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Supplementary Information for

Navigating into chemical space between MGCD0103 and SAHA: Novel Histone Deacetylase Inhibitor as a Promising Lead

Navigating into chemical space between MGCD0103 and SAHA: Novel Histone Deacetylase Inhibitor as a Promising Lead

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Chemistry Section Details

All chemicals and reagents of analytical grade used were purchased from Aldrich (USA). Melting points (uncorrected) were determined on a XT4MP apparatus (Taike Corp, Beijing, China). All the ^{1}H NMR spectra were recorded on a Bruker DPX 300 model Spectrometer at 25 ℃ with TMS and solvent signals allotted as internal standards, and chemical shifts were reported in ppm (d). ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument and were within 0.4% of the theoretical values. TLC was performed on the glass-backed silica gel sheets (Silica Gel 60 GF254) and visualized in UV light (254 nm). Column chromatography was performed by silica gel (200-300 mesh) eluting with ethyl acetate and petroleum ether.

Sythesis details of compounds in Scheme 1

4-(Pyridin-3-yl) pyrimidin-2-amine (compound **B)**:

In a round-bottom flask with a stir bar and reflux condenser were charged 3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one (3.52 g, 20mmol), guanidine hydrochloride (1.91 g, 20mmol), NaOH (0.8 g, 20mmol), and nBuOH (30 ml). The reaction was heated to reflux overnight, and then cooled to room temperature. The precipitate was collected by filtration and washed by cold water and dried in vacuo to get the title compound. 4-(pyridin-3-yl) pyrimidin-2-amine, off-white solid, yield 2.82 g, 85% Mp 187-189 ℃.

N-(4-Iodobenzyl)-4-(pyridin-3-yl) pyrimidin-2-amine (compound **C)**:

4-(pyridin-3-yl) pyrimidin-2-amine **B** (1.72 g, 10mmol) was dissolved in dry THF (40 ml), and the solution was cooled in an ice bath. NaH (0.96 g, 40mmol) was then added. The mixture was stirred in the ice bath for 15 min before adding the 1-(bromomethyl)-4-iodobenzene dropwise. After stirring for 6h, the suspension was filtrated to remove NaH and evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc = 1/1) to

give N-(4-iodobenzyl)-4-(pyridin-3-yl) pyrimidin-2-amine, off-white solid, yield 2.09g, 54%. Mp 164-165 ℃.

Methyl 4-((4-(pyridin-3-yl) pyrimidin-2-ylamino) methyl) benzoate (compound **D**):

4-(pyridin-3-yl) pyrimidin-2-amine **B** (1.72 g, 10mmol) was dissolved in dry THF (40 ml), and the solution was cooled in an ice bath. NaH (0.96g, 40mmol) was then added. The mixture was stirred in the ice bath for 15 min before adding methyl 4-(bromomethyl) benzoate dropwise. After stirring for 6h, the suspension was filtrated to remove NaH and evaporated in vacuo. The crude residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc = 1/1.) methyl 4-((4-(pyridin-3-yl) pyrimidin-2-ylamino) methyl) benzoate, off-white solid, yield 1.60 g, 50%. Mp 174-175 ℃.

(E)-3-(4-((4-(pyridin-3-yl) pyrimidin-2-ylamino) methyl) phenyl) acrylic acid (compound **E**):

N-(4-iodobenzyl)-4-(pyridin-3-yl)pyrimidin-2-amine **C** (540 mg, 1.39 mmol) was dissolved in DMF (20 ml), into which the solution was added tris(dibenzylideneacetone)dipalladium (38 mg, 0.091 mmol), tri-o-tolylphosphine (25 mg, 0.083 mmol), Et_3N (483 μl, 3.48 mmol), and finally acrylic acid (114 μl, 1.67mmol). The mixture was degassed and purged with nitrogen, then heated to 100 ℃ overnight. The solution was filtered through a Celite pad, and followed by being evaporated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH = 95/5). Yellow solid, yield 415 mg, 90%, Mp 211-213℃.

4-((4-(Pyridin-3-yl) pyrimidin-2-ylamino) methyl) benzoic acid (compound **F**):

Methyl 4-((4-(pyridin-3-yl) pyrimidin-2-ylamino) methyl) benzoate **D** (640 mg, 2 mmol) was dissolved in water and MeOH (1/1) mixture with 2M NaOH. After stirring for 2h at room temperature, an aqueous solution of HCl (2M) was added to make the pH of the solution at 4~5. The precipitate was collected by filtration to get the title compound: off-white solid, yield 550 mg, 90%. Mp 223-224 ℃.

(E)-N-hydroxy-3-(4-((4-(pyridin-3-yl) pyrimidin-2-ylamino) methyl) phenyl) acrylamide (compound **1**):

(E)-3-(4-((4-(pyridin-3-yl) pyrimidin-2-ylamino) methyl) phenyl) acrylic acid **E** (219 mg, 0.66mmol) was dissolved in DMF (20 ml). To the solution 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (151 mg, 0.79mmol) and hydroxybenzotriazole hydrate (134 mg, 0.99mmol) were added. The mixture was stirred for 20 min at room temperature, and then NH_2OTHP (116 mg, 0.99mmol) was added. The resulting mixture was heated to 50 ℃ for 6h. DMF was removed under reduced pressure. The crude residue was purified by flash chromatography on silica gel (hexane/acetone = 7/3).The product was dissolved in CH_2Cl_2 (15 ml) and treated with 1M HCl in Et_2O (3.4 ml) for 20min. The precipitating white solid was filtered to get title compound as its hydrochloride: white solid, yield 116 mg, 51%. Mp 217-218 ℃. ^{1}HNMR (400 MHz, DMSO-*d*6) δ: 9.45(s, 1H), 8.97 (d, 2H, J = 8.0 Hz), 8.55 (d, 1H, J = 4.8Hz), 8.37 (s, 1H), 8.02 (s, 1H), 7.48-7.53 (m, 5H), 6.45 (d, 1H, J = 16 Hz), 4.65 (s, 2H).

