Supporting Information

Activation of Nrf2 and Hypoxic Adaptive Response Contribute to Neuroprotection Elicited by Phenylhydroxamic Acid Selective HDAC6 Inhibitors

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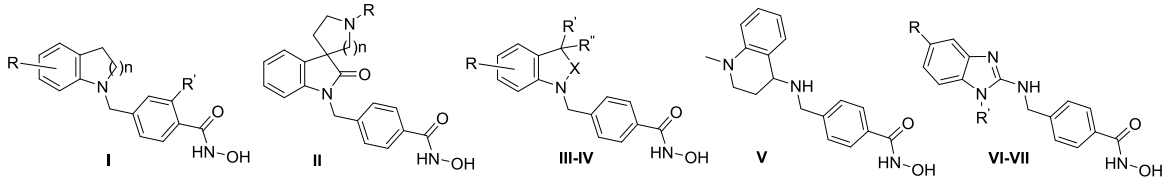
Table 1S. *In vitro* Nrf2 and HIF-1α Activation Screening Results.

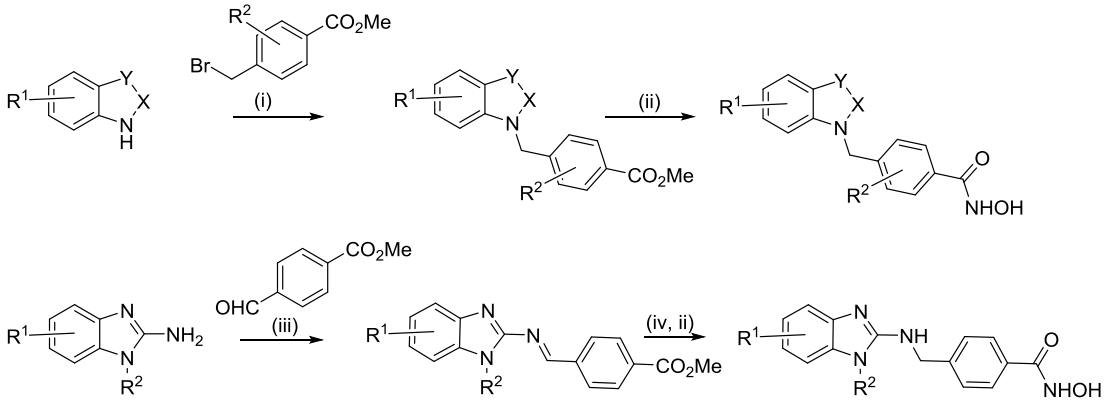


Type of CAP	Compd #	R	R'	R''	n	X	Nrf2 fold activation[a]	HIF-1α fold activation[a]
I	1a	H	H		1	CH_2	2.3	1.4
I	1b	5-Cl	H		1	CH_2	6.5	7.0
I	1c	5-Cl	F		1	CH_2	1.6	7.2
I	1d ***(ING-6)***	6-Cl	H		1	CH_2	5.6	7.5
I	1e	5-F	H		1	CH_2	7.7	1.4
I	1f	6-F	H		1	CH_2	7.8	3.6
I	1g	5-F,6-Cl	H		1	CH_2	5.5	7.8
I	1h	5-CH_3O	H		1	CH_2	2.4	1.0
I	1i	5-NO_2	H		1	CH_2	6.6	3.9
I	1j	6-BnO	H		1	CH_2	2.2	7.0
I	1k	6-Cl	H		2	CH_2	8.9	3.5
III	1l	H	CH_3	CH_3		CH_2	1.8	2.8
III	1m	5-F	cyclopropyl			CH_2	6.2	5.8
IV	1n	H	CH_3	CH_3		C=0	3.4	1.0
IV	1o ***(ING-66)***	H	F	F		C=0	9.9	5.6
II	1p	H	H		1	C=0	2.0	1.3
II	1q	CH_3	H		2	C=0	1.5	1.0
VI	1r	H	CH_3				1.8	2.6
VI	1s	Cl	CH_3				1.3	1.1
VII	1t	H	Ph				1.2	1.0
V	1u						1.3	1.0
	TSA						1.2	1.1
	SAHA						1.6	1.0
	tuba statin A						2.8	2.4

[a]measured at 10 μM. Mean from at least three determinations by the SH-SY5Y/Neh2-luc [1] and SH-SY5Y/HIF-ODD-luc [2] reporters: standard error ~5-10%

Chemistry. General Methods.

All reactions were conducted under argon and stirred magnetically. Starting materials, reagents, and solvents were purchased from commercial suppliers and used without further purification unless stated otherwise.^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometer at 400 MHz and 100 MHz respectively with TMS as an internal standard. HRMS experiments were performed on LTO-FTICR or Shimadzu IT-TOF Mass Spectrometers. TLC was performed with Merck 250-mm $60F_{254}$ silica gel plates. Preparative TLC was performed with Analtech 1000-mm silica gel GF plates. Column chromatography was performed using Merck silica gel (40-60 mesh). The final compounds were purified by preparative HPLC, which was carried out on an ACE 5 AQ column (150 mm × 20 mm), with detection at 254 and 280 nm on a Shimadzu SPD-10A VP detector; flow rate = 17.0 mL/min; gradient of 0−50% methanol in water (both containing 0.05 vol% of TFA) in 30 min. Analytical HPLC was carried out on an ACE 3AQ C_{18} column (100 × 4.6 mm), with a Shimadzu 10 VP Series HPLC with a diode array detector; flow rate = 2.0 mL/min; from 10% acetonitrile in water to 50% in 10 min and to 100% acetonitrile in 5 min with 0.05% TFA (method 1), or from 30% acetonitrile in water to 100% of acetonitrile in 15 min with 0.05% TFA (method 2). Purity of final compounds was ≥96%, as determined by HPLC. Unless otherwise stated, final compounds were isolated as amorphous solids without collection of melting point data.



Reagents and conditions: (i) NaH, DMF, 0 °C; (ii) NH_2OH, NaOH, THF/MeOH; (iii) toluene, reflux; (iv) $NaBH_4$, MeOH/THF;

General procedure A: To a solution of indoline/indolinone (1 eq) in DMF (2 ml per 1 mmol) 55% NaH (2 eq) was added at 0 °C. The reaction mixture was stirred for 20 min, and methyl 4-(bromomethyl)benzoate (1 eq) was added. The reaction was quenched with 1N HCl and CH_2Cl_2 was added. The resulting solution was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography to provide ester.

