 mg of the title compound (yield: 61%).

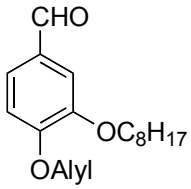
^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.97 (t, *J* = 7.4 Hz, 3H, CH_3), 1.79 (qn, *J* = 7.4 Hz, 2H, CH_2), 3.95 (t, *J* = 7.4 Hz, 2H, CH_2), 4.59 (d, *J* = 5 Hz, 2H, CH_2), 5.23 (d, *J* = 18 Hz, 1H, CH), 5.36 (d, *J* = 18 Hz, 1H, CH), 5.97 (m, 1H, CH), 6.88 (d, *J* = 8 Hz, 1H, CH), 7.31 (s, 2H, 2 CH), 9.74 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.5

(CH_3), 22.4 (CH_2), 69.6 (CH_2), 70.5 (CH_2), 110.9 (CH), 112.4 (CH), 118.0 (CH_2), 126.3 (CH), 130.2 (C), 132.5 (CH), 149.5 (C), 153.9 (C), 190.9 (CHO).



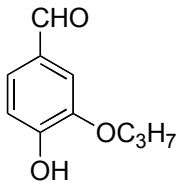
4-allyloxy-3-pentyloxybenzaldehyde 37b: The same procedure as described above with 1-bromopentane afforded the title compound in 51% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.92 (t, *J* = 7.3 Hz, 3H, CH_3), 1.40 (m, 4H, 2 CH_2), 1.85 (t, *J* = 7.3 Hz, 2H, CH_2), 4.06 (t, *J* = 7.4 Hz, 2H, CH_2), 4.67 (d, *J* = 5 Hz, 2H, CH_2), 5.31 (d, *J* = 18 Hz, 1H, CH), 5.44 (d, *J* = 18 Hz, 1H, CH), 6.05 (m, 1H, CH), 6.96 (d, *J* = 8 Hz, 1H, CH), 7.39 (s, 2H, 2 CH), 9.82 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (CH_3), 22.4 (CH_2), 28.1 (CH_2), 28.7 (CH_2), 69.1 (CH_2), 69.6 (CH_2), 110.8 (CH), 112.4 (CH), 118.0 (CH_2), 126.3 (CH), 130.2 (C), 132.5 (CH), 149.5 (C), 153.9 (C), 191.0 (CHO).



4-allyloxy-3-octyloxybenzaldehyde 37c: The same procedure as described above with 1-bromoctane afforded the title compound in 70% yield.

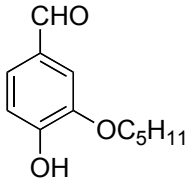
^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.84 (t, *J* = 7.4 Hz, 3H, CH_3), 1.24 (m, 8H, 4 CH_2), 1.43 (m, 2H, CH_2), 1.80 (qn, *J* = 7.4 Hz, 2H, CH_2), 4.01 (t, *J* = 7.4 Hz, 2H, CH_2), 4.64 (s, 2H, CH_2), 5.27 (d, *J* = 18 Hz, 1H, CH), 5.40 (d, *J* = 18 Hz, 1H, CH), 6.01 (m, 1H, CH), 6.92 (d, *J* = 8 Hz, 1H, CH), 7.36 (s, 2H, 2 CH), 9.78 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (CH_3), 22.7 (CH_2), 26.0 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 31.8 (CH_2), 69.1 (CH_2), 69.6 (CH_2), 110.9 (CH), 112.4 (CH), 118.0 (CH_2), 126.3 (CH), 130.2 (C), 132.5 (CH), 149.5 (C), 153.9 (C), 190.4 (CHO).



3-propyloxy-4-hydroxybenzaldehyde 38a: 1.49mmol of **37a** was heated at 90°C for 5 hours in acetic acid (10mL) with 0.1 equivalent of $Pd(OAc)_2$ (41mg, 0.182mmol)

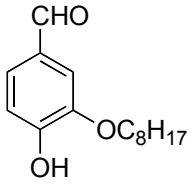
and 0.5 equivalent of PPh_3 (239mg, 0.91mmol). After cooling down, water was added and the reaction mixture was extracted twice with diethyl ether. The combined organic layers were extracted with NaOH 2M (3x20ml). The sodium hydroxide extracts were neutralised with concentrated HCl and finally extracted twice with dichloromethane. The combined dichloromethane layers were dried with $MgSO_4$, filtered and concentrated under reduced pressure to afford 100 mg of the title compound (yield: 61%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.98 (t, *J* = 7.4 Hz, 3H, CH_3), 1.79 (qn, *J* = 7.4 Hz, 2H, CH_2), 4.01 (t, *J* = 7.4 Hz, 2H, CH_2), 6.15 (brs, 1H, OH), 6.96 (d, *J* = 8 Hz, 1H, CH), 7.32 (s, 2H, 2 CH), 9.74 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 10.4 (CH_3), 22.4 (CH_2), 70.7 (CH_2), 109.7 (CH), 114.4 (CH), 127.3 (CH), 129.8 (C), 146.6 (C), 151.9 (C), 191.0 (CHO).