N-hydroxy-4-((4-(pyridin-3-yl) pyrimidin-2-ylamino) methyl) benzamide (compound **2**):

4-((4-(pyridin-3-yl) pyrimidin-2-ylamino) methyl) benzoic acid **F** (201 mg, 0.66mmol) was dissolved in DMF (20 ml). To the solution 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (151 mg, 0.79mmol) and hydroxybenzotriazole hydrate (134 mg, 0.99mmol) were added. The mixture was stirred for 20 min at room temperature, and then NH_2OTHP (116 mg, 0.99mmol) was added. The resulting mixture was heated to 50 ℃ for 6h. DMF was removed under reduced pressure. The crude residue was purified by flash chromatography on silica gel (hexane/acetone = 7/3).The product was dissolved in CH_2Cl_2 (15 ml) and treated with 1M HCl in Et_2O (3.4 ml) for 20 min. The precipitating white solid was filtered to get title compound as its hydrochloride: white solid, yield 123 mg, 58%. Mp 224-226 ℃. ^{1}HNMR (400 MHz, DMSO-*d*6) δ: 11.20 (s, 1H), 9.42 (s, 1H), 8.95 (d, 2H, J = 6.4 Hz), 8.53 (s, 1H), 8.38 (s, 1H), 8.00 (s, 1H), 7.70 (d, 2H, J = 8.0 Hz), 7.45-7.46 (m, 3H), 4.67 (s, 2H).