General procedure B: To a stirred solution of NaOH (4 eq) in MeOH (3 ml per 2 mmol of NaOH) a solution of NH_2OH (50% sol. in water) was added at 0 °C. After 10 min, a solution of ester (1 eq) in THF (3 ml) was added at 0 °C. After 1-2 h, the reaction mixture was acidified with 1N HCl (pH~ 4). Ice and water were added and a precipitate was formed. The precipitate was filtered and washed with H_2O and hexane. The sample was further purified by HPLC for biological test.

General procedure C: A mixture of benzoimidazol-2-amine/ tetrahydroquinolin-4-amine (1 eq) and methyl 4-formylbenzoate (1 eq) in toluene (5 ml/mmol) was refluxed for 6 h. The solvent was evaporated, and the product was dried in vacuo and used in next step.

General procedure D: To a solution of the imine (1 eq) in 10 ml of a mixture of MeOH-THF (1:1) sodium borohydride (1.5 eq) was added at 0 °C. The reaction mixture was stirred for 30 min, quenched with ice water, and extracted with ethyl acetate. Combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The product was used in next step without additional purification.

***N*-Hydroxy-4-(indolin-1-ylmethyl)benzamide (1a):** ^{1}H NMR (Acetone-d_6, 400 MHz) δ 2.96 (t, *J* = 8.2 Hz, 2H), 3.38 (t, *J* = 8.2 Hz, 2H), 4.38 (s, 2H), 6.60 (d, *J* = 7.8 Hz, 1H), 6.68 (t, *J* = 7.7 Hz, 1H), 7.03 (t, *J* = 7.7 Hz, 1H), 7.08 (d, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 8.1 Hz, 2H), 7.83 (d, *J* = 8.1 Hz, 2H); ^{13}C NMR (Acetone -d_6, 100 MHz) δ 28.1, 53.3, 107.9, 118.6, 124.4, 126.7, 127.1, 127.10, 127.14, 128.1, 128.6, 130.2, 131.0, 142.1, 151.5, 164.9; FAB-HRMS calcd for $C_{16}H_{16}N_2O_2$ $[M + H]^+$: 269.1285; found: 269.1295. HPLC (method 1) 98%.

4-((5-Chloroindolin-1-yl)methyl)-*N*-hydroxybenzamide (1b): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.91 (t, *J* = 8.3 Hz, 2H), 3.31 (t, *J* = 8.3 Hz, 2H), 4.30 (s, 2H), 6.51 (d, *J* = 8.3 Hz, 1H), 6.99 (dd, *J* = 1.6, and 8.3 Hz, 1H), 7.06 (s, 1H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.71 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 28.1, 52.3, 53.3, 108.1, 120.9, 124.7, 127.4, 128.2, 132.0, 132.4, 141..7, 1515.4, 164.4; FAB-HRMS calcd for $C_{16}H_{15}ClN_2O_2$ $[M + H]^+$: 303.0895; found: 303.0903. HPLC (method 1) 98%.

4-((5-Chloroindolin-1-yl)methyl)-2-fluoro-*N*-hydroxybenzamide (1c): ^{1}H NMR (acetone-d_6, 400 MHz) δ 2.28 (t, *J* = 8.3 Hz, 2H), 3.30 (t, *J* = 8.3 Hz, 2H), 4.24 (s, 2H), 6.35 (d, *J* = 8.4 Hz, 1H), 6.85 (dd, *J* = 2.0, 8.2 Hz, 1H), 6.93 (s, 1H), 7.10 (d, *J* = 12.1 Hz, 1H), 7.19 (d, J = 7.6 Hz, 1H), 7.68 (t, *J* = 7.2 Hz, 1H), 10.28 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.9, 52.3, 53.4, 107.8, 107.9, 115.0, 121.9, 123.9, 126.7, 130.7, 132.5, 150.8, 161.2, 161.4; FAB-HRMS calcd for $C_{16}H_{14}ClFN_2O_2$ $[M + H]^+$: 321.0801; found: 321.0811. HPLC (method 1) 98%.

4-((6-Chloroindolin-1-yl)methyl)-*N*-hydroxybenzamide (1d, ING-6): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.88 (t, *J* = 8.6 Hz, 2H), 3.34 (t, *J* = 8.6 Hz, 2H), 4.32 (s, 2H), 6.53 (m, 3H), 6.98 (d, *J* = 7.5 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.70 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.7, 53.9, 54.8, 110.5, 121.2, 125.6, 127.5, 128.7, 129.9, 130.2, 133.3, 139.8, 149.9, 160.9, 161.3, 166.3; FAB-HRMS calcd for $C_{16}H_{15}ClN_2O_2$ $[M + H]^+$: 303.0895; found: 303.0889. HPLC (method 1) 97%.

4-((5-Fluoroindolin-1-yl)methyl)-*N*-hydroxybenzamide (1e): ^{1}H NMR (Acetone -d_6, 400 MHz) δ 2.95 (t, *J* = 8.2 Hz, 2H), 3.34 (t, *J* = 8.2 Hz, 2H), 4.31 (s, 2H), 6.49 (m, 1H), 6.75 (m, 1H), 6.89 (dd, *J* = 1.2, and 8.5 Hz, 1H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.82 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (Acetone -d_6, 100 MHz) δ 24.8, 53.3, 53.6, 106.9, 107.0, 111.3, 111.5, 112.0, 112.3, 126.6, 127.5, 130.7, 131.6, 131.7, 141.9, 148.4, 164.9, 157.3, 164.3; FAB-HRMS calcd for $C_{16}H_{15}FN_2O_2$ $[M + H]^+$: 287.1190; found: 287.1200. HPLC (method 1) 98%.

4-((6-Fluoroindolin-1-yl)methyl)-*N*-hydroxybenzamide (1f): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.91 (t, *J* = 8.2 Hz, 2H), 3.43 (t, *J* = 8.2 Hz, 2H), 4.38 (s, 2H), 6.29 (m, 2H), 7.00 (m, 1H), 7.47 (d, *J* = 8.3 Hz, 2H), 7.83 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.3, 52.2, 53.7, 94.4, 94.7, 102.5, 102.7, 124.5, 124.6, 125.3, 127.0, 127.8, 131.1, 142.0, 154.0, 154.1, 162.2, 164.5; FAB-HRMS calcd for $C_{16}H_{15}FN_2O_2$ $[M + H]^+$: 287.1190; found: 287.1197. HPLC (method 1) 97%.