3-pentyloxy-4-hydroxybenzaldehyde 38b: Using the procedure described above with 0.64mmol of **37b** afforded the title compound in 75% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (t, *J* = 7.4 Hz, 3H, CH_3), 1.32 (m, 4H, 2 CH_2), 1.76 (m, 2H, CH_2), 4.01 (t, *J* = 7.4 Hz, 2H, CH_2), 6.36 (brs, 1H, OH), 6.96 (d, *J* = 8 Hz, 1H, CH), 7.32 (s, 2H, 2 CH), 9.73 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (CH_3), 22.4 (CH_2), 28.1 (CH_2), 28.7 (CH_2), 69.2 (CH_2), 109.6 (CH), 114.4 (CH), 127.3 (CH), 129.8 (C), 146.6 (C), 151.9 (C), 191.0 (CHO).



3-octyloxy-4-hydroxybenzaldehyde 38c: Using the same procedure as described above with 1.27mmol of **37c** afforded the title compound in 25% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.81 (t, *J* = 7.4 Hz, 3H, CH_3), 1.24 (m, 8H, 4 CH_2), 1.38 (m, 2H, CH_2), 1.97 (m, 2H, CH_2), 4.04 (t, *J* = 7.4 Hz, 2H, CH_2), 6.23 (brs, 1H, OH), 6.96 (d, *J* = 8 Hz, 1H, CH), 7.33 (s, 2H, 2 CH), 9.74 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (CH_3), 22.6 (CH_2), 25.9 (CH_2), 29.0 (CH_2), 29.2 (CH_2),

29.3 (CH_2), 31.8 (CH_2), 69.3 (CH_2), 109.6 (CH), 114.3 (CH), 127.3 (CH), 129.8 (C), 146.6 (C), 151.8 (C), 191.0 (CHO).

2. Screening compounds for inherent toxicity and protective effects against SLTx and DTx.[1]

HeLa cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum (DMEM/FCS). Cells were seeded at 2.10^4/well of a 96-well tissue culture plate and allowed to settle overnight. They were then pretreated for 30 mins with DMEM/FCS containing either DMSO vehicle alone or 50 μM compound diluted in DMSO. Subsequently they were treated for an hour with 50 $ng.ml^{-1}$ Shiga–like toxin (SLTx) in DMEM/FCS containing vehicle or compound as appropriate, after which protein synthesis ability was determined by measuring incorporation of [^{35}S]-met into acid-precipitable material, using a previously published assay.[1] Results were normalized for each compound to coeval controls measuring protein synthesis ability of non-toxin-treated cells, which reflects the toxicity of each compound to the protein synthesis machinery. All experiments were carried out at least three times; the standard deviation was within 5% for each experiment.

3. Immunofluorescence.[1]

Cells grown on glass coverslips were fixed either with methanol for 4 minutes at -20˚C followed by permeabilization with 0.1% Triton X-100 in PBS for 5 minutes, blocked with PBS containing 3% BSA and probed with primary antibodies as indicated and detailed below and Alexa-Fluor™ conjugated secondary antibodies (Invitrogen) or Cy-dye conjugated secondary antibodies (Jackson Immunoresearch/Stratech Scientific, Newmarket, UK). The antibodies used are as followed: rabbit polyclonal anti-giantin (Covance); monoclonal mouse anti-GM130 (BD Transduction Laboratories); polyclonal sheep anti-TGN46 (AbD Serotec); mouse monoclonal anti-58K Golgi protein antibody [58K-9] (Abcam); Cy-dye secondary antibodies were from Jackson Immunoresearch. Nuclei were counterstained using DAPI (Invitrogen) and coverslips were mounted in Mowiol.

A plasmid encoding for TGN46-GFP was kindly provided by Vas Ponnambalam (University of Leeds, UK). The transferrin-receptor in fusion with GFP (TfnR-GFP)

was kindly provided by Gary Banker (Oregon Health and Science University, Oregon, USA). The TfnR-GFP cassette was excised from a pJPA5-TfnR-GFP vector using EcoRI and XbaI restriction sites and cloned into a pLVX-Puro (Clontech) using the same sites. The resulting vector (pLVXPuro- TfnR-GFP) was verified by sequencing and used in transient overexpression.

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