Figures for NMR^{1}H, ESI and Vitro Antiproliferative Assay

Figure s1: NMR^{1}H of Compound **1**

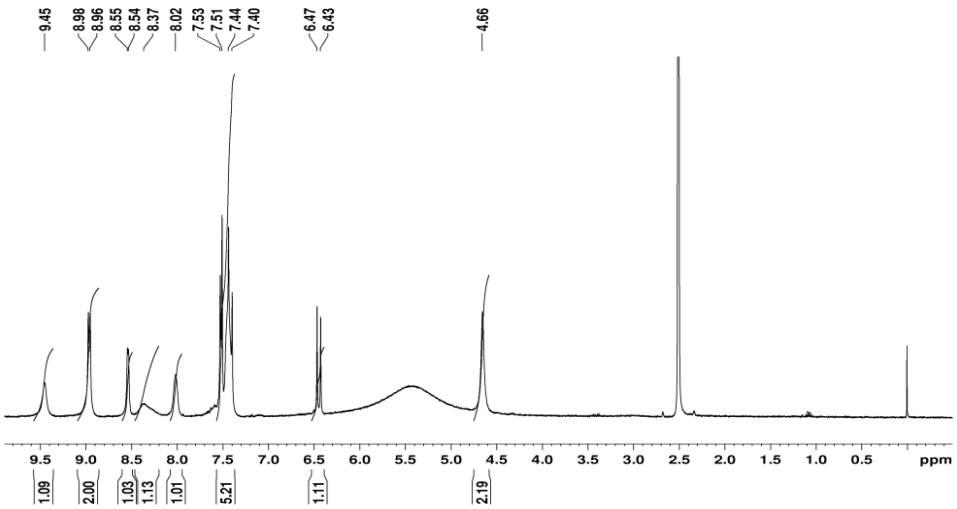


Figure s2: ESI of compound **1**

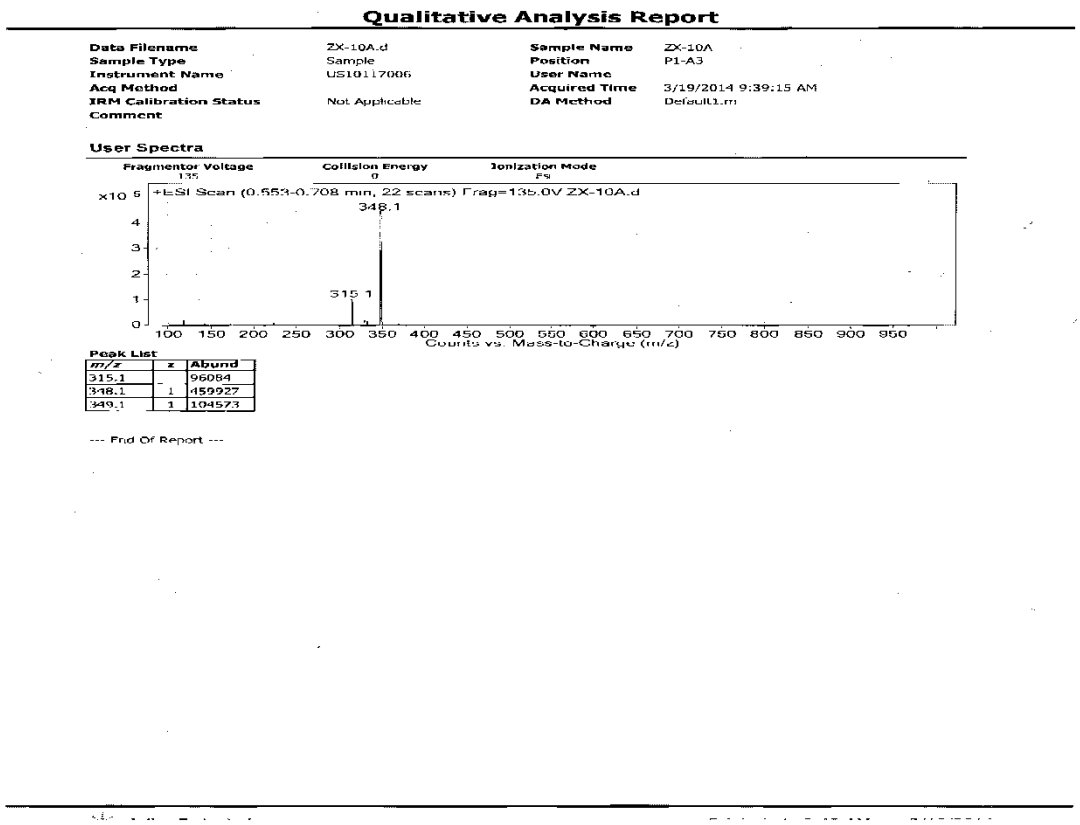


Figure s3: NMR[1]H of Compound **2**

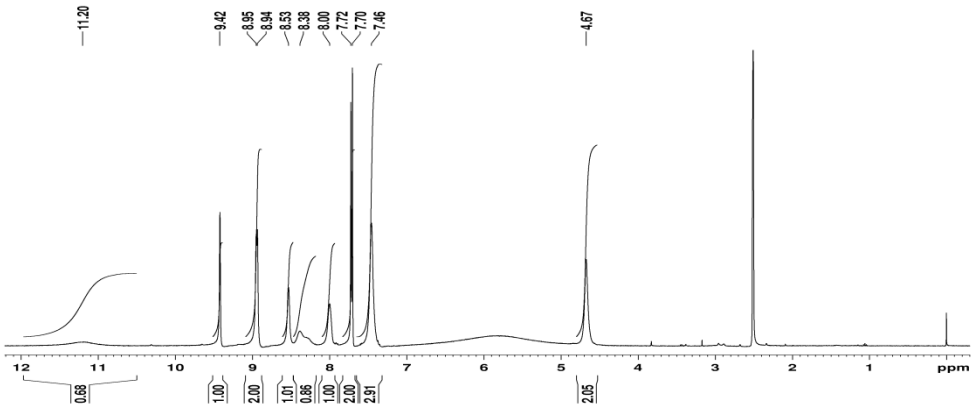
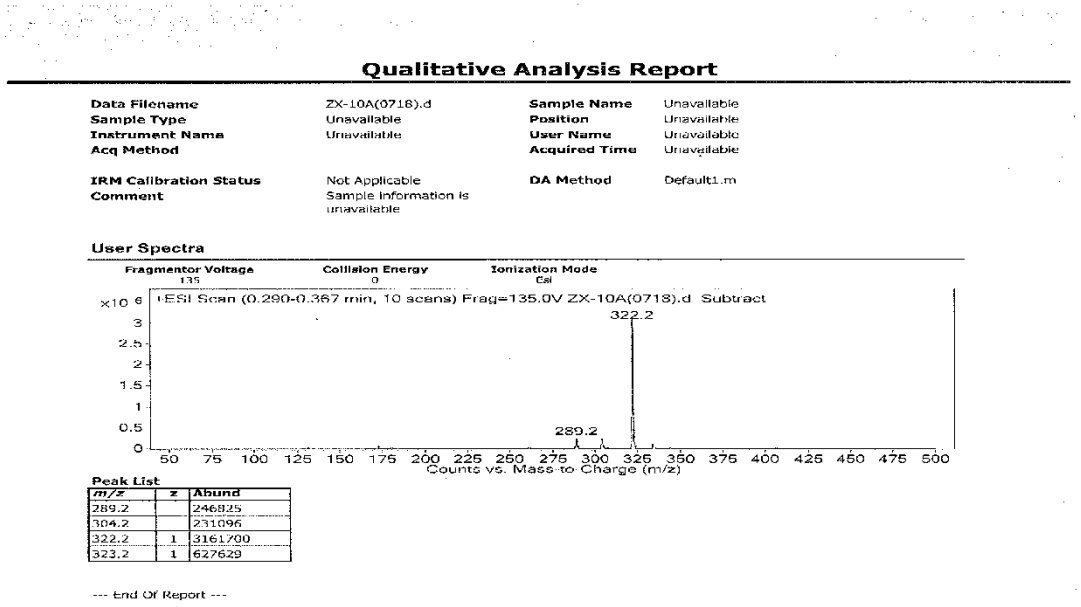


Figure s4: ESI of compound **2**



Qualitative Analysis Report

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Sample Type	Unavailable	**Position**	Unavailable
Instrument Name	Unavailable	**User Name**	Unavailable
Acq Method		**Acquired Time**	Unavailable
IRM Calibration Status	Not Applicable	**DA Method**	Default1.m
Comment	Sample information is unavailable		

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

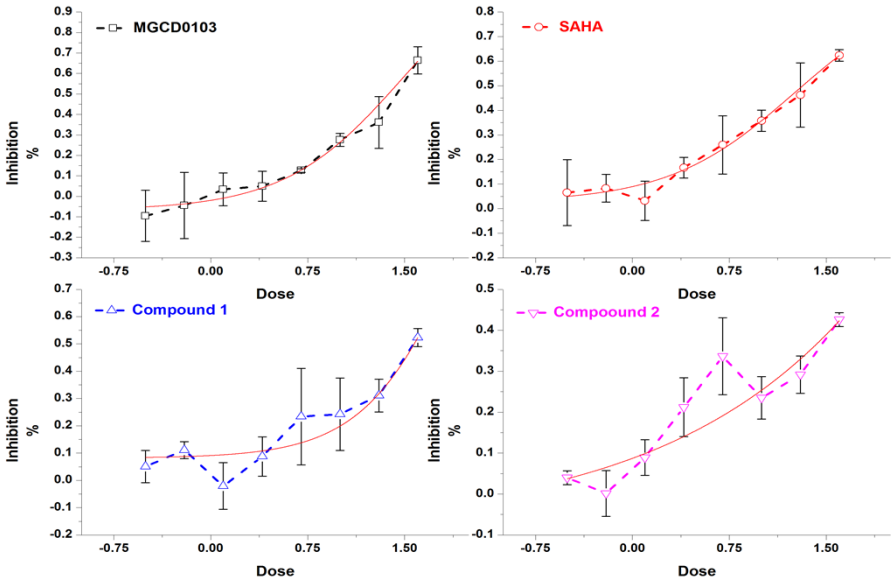
Peak List

m/z	z	Abund
289.2		246825
304.2		231096
322.2	1	3161700
323.2	1	627629

--- End Of Report ---

Figure s5: All the figures are based on the MTT assay, the inhibition in Y-axis is calculated by $Inhibition=1-(OD_{control}-OD_{blank})/(OD_{experiments}-OD_{blank})$ and the dose in X-axis is calculated by $Dose=ln\,compound\ concentrations$.