4-((6-Chloro-5-fluoroindolin-1-yl)methyl)-*N*-hydroxybenzamide (1g): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.50 (t, J = 8.3 Hz, 2H), 3.28 (t, J = 8.3 Hz, 2H), 4.30 (s, 2H), 6.64 (d, J = 6.1 Hz, 1H), 7.10 (d, J = 8.9 Hz, 1H), 7.38 (d, J = 8.1 Hz, 2H), 7.71 (d, J = 8.1 Hz, 2H), 11.20 (bs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 28.1, 52.5, 53.6, 107.3, 113.5, 113.7, 117.2, 117.5, 127.5, 128.2, 130.9, 131.0, 132.0, 141.5, 149.4, 149.7, 151.7, 164.5; FAB-HRMS calcd for $C_{16}H_{14}ClFN_2O_2$ [M + H]$^+$: 321.0801; found: 321.0813:. HPLC (method 1) %.

***N*-Hydroxy-4-((5-methoxyindolin-1-yl)methyl)benzamide (1h)**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.84 (t, J = 8.0 Hz, 2H), 3.17 (t, J = 8.0 Hz, 2H), 3.64 (s, 3H), 4.19 (s, 2H), 6.45 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 8.2 Hz, 1H), 6.73 (s, 1H), 7.41 (d, J = 7.8 Hz, 2H), 7.70 (d, J = 7.8 Hz, 2H), 9.02 (s, 1H), 11.18 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 28.7, 54.0, 54.2, 55.9, 108.1, 112.0, 112.08, 127.3, 128.3, 131.6, 131.8, 142.3, 146.9, 152.8, 164.6; FAB-HRMS calcd for $C_{17}H_{18}N_2O_3$ [M + H]$^+$: 299.1390; found: 299.1398. HPLC (method 1) 98%.

***N*-Hydroxy-4-((5-nitroindolin-1-yl)methyl)benzamide (1i):** ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.08 (t, J = 8.6 Hz, 2H), 3.65 (t, J = 8.6 Hz, 2H), 4.58 (s, 2H), 6.58 (d, J = 8.9 Hz, 1H), 7.35 (d, J = 8.1 Hz, 2H), 7.72 (d, J = 8.1 Hz, 2H), 7.84 (s, 1H), 7.97 (dd, J = 2.2, 8.9 Hz, 1H), 11.20 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 26.8, 49.9, 52.4, 104.2, 120.7, 126.9, 127.6, 127.9, 130.7, 132.3, 137.1, 140.4, 157.5; FAB-HRMS calcd for $C_{16}H_{15}N_3O_4$ [M + H]$^+$: 314.1135; found: 314.1143. HPLC (method 1) 98.9%.

4-((6-(Benzyloxy)indolin-1-yl)methyl)-*N*-hydroxybenzamide (1j): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.81 (t, J = 8.0 Hz, 2H), 3.27 (t, J = 8.0 Hz, 2H), 4.28 (s, 2H), 4.99 (s, 2H), 6.19 (d, J = 7.8 Hz, 1H), 6.23 (s, 1H), 6.88 (d, J = 7.8 Hz, 1H), 7.34 (m, 7H), 7.70 (d, J = 8.0 Hz, 2H), 9.0 (b.s, 1H), 11.18 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.6, 52.3, 53.7, 69.9, 95.7, 103.1, 122.3, 124.8, 127.4, 127.9, 128.0, 128.2, 128.7, 131.9, 137.8, 142.0, 153.7, 159.9, 154.6; FAB-HRMS calcd for $C_{23}H_{22}N_2O_3$ [M - H]$^-$: 373.1558; found: 373.1562. HPLC (method 1) 98.2%.

4-((6-Chloro-3,4-dihydroquinolin-1(2*H*)-yl)methyl)-*N*-hydroxybenzamide (1k): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.91 (m, 2H), 2.73 (t, J = 6.2 Hz, 2H), 3.37 (t, J = 5.6 Hz, 2H), 4.51 (s, 1H), 6.35 (d, J = 8.8 Hz, 1H), 6.86 (dd, J = 2.5, and 8.8 Hz, 1H), 6.94 (d, J = 2.5 Hz, 1H), 7.27 (d, J = 8.2 Hz, 2H), 7.68 (d, J = 8.2 Hz, 2H), 8.99 (bs, 1H), 11.15 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.8, 27.7, 49.9, 54.3, 112.3, 119.1, 124.3, 126.7, 126.8, 127.5, 128.5, 131.8, 142.3, 144.1, 164.5.HRMS (ESI): *m/z* [M+H]$^+$ calculated for $C_{17}H_{17}ClN_2O_2$: 317.1051, found: 317.1056. HPLC (method 2) 97.6%.

4-((3,3-Dimethylindolin-1-yl)methyl)-*N*-hydroxybenzamide (1l): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.22 (s, 6H), 3.04 (s, 2H), 4.30 (s, 2H), 6.51 (d, J = 7.8 Hz, 1H), 6.62 (t, J = 7.3 Hz, 1H), 6.97 (m, 2H), 7.39 (d, J = 7.9 Hz, 2H), 7.71 (d, J = 7.9 Hz, 2H), 11.15 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.8, 52.2, 67.5, 107.4, 117.9, 122.0, 127.4, 127.6, 128.1, 131.9, 138.9, 142.2, 150.9, 164.5; FAB-HRMS calcd for $C_{18}H_{20}N_2O_2$ [M + H]$^+$: 297.1598; found: 297.1606. HPLC (method 1) 98.2%.

4-((5'-Fluorospiro[cyclopropane-1,3'-indolin]-1'-yl)methyl)-*N*-hydroxybenzamide (1m): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.94 (d, J = 6.0 Hz, 4H), 4.32 (s, 2H), 6.51(m, 2H), 6.73 (dt, J = 2.5, and 9.0 Hz, 1H), 7.39 (d, J = 8.0 Hz, 2H), 7.74 (d, J = 8.0 Hz, 2H), 11.18 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 16.6, 23.5, 23.6, 53.0, 61.5, 106.6, 106.9, 107.0, 107.1, 112.4, 112.6, 127.4, 128.3, 132.0, 137.2, 137.2, 141.9, 149.2, 155.4, 157.7, 158.8, 164.4; FAB-HRMS calcd for $C_{18}H_{17}N_2O_2F$ [M + H]$^+$: 313.1347; found: 313.1359. HPLC (method 2) 97.4%.

