Supporting Information for

Original article

Evodiamine-inspired dual inhibitors of histone deacetylase 1 (HDAC1) and topoisomerase 2 (TOP2) with potent antitumor activity

Yahui Huang[a], Shuqiang Chen[a], Shanchao Wu[a], Guoqiang Dong[a,*], Chunquan Sheng[a,b,*]

[a]*Department of Medicinal Chemistry, School of Pharmacy, Second Military Medical University, Shanghai 200433, China*

[b]*School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou 450001, China*

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[*]Corresponding authors. Tel./fax: +86 21 81871239 (Chunquan Sheng), +86 21 81871242 (Guoqiang Dong).

E-mail addresses: shengcq@smmu.edu.cn (Chunquan Sheng), dgq-81@163.com (Guoqiang Dong).

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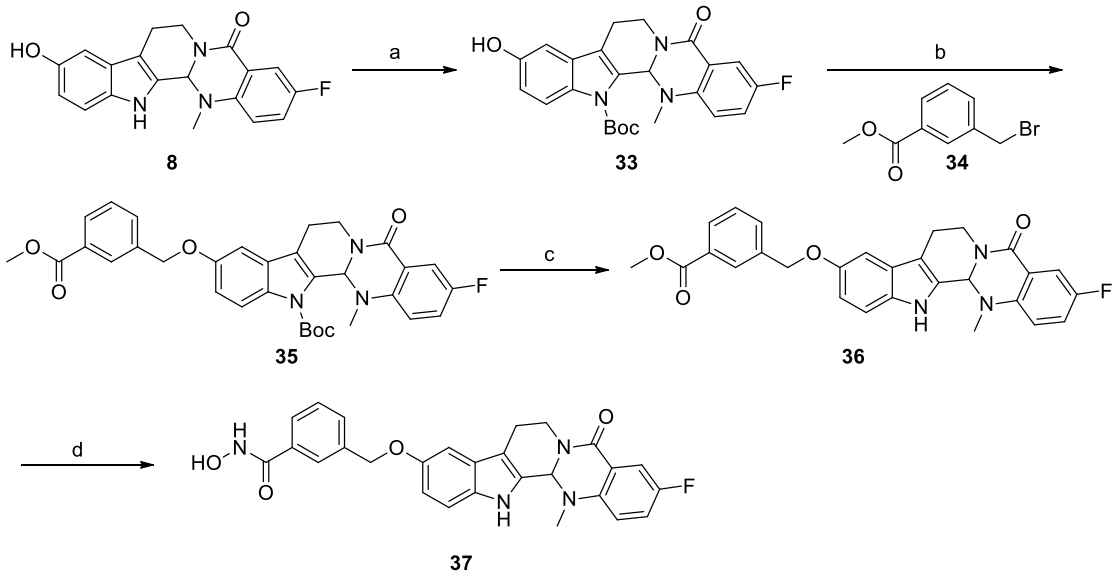
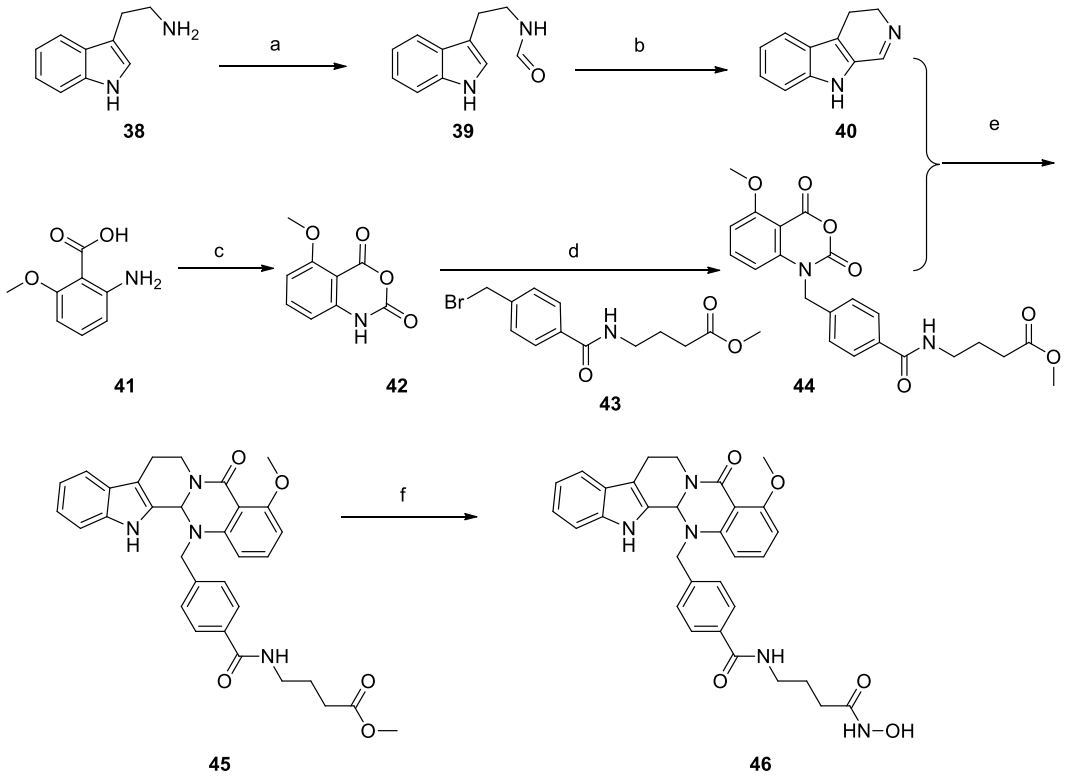