A549-24 h



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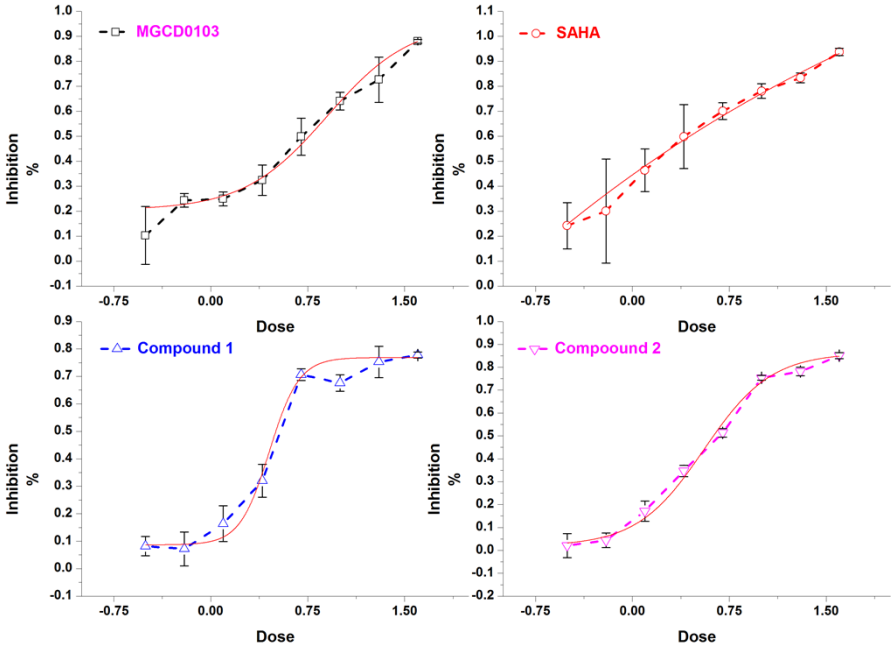

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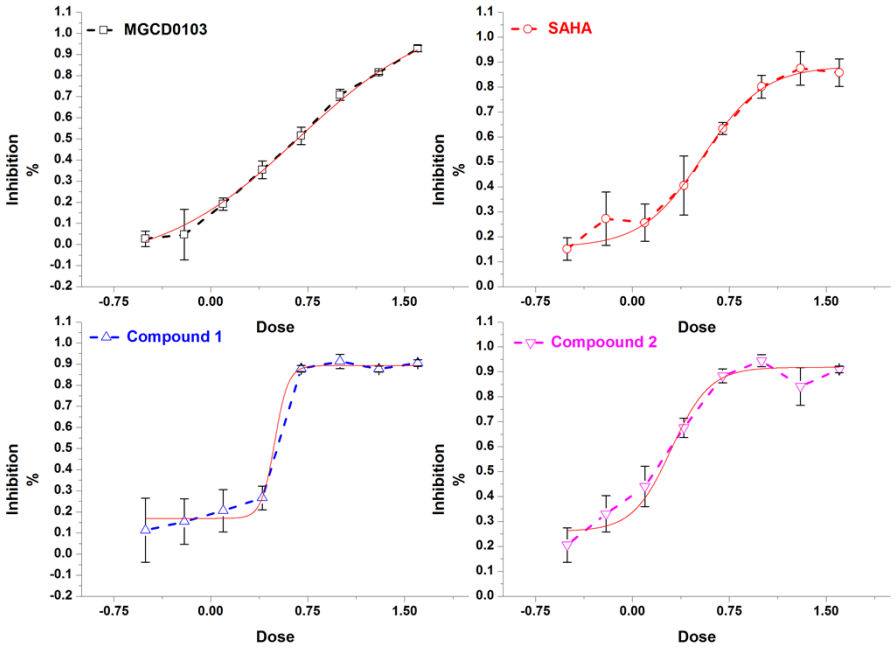

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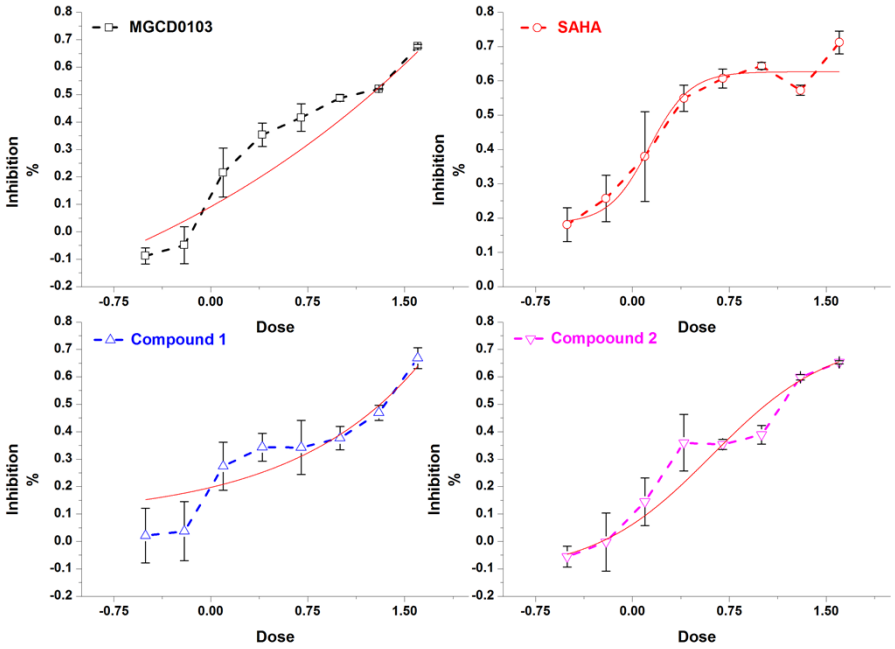