4-((3,3-Dimethyl-2-oxoindolin-1-yl)methyl)-*N*-hydroxybenzamide (1n): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.33 (s, 6H), 4.94 (s, 2H), 6.97 (d, J = 7.5 Hz, 1H), 7.00 (t, J = 7.5 Hz, 1H), 7.16 (t, J = 7.5 Hz, 1H), 7.36 (m, 3H), 7.69 (d, J = 8.1 Hz, 1H), 9.01 (s, 1H), 11.15 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 26.6, 40.6, 43.9, 109.4, 122.8, 123.0, 127.4, 127.7, 128.0, 132.4, 135.7, 140.2, 141.6, 164.4, 180.9; FAB-HRMS calcd for $C_{18}H_{18}N_2O_3$ [M + H]$^+$: 311.1390; found: 311.1377. HPLC (method 1) 99.7%.

4-((3,3-Difluoro-2-oxoindolin-1-yl)methyl)-*N*-hydroxybenzamide (1o, ING-66): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.00 (s, 2H), 7.16 (d, J = 7.7 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.39 (d, J = 7.9 Hz, 2H), 7.55 (t, J = 7.6 Hz, 1H), 7.72 (d, J = 7.9 Hz, 2H), 11.18 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 43.3, 111.5, 111.6, 113.9, 118.9, 119.2, 119.4, 124.6, 125.2, 127.6, 127.9, 132.8, 134.7, 138.6, 143.2, 143.3, 143.4, 164.2, 164.7, 165.0, 165.3; FAB-HRMS calcd for $C_{16}H_{12}N_2O_3F_2$ [M + H]$^+$: 319.0889; found: 319.0904. HPLC (method 2) 97.6%.

***N*-hydroxy-4-((2-oxospiro[indoline-3,3'-pyrrolidin]-1-yl)methyl)benzamide (1p).** ^{1}H NMR (MeOD, 400 MHz) δ 2.45-2.53 (m, 2H), 3.63 (d, J = 2.4 Hz, 1H), 3.66-3.90 (m, 3H), 4.97 (d, J = 14.1, 1Hz), 5.04 (d, J = 14.1, 1Hz), 6.90 (d, J = 7.8 Hz, 1H), 7.15 (t, J = 7.5 Hz, 1H), 7.28 (t, J = 7.8 Hz, 1H), 7.44 (m, 3H), 7.72 (d, J = 8.2 Hz, 2H); ^{13}C NMR (MeOD, 100 MHz) δ 35.8, 42.8, 45.3, 52.0, 52.1, 109.5, 122.7, 123.3, 127.1, 127.2, 128.1, 129.0, 131.6, 139.6, 142.4, 161.1, 161.5, 178.6; FAB-HRMS calcd for $C_{19}H_{18}N_3O_3$ [M - H]$^-$: 338.1348; found: 338.1360. HPLC (method 2) 98.5%.

1-(4-(Hydroxycarbamoyl)benzyl)-1',1'-dimethyl-2-oxospiro[indoline-3,4'-piperidin]-1'-ium (1q): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.03 (d, J = 15.2 Hz, 2H), 2.38 (t, J = 2.2 Hz, 2H), 3.25 (s, 3H), 3.35 (s, 3H), 3.55 (d, J = 13.0 Hz, 2H), 3.93 (t, J = 11.3 Hz, 2H), 4.95 (s, 2H), 6.92 (d, J = 7.5 Hz, 1H), 7.10 (t, J = 7.5 Hz, 1H), 7.25 (t, J = 7.5 Hz, 1H), 7.37 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H), 7.75 (d, J = 7.5 Hz, 1H), 9.04 (s, 1H), 11.17 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.3, 41.6, 42.4, 54.9, 57.7, 109.3, 122.7, 123.1, 127.0, 127.2, 128.5, 131.4, 131.6, 139.7, 141.4, 166.2, 178.4; FAB-HRMS calcd for $C_{22}H_{25}N_3O_3$ [M + H]$^+$: 380.1975; found: 380.1969. HPLC (method 2) 99.8%.

***N*-Hydroxy-4-(((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)amino)methyl)benzamide (1r):** ^{1}H NMR (DMSO-d_6, 400 MHz) δ 4.63 (d, J = 5.9 Hz, 2H), 11.12 (s, 1H), 6.92 (m, 2H), 7.15 (m, 2H), 7.29 (t, J = 6.0 Hz, 1H), 7.45 (d, J = 8.3 Hz, 2H), 7.70 (d, J = 8.3 Hz, 2H), 8.97 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 26.8, 45.2, 106.5, 114.2, 118.9,120.3, 126.4, 126.6, 130.4, 134.3, 140.8, 143.2, 154.5, 166.2.FAB-HRMS calcd for $C_{16}H_{16}N_4O_3$[M+H]$^+$: 297.1346, found: 297.1352. HPLC (method 2): 99.9%.

4-(((5-chloro-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)amino)methyl)-N-hydroxybenzamide (1s): ^{1}H NMR (MeOD, 400 MHz) δ 4.81 (s, 2H), 7.34 (dd, J = 1.8, 8.6 Hz, 1H), 7.49 (d, J = 1.8 Hz, 1H), 7.47 (d, J = 8.6 Hz, 1H), 7.54 (d, J = 8.2 Hz, 2H), 7.78 (d, J = 8.2 Hz, 2H). ^{13}C NMR (MeOD, 100 MHz) δ 28.4, 46.0, 110.7, 111.3, 123.7, 126.9, 127.3, 129.1, 129.7, 130.3, 131.8, 139.5, 150.6, 166.1.FAB-HRMS calcd for $C_{16}H_{15}N_4O_2$ Cl[M+H]$^-$: 329.0811, found: 329.0799. HPLC (method 2): 98.7%.

***N*-hydroxy-4-(((1-phenyl-1*H*-benzo[*d*]imidazol-2-yl)amino)methyl)benzamide (1t).** ^{1}H NMR (MeOD, 400 MHz) δ 7.75 (m, 5H), 7.67 (m, 2H), 7.50 (m, 3H), 7.31 (m, 2H), 7.02 (d, J = 8.1 Hz, 1H), 4.78 (s, 2H). ^{13}C NMR (MeOD, 100 MHz) δ 166.1, 149.9, 139.8, 132.2, 131.2, 130.9, 130.8, 130.6, 130.5, 127.6, 127.3, 127.2, 126.6, 124.2, 124.0, 123.5, 111.5, 111.3, 109.8, 45.8.FAB-HRMS calcd for $C_{21}H_{18}N_4O_2$[M+H]$^+$: 359.1503, found: 359.1518. HPLC (method 2): 99.8%.