Scheme S1 Synthesis of compound **37**. Reagents and conditions: (a) $(Boc)_2O$, DMAP, NaOH, THF, K_2CO_3, reflux, r.t., 4 h, 69%; (b) K_2CO_3, DMF, 12 h, 66%–88%; (c) TFA, DCM, r.t., 6 h, 64%–69%; (d) NH_2OH, CH_3OH, 45 °C, 1 h, 52%–81%.



Scheme S2 Synthesis of compound **46**. Reagents and conditions: (a) ethyl formate, reflux, 12 h, 90%; (b) $POCl_3$, DCM, 0 °C, 6 h, 68%; (c) triphosgene, THF, reflux, 12 h, 75%; (d) K_2CO_3, DMF, 2.5 h, 59%–68%; (e) DCM, r.t., 12 h, 39%–56%; (f) $NH_2OH{\cdot}HCl$, KOH, MeOH, 1 h, 39%–48%.

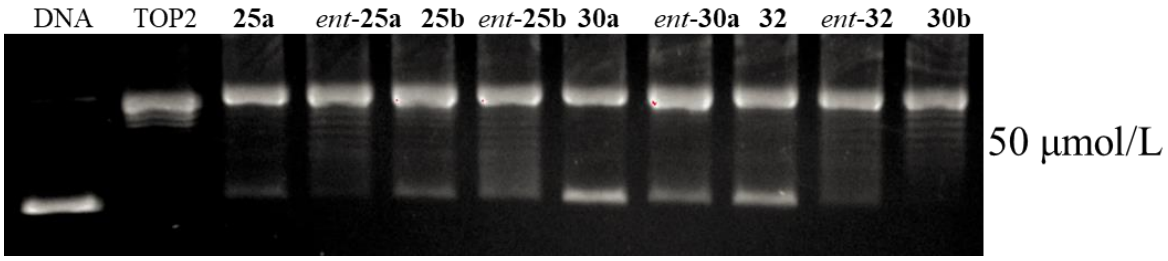


Figure S1 TOP2 inhibitory potency of the target compounds at 50 μmol/L. Lane 1, supercoiled plasmid DNA; lane 2, DNA + TOP2; lanes 3–11, DNA + TOP2 + compound.