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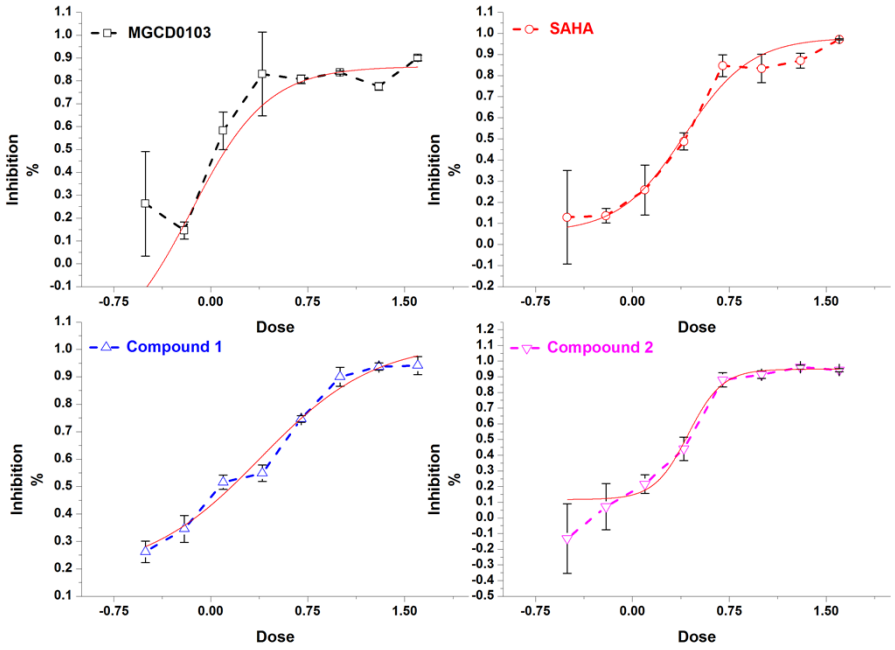

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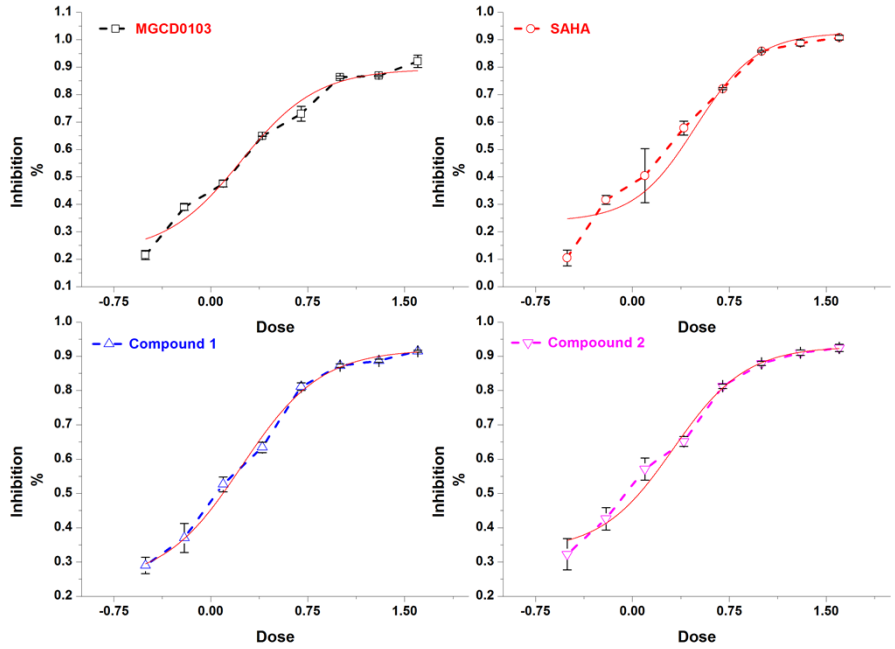

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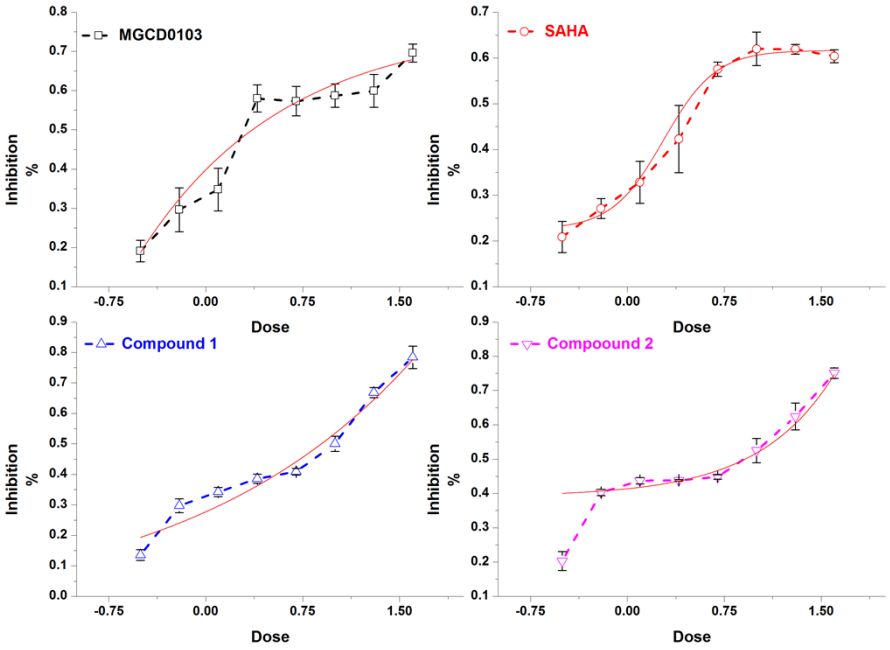