***N*-Hydroxy-4-(((1-methyl-1,2,3,4-tetrahydroquinolin-4-yl)amino)methyl)benzamide (1u):** ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.07 (m, 1H), 2.37 (m, 1H), 2.92 (s, 3H), 3.26 (m, 1H), 3.38 (m, 1H), 4.19 (m, 1H), 4.4 (m, 2H), 6.62 (t, *J* = 7.1 Hz, 1H), 6.73 (d, *J* = 8.6 Hz, 1H), 7.22 (m, 2H), 7.59 (d, *J* = 8.2, 2H), 7.8 (d, *J* = 8.2 Hz, 2H), 8.9 (s, 1H), 9.02 (s, 1H), 11.29 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.7, 38.2, 44.9, 48.2, 55.3, 112.1, 113.5, 116.1, 127.4, 129.8, 130.4, 131.3, 134.3, 146.6, 164.8.HRMS (ESI): *m/z* $[M+H]^-$ calculated for $C_{18}H_{21}N_3O_2$: 310.1561, found: 310.1564. HPLC (method 2): 98.9%.

Table 2S. *In vitro* HDAC isoform selectivity profile of compound **ING-6**.

Compound	HDAC isoforms inhibition IC_{50} (µM)										
	1	2	3	4	5	6	7	8	9	10	11
ING-6	3.77	6.24	4.34	3.48	2.70	0.009	0.98	0.53	2.06	6.39	4.52

[a] Performed by Reaction Biology Corporation (http://www.reactionbiology.com)

Neh2- and ODD-luc reporter assays.

The assay was performed as previously described.[1, 2] In brief, the studied compounds, with varied concentrations in the range 2.5-20 µM, were tested in 96-format white flat-bottom plates. SH-SY5Y/Neh2-luc and SH-SY5Y/HIF1 ODD-luc cells were plated at the density of 25,000 cell per well using a WellMate multichannel dispenser from Matrix (ThermoFisher Scientific) and grown overnight on DMEM/F12+GlutaMAX (100 µl per well). Then the activator (2 µl of 50x stock solutions in DMSO prepared in a master plate, right before use) was added, and the plates were incubated for 3 h; the medium was removed, cells lysed, and luciferase activity was measured on a SpectraMax M5^e Microplate Reader with BrightGlo^{TM} reagent (Promega). The reporter activation was normalized to the background luminescence. The experiments were performed in triplicate. TBHQ and ciclopirox were used as positive controls for SH-SY5Y/Neh2-luc and SH-SY5Y/HIF1 ODD-luc reporter lines, respectively.

Cell culture studies.

Mouse N2a neuroblastoma cells (ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, CA) supplemented with 10% fetal bovine serum in 100U/ml penicillin, and 100 µg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2. Cells were cultured up to 80% confluence in 6-well plates and treated with the respective concentrations of drugs for times indicated in the figure legends for real-time PCR. For cytotoxicity studies, cells were plated in 96-well plates with 5000 cells per well seeding ratio. After overnight incubation, cells were treated with different concentrations of drugs for 4 hours. Cytotoxic challenge was induced by addition of MPP^+, which was assessed at 24 h after MPP+ by Presto-Blue cell proliferation assay (Life Technologies, CA).

RNA isolation and real-time RT-PCR.

Total RNA from mouse N2a neuroblastoma cells or mice liver, whole brain, ventral midbrain and striatum were isolated and processed according to the manufacturer's protocol using TRIzol reagent (Life Technologies). Reverse transcription of about 2 µg of total RNA was performed using a High-Capacity cDNA Reverse Transcription Kit (Life Technologies). The cDNA was diluted, and about 100 ng was used to amplify in an ABI prism 7900 HT Real-time PCR system (Applied Biosystems) for various genes using primers (Table 2S) and Fast SYBR® Green Master Mix (Life Technologies) in a 10 µL reaction mixture. Cycling parameters were 95 °C for 10 s, followed by 60 °C for 1 min. Relative expression was calculated using the ΔΔCt method.[3] Values are expressed as a fold of control reaction and normalized to glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) expression or β-actin expression.

Table 3S. **List of mouse primer sequences used for the qRT-PCR**

Gene	***Left primer***	***Right primer***
NAD(P)H dehydrogenase, quinone 1 (*Nqo1*)	5'AGCGTTCGGTATTACGATCC3'	5'AGTACAATCAGGGCTCTTCTCG3'
Heme Oxygenase 1 *HMOX1*	5'GTCAAGCACAGGGTGACAGA3'	5'ATCACCTGCAGCTCCTCAAA3'

Administration of ING-66 (1o) in mice to test Nrf2 and HIF-1 target genes activation.

C57Bl6 male mice (8−10 weeks old at testing) were obtained from Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed in designated animal suites maintained at 22 ± 2 °C on a 12 h light−dark cycle with 4-5 mice per cage. All animal experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and use Committee of the Augusta University. Mice were administered with two doses of **1o** 25 mg/kg 12 hours apart by intraperitoneal route dissolved in (1:8, DMSO: Neobee oil) in a volume of 10 mL/kg; control mice were administered with vehicle (1:8, DMSO:Neobee oil) at the same frequency and volume as the drug. Mice were sacrificed 3 h after the last dose of the drug or vehicle and the striatum, ventral midbrain and liver were dissected and snap-frozen in liquid nitrogen until processed for RNA analysis.

Measurement of glutathione adducts formation.

Reactivity of TBHQ[4] and compounds **1d (ING-6)** and **1o (ING-66)** toward GSH was monitored by LC-MS as described.[5] 100 µl of a 10 mM solution of GSH in a phosphate-saline buffer and 100 µl of a 10 mM solution of a tested compound in acetonitrile were added to 800 µl of phosphate-saline buffer, pH 7.4. The reaction mixture was kept at ambient temperature, and the reaction was monitored by MS spectroscopy (a Waters Acquity UPLC/MS system) by taking aliquots at fixed time intervals. Chromatographic separation was achieved on an Acquity UPLC BEH C18 (2.1 x 50 mm, particle size 1.7 µm) column with a flow rate of 0.75 mL/min and mobile phases as follows: (A) water with 0.1% v/v formic acid; (B) acetonitrile with 0.1% v/v formic acid, with the mobile phase gradient from 5% to 95% of B over 2 min. An analytical method employing positive ion electrospray ionization (ESI) was developed to detect GSH (*m/z* 308), compounds **1d** (*m/z* 302), and **1o** (*m/z* 318) and GS-TBHQ adduct (*m/z* 471).