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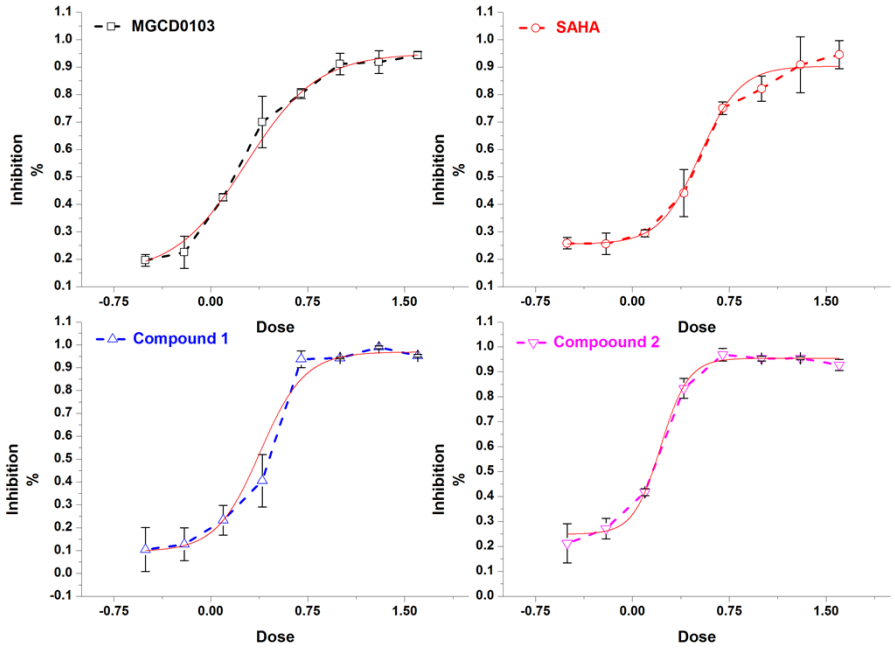

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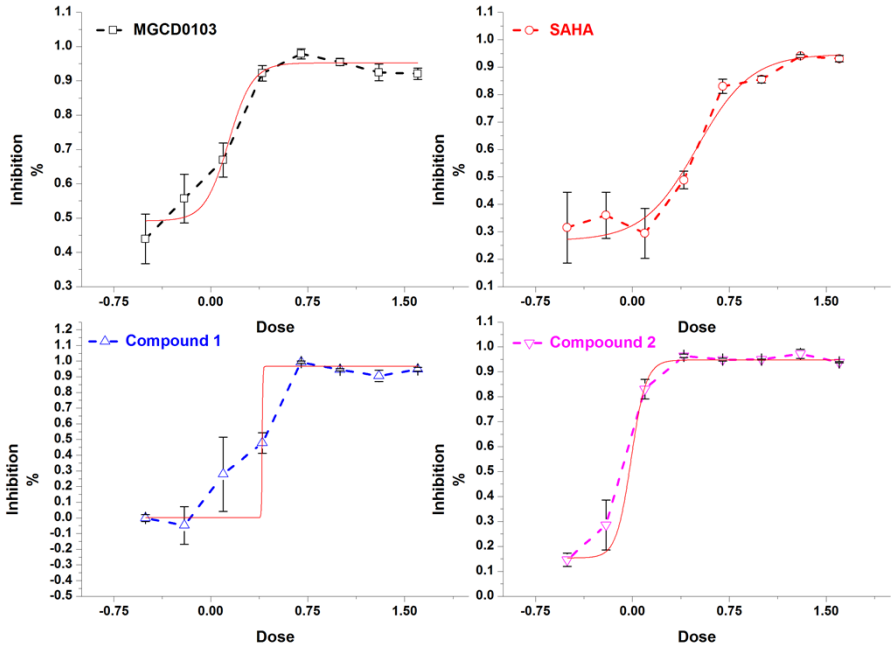

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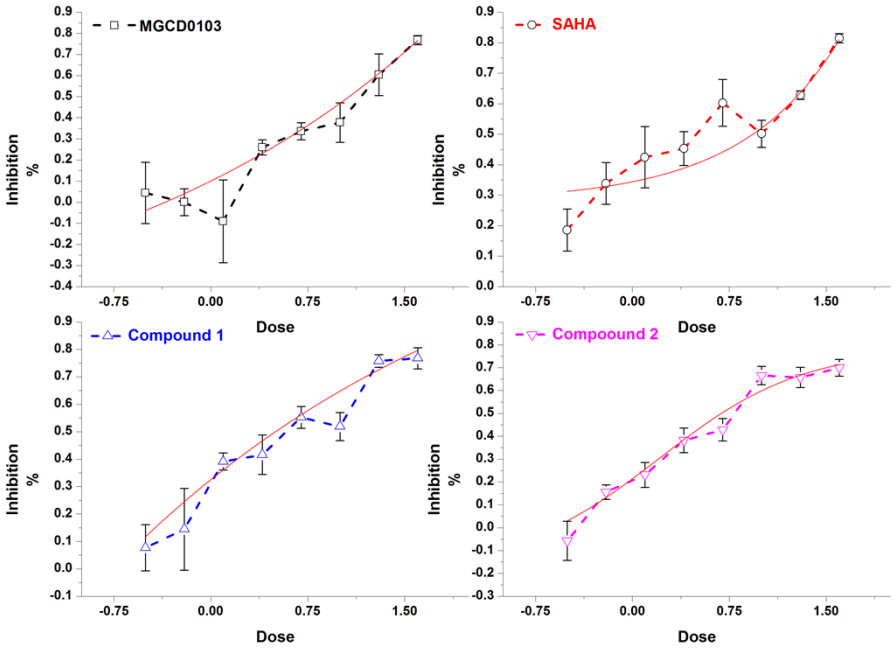