Glutathione depletion model and Viability Assay.

Primary neuronal cultures were prepared from the forebrains of Sprague-Dawley rat embryos (E17) and plated on 96 well plates at a 10^6 cells/ml density. After 24 hours, cells were rinsed with warm PBS and then placed in minimum Essential Medium (MEM; Life Technologies, Grand Island, NY) containing 5 mM HCA in the presence of a compound of interest at varied concentrations. Cells were incubated for 24 h or longer to see 90% cell death in HCA treated controls. Viability was assessed by the MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

H_2O_2-Inudced cytotoxicity model.

Human SHSY-5Y neuroblastoma cells were cultured in Dulbecco's modified Eagle's medium/F12 (DMEM/F12) (Life Technologies, CA) supplemented with 10% fetal bovine serum in 100U/ml penicillin at 37°C in a humidified atmosphere of 5% CO_2. For H_2O_2 cytotoxicity studies, cells were plated in 96-well plates with 20,000 cells per well seeding ratio. After overnight incubation, cells were pretreated with different concentrations of drugs for 1- or 24 h prior to insult induced by addition H_2O_2, which was assessed at 24 h post insult by MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay.

Oxygen glucose deprivation (OGD).

For OGD studies, cells were plated in 96-well plates with 40,000 cells per well seeding ratio. After overnight incubation, cells were treated with different concentrations of drugs for 24 h prior to OGD (Paradigm 1) and at the end of OGD [6]. To perform oxygen glucose deprivation, cells were replaced with DMEM no glucose media and then transferred to a sealed hypoxic chamber with an atmosphere of 5% CO_2/95% N_2 for 2 h. After 2 h, cells were removed from the hypoxic chamber and resupplied with growth media for reperfusion/recovery for 24 h until MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay was performed.

Immunofluorescence staining of Nrf2.

HeLa cells were grown on chamber slides and treated with 10 μM of compound for 3 hrs. Cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% triton/PBS, and blocked with 5% BSA in PBST at room temperature. The slides were then probed with rabbit monoclonal to Nrf2 (ab62352, Abcam) overnight at 4 °C and secondary with anti-rabbit Alexa Fluor 488 (Jackson Laboratories) and Hoechst staining. Slides were mounted and visualized using a BZ-X700 microscope (Keyence). Band intensity was measured and normalized against GAPDH. n=3, * $p<0.05$ as measured by students t-test

Hepatotoxicity.

"Liver-on-a-chip" device "Homunculus" (developed in Bioclinicum Ltd, Moscow, Russia) with differentiated *HepaRG* cell spheroids was used as described.[7, 8] In brief, *HepaRG* cells were cultivated in Williams' Medium E medium containing 2 mM L-glutamine, 10% FBS, 5 μg/ml human recombinant insulin, 0.05 mM hydrocortisone hemisuccinate, 100 U/ml penicillin, 100 U/ml streptomycin; 2% DMSO for 14 days was used to differentiate the cells. The cells were collected, suspended (5,000 cell/45 μL) and placed into Perfecta3D™ Hanging Drop Plates (3D Biomatrix, USA) supporting spheroid formation within 3 days. The differentiated *HepaRG* cell spheroids were then placed into a 96-well microplate (low attachment plates, Costar, USA), and cultivated in 250 μL media for 2 days in a CO_2-incubator (37°C, 95% air, 5% CO_2, 98% humidity). To study hepatotoxicity 10 samples of *HepaRG* spheroids (50,000 cells in total) were placed into the chip, 125 μL media removed and the same volume of the compound under study (serial dilution within the range of concentrations of interest) in the FBS-free cultural media injected back; plain serum-free media was used as a control. The compounds were incubated in the chip for 48 h under the optimized circulation regime (5 Hz, 10 kPa [7]). Cell viability was tested with MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide - assay: upon removal of the cultural media, cells were washed twice with 250 μL PBS, and then incubated with 0.5 mg/mL MTT dissolved in the serum-free media for 3 h. The formation of formazane product was measured at 570 nm. The viability was calculated as (viable cells)= (OD of drug treated sample/OD of drug untreated sample). Mean ± SD, n=5.