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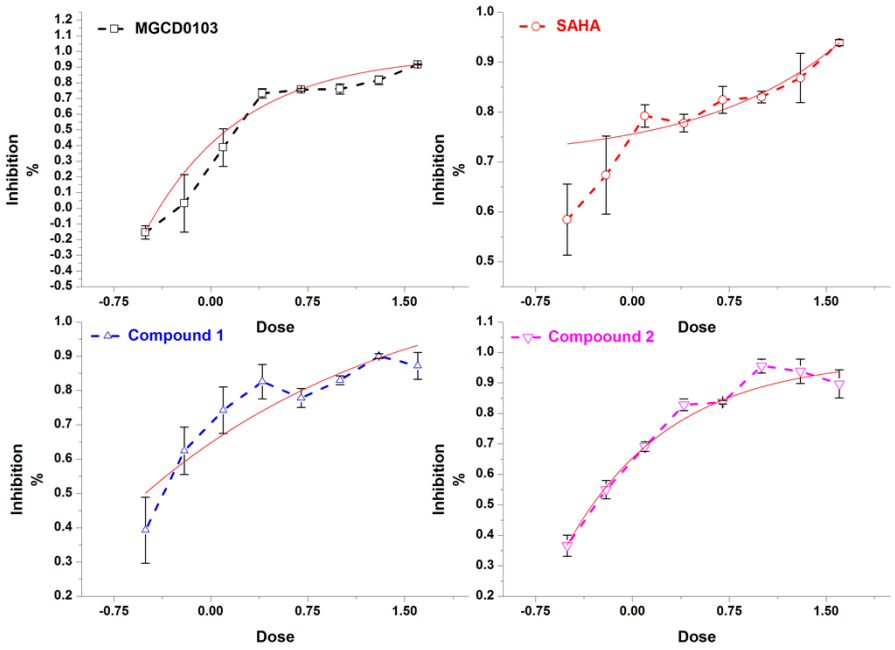

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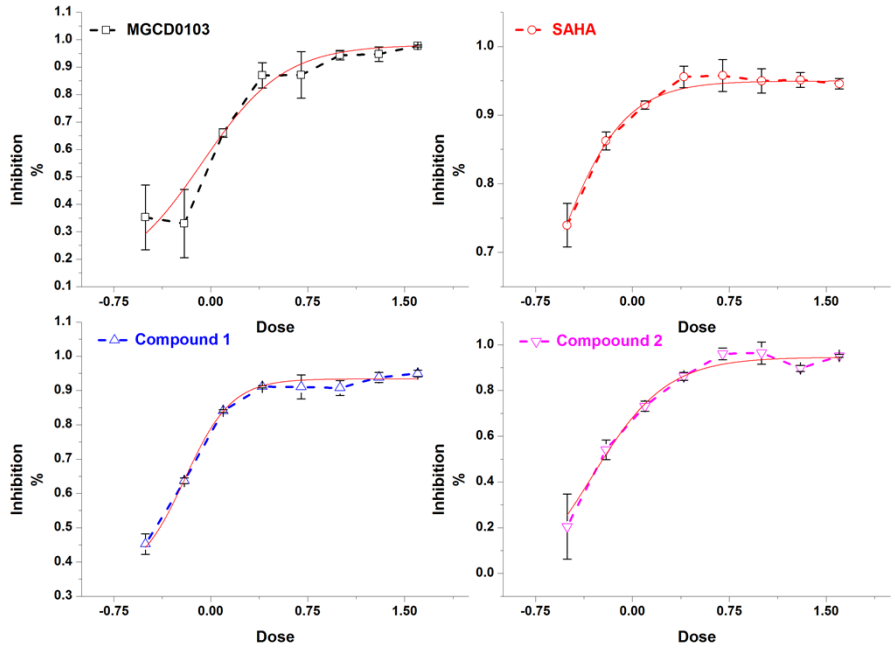

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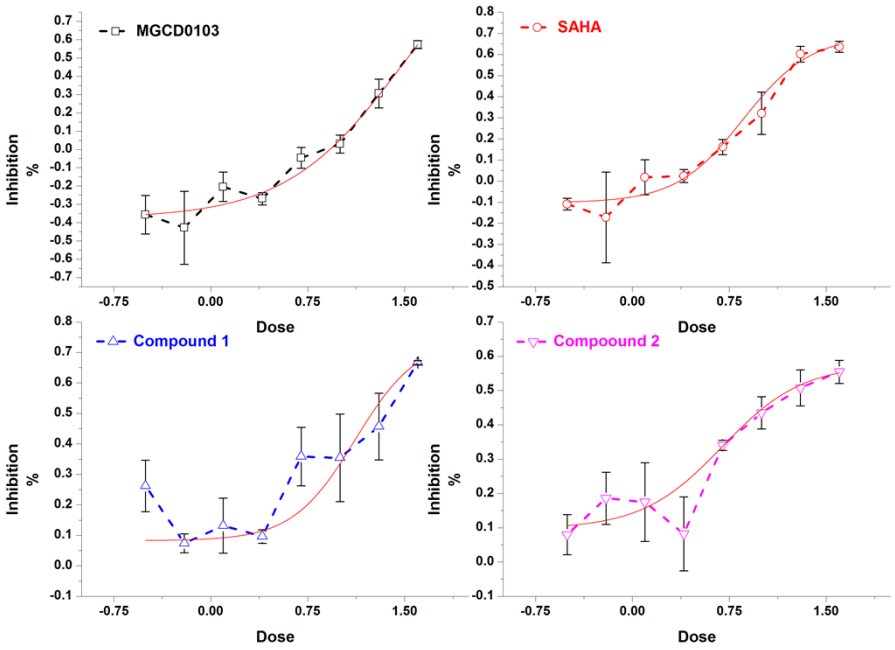

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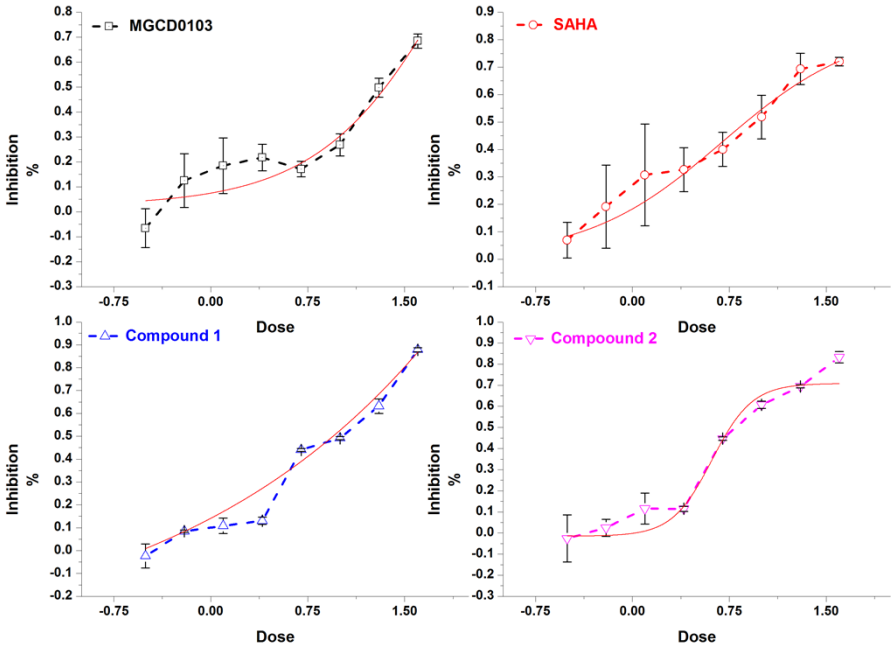

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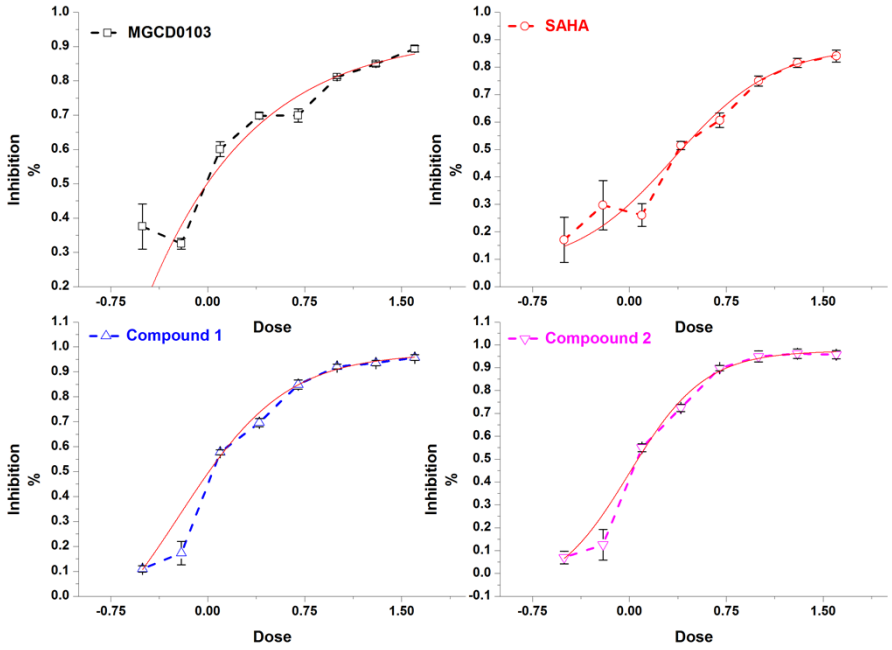

Table 4 Results of virtual screening by molecular docking based on three human HDAC protein crystal structures (HDAC2, pdb code: 3MAX; HDAC4, pdb code: 2VQW; HDAC8, pdb code: 1W22)

Designed Compounds	CDOCKER_INTERACTION_ENERGY (kcal/mol)[a]			Consensus Score [b]
	3MAX	2VQW	1W22	
NH_6	-44.8109	nd [c]	-42.5724	2
NH_9	-43.7696	-63.3461	-52.8084	2
NH_13	-55.5114	nd	-45.9295	2
NH_16	-40.0754	-57.4949	-47.1285	2
S_6	-44.7454	nd	-48.3771	2
S_13	-44.9942	-63.5364	-38.4227	2
NH_4	-31.5481	-57.4348	-35.4223	1
NH_10	-44.9379	-56.2177	-35.7044	1
NH_11	-45.1783	-11.9014	-38.7428	1
NH_12	-53.3811	nd	-40.5981	1
NH_17	-40.5589	-43.6633	-46.5146	1
S_1	-36.9707	-58.1268	-30.5661	1
S_9	-44.7117	-56.9281	-43.222	1
S_16	-33.3572	-57.5656	-33.4615	1
NH_1	-33.4412	-52.4794	-34.4908	0
NH_2	-34.5862	nd	-37.0525	0
NH_3	-37.8038	-53.6372	-37.1858	0

NH_5	-35.2228	-54.1914	-38.3205	0
NH_7	-41.5533	-22.9162	-37.4001	0
NH_8	-33.7839	-53.1635	-35.1557	0
NH_14	-43.8521	nd	-36.3722	0
NH_15	-30.7174	-52.1275	-41.3081	0
S_2	-32.5077	-56.2285	-30.3053	0
S_3	-37.6851	-43.3897	-35.9425	0
S_4	-30.8318	-27.3182	-33.1053	0
S_5	-38.9497	nd	-36.1872	0
S_7	-42.9461	-56.2321	-32.92	0
S_8	-26.8635	-50.047	-28.6409	0
S_10	-31.983	-49.8078	-34.0877	0
S_11	-41.6823	nd	-31.5389	0
S_12	-38.6814	-2.01496	-37.4977	0
S_14	-37.6577	-1.67183	-34.8718	0
S_15	-33.41	-47.7369	-39.4122	0
S_17	-29.3918	-46.6355	-33.2257	0

[a] Molecular docking assays was performed on the Discovery Studio 3.5 software suite, in which the CDOCKER algorithm is the main docking protocol. [b] The Consensus Score protocol in Discovery Studio uses the entire set of poses across all molecules to rank order your output; simply saying, if the value of docking energy for each target protein is ranked into the top 20% of the entire molecules, the value of Consensus score will be added one. [c]nd: no data.

Table 5 The docking energy of MGCD0103, SAHA, compound **1** and **2** on the homology modeling of HDAC6

Compounds	CDOCKER_ENERGY (kcal/mol) [a]	
	Model 1 protein	Model 2 protein
SAHA	-38.45	-46.34
1	-32.75	-36.01
2	-32.55	-30.87
MGCD0103	-25.69	-24.12

[a] Molecular docking assays was performed on the Discovery Studio 3.5 software suite, in which the CDOCKER algorithm is the main docking protocol.