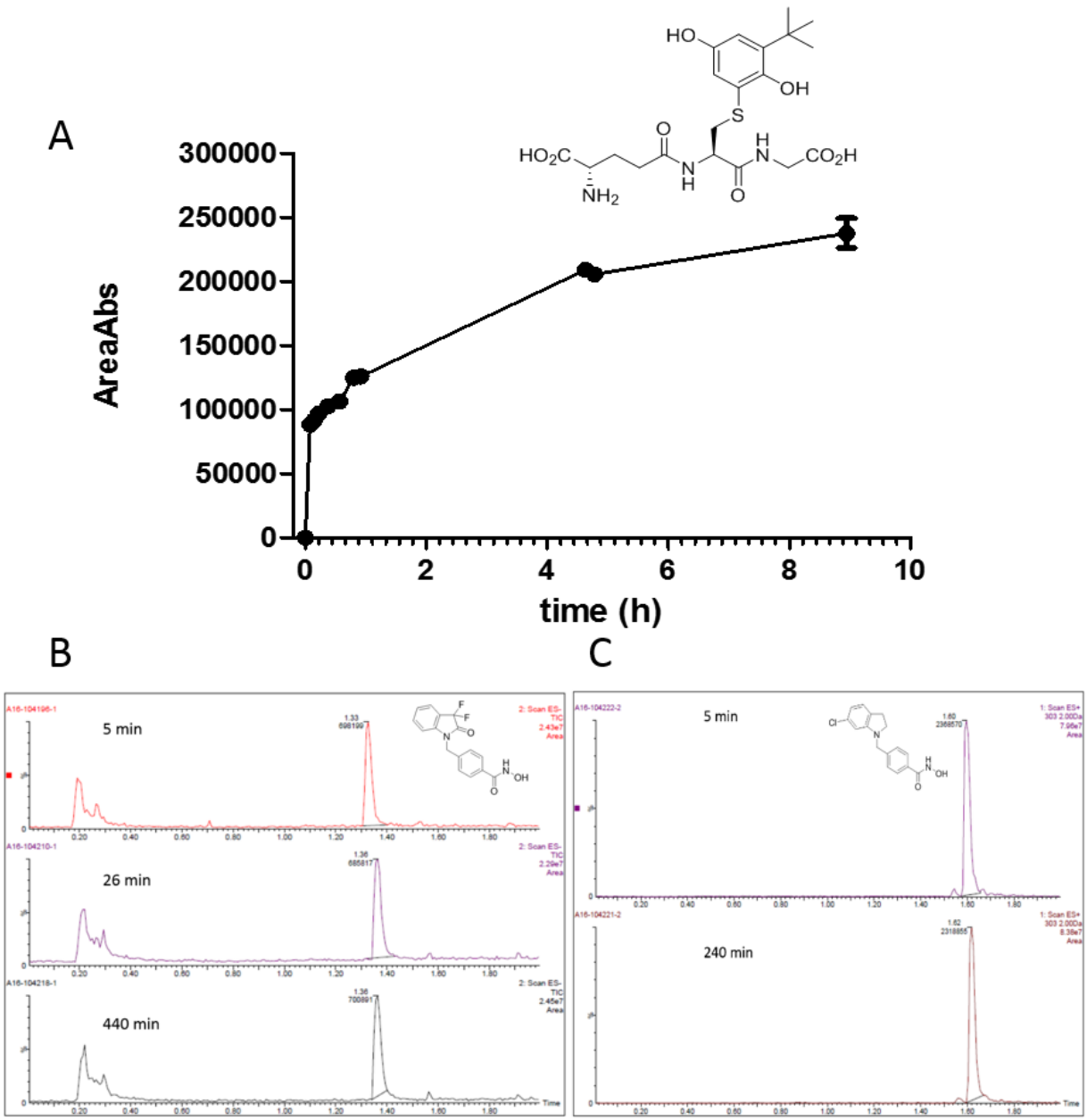


Figure 1S. Reactivity of TBHQ and stability of compounds **1d** (**ING-6)** and **1o** (**ING-66)** toward GSH. **(A)** Time course of the S-alkylation reactions between 1 mM TBHQ and 1 mM GSH in PBS at pH 7.4 was measured as described above. **(B)** Representative TIC LC-MS ES- scans of the reaction mixture of compound **ING-66** with GSH at 5, 26, and 440 min; monitoring of $[M+H]^+$ 319 for detection of **ING-66**; **(C)** Representative LC-MS ES+ scans of the reaction mixture of compound **ING-6** with GSH at 5, and 240 min; monitoring of $[M+H]^+$ 303 for detection of **ING-6**.

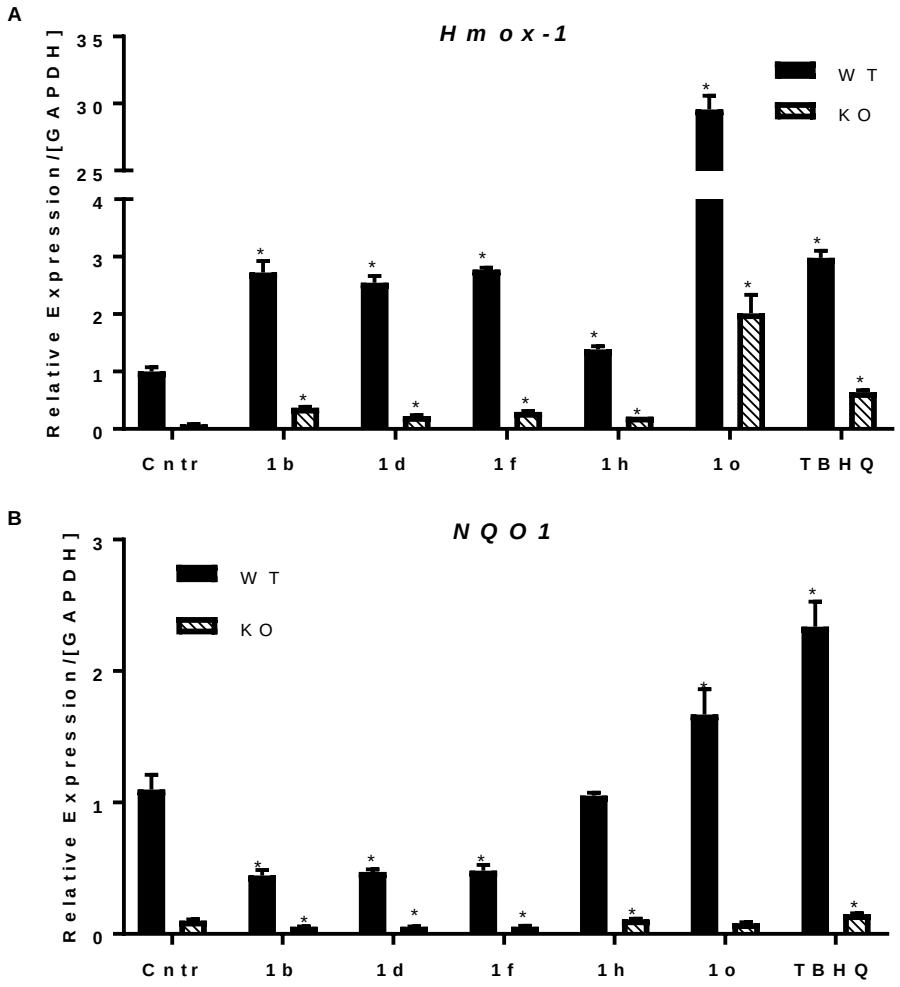


Figure 2S. Selective activation of Nrf2 by novel phenylhydroxamates and TBHQ. Quantitative RT-PCR analysis showing relative mRNA levels of ARE target genes in WT and Nrf2 KO mouse embryonic fibroblasts **(A)** at 3 h for *Hmox-1*, and **(B)** at 8 h for *NQO1* after treatment with test compounds (20 μM). TBHQ was used as a canonical Nrf2 activator and DMSO was used as control. Compound **1o (ING-66)** was the most effective among all tested compounds, showing a thirtyfold increase in HO-1 mRNA levels and almost twofold increase in *NQO1*. As anticipated, the effects of TBHQ and phenylhydroxamates were largely dependent on Nrf2.Bars represent the mean ± SEM of relative mRNA levels (relative to GAPDH). $^{*}p < 0.05$ compared to controls, (n = 3).

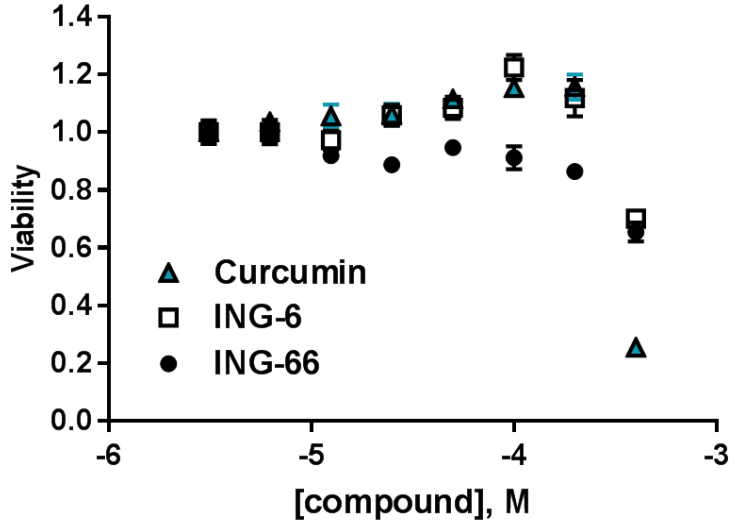


Figure 3S. Hepatotoxicity of ING-6, ING-66, and curcumin in a liver-on-a-chip device "Homunculus" with differentiated *HepaRG* cells spheroids at 48 h circulation. Viability determined using MTT assay. Dot plot represents percentage control mean ± SEM compared to the control of viable cells (n=6).

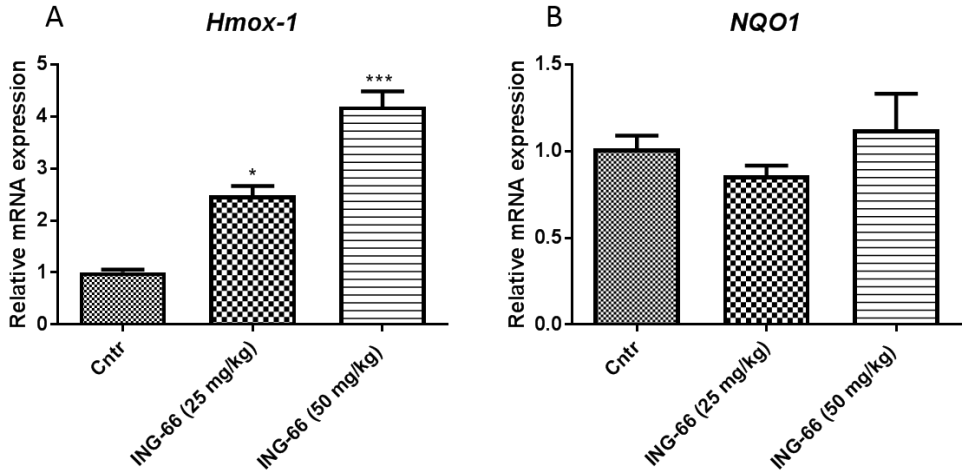
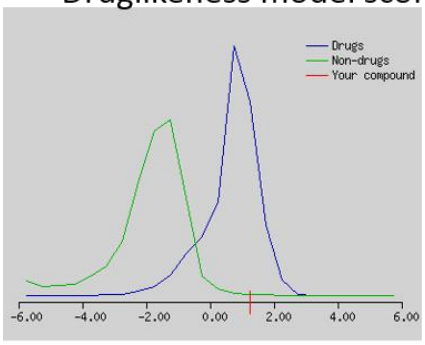
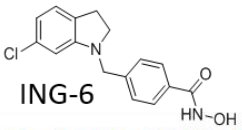


Figure 4S. *In vivo* activation of Nrf2 pathway by ING-66. Quantitative RT-PCR analysis showing relative mRNA levels of (A) *Hmox-1* and (B) *NQO1* in the liver of WT mice treated with two doses, 25 and 50 mg/kg, of ING-66 or vehicle control (i.p.). Bars represent the mean ± SEM of relative mRNA levels (relative to GAPDH). *p < 0.05, ***p<0.001 compared to controls, (n = 3-5).

Druglikeness model score: 1.26

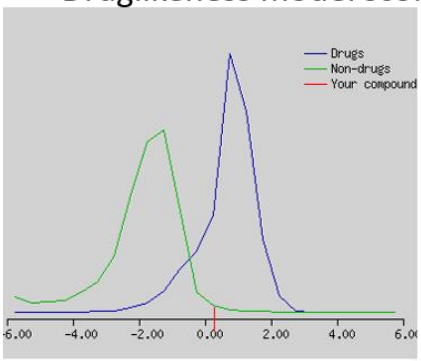


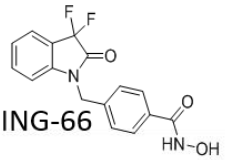


ING-6

Molecular formula: C16 H15 Cl N2 O2
Molecular weight: 302.08
Number of HBA: 2
Number of HBD: 2
MolLogP : 2.80
MolLogS : -5.02 (in Log(moles/L)) 2.89 (in mg/L)
MolPSA : 49.09 A^2
MolVol : 277.02 A^3
Number of stereo centers: 0

Druglikeness model score: 0.27

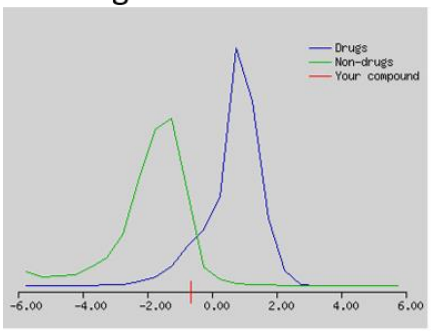


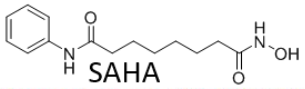


ING-66

Molecular formula: C16 H12 F2 N2 O3
Molecular weight: 318.08
Number of HBA: 3
Number of HBD: 2
MolLogP : 2.88
MolLogS : -4.53 (in Log(moles/L)) 9.37 (in mg/L)
MolPSA : 61.63 A^2
MolVol : 293.16 A^3
Number of stereo centers: 0

Druglikeness model score: -0.65





SAHA

Molecular formula: C14 H20 N2 O3
Molecular weight: 264.15
Number of HBA: 3
Number of HBD: 3
MolLogP : 1.97
MolLogS : -3.25 (in Log(moles/L)) 147.61 (in mg/L)
MolPSA : 69.10 A^2
MolVol : 273.39 A^3
Number of stereo centers: 0

Figure 5S. MolSoft, L.L.C. "Molecules *in silico*" (Molsoft.com/mprop/) predictions of molecular properties and drug-likeness of ING-6, ING-66 and SAHA.

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