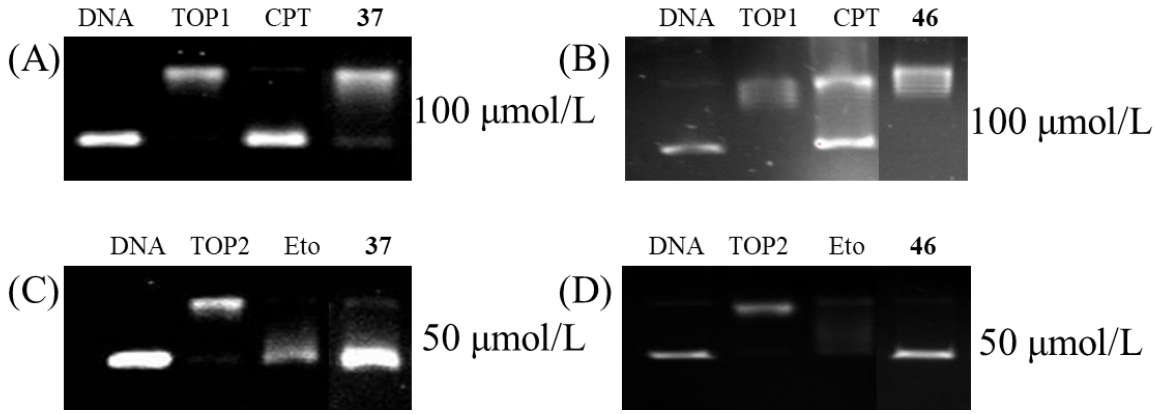


Figure S2 TOP inhibitory potency of the compounds **37** and **46**. TOP1 inhibitory activity of compounds **37** (A) and **46** (B) at 100 μmol/L; TOP2 inhibitory activity of compounds **37** (C) and **46** (D) at 50 μmol/L.

Table S1 *In vitro* antiproliferative activity of compounds **37** and **46**.

Compd.	IC_{50} (μmol/L)			
	HDAC1	HCT116	MCF-7	A549
37	1.9	> 20	> 20	> 20
46	> 1	> 10	> 10	> 10

Table S2 *In vitro* antiproliferative activity of compounds **25a** and **30a.**

Compd.	IC_{50} (μmol/L)			
	HEL	HL60	K562	HUVEC
25a	0.34 ± 0.04	0.63 ± 0.15	1.0 ± 1.2	7.4 ± 1.1
30a	0.66 ± 0.05	0.56 ± 0.06	1.9 ± 0.65	48 ± 3.2
1	0.21 ± 0.03	0.49 ± 0.04	0.58 ± 0.20	5.9 ± 2.5
6	1.61 ± 0.16	1.30 ± 0.05	1.34 ± 0.42	3.7 ± 2.2
1 + 6 (1:1)	0.19 ± 0.06	0.61 ± 0.11	0.47 ± 0.08	1.0 ± 0.1

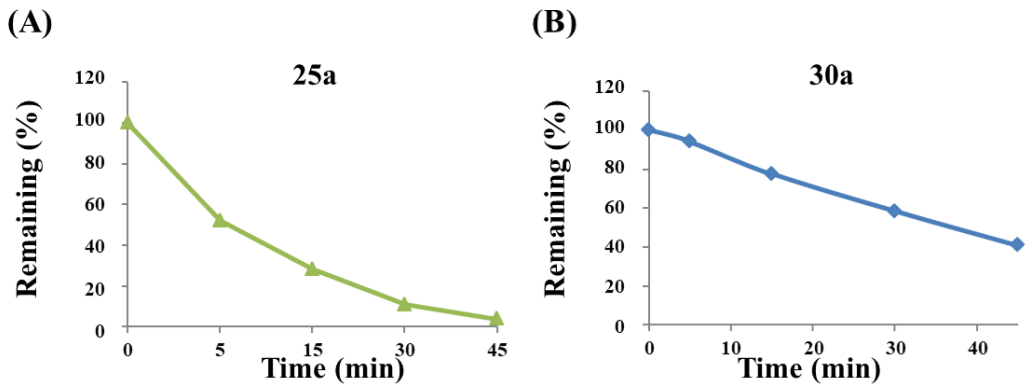


Figure S3 The mice microsomal stability of compounds **25a** and **30a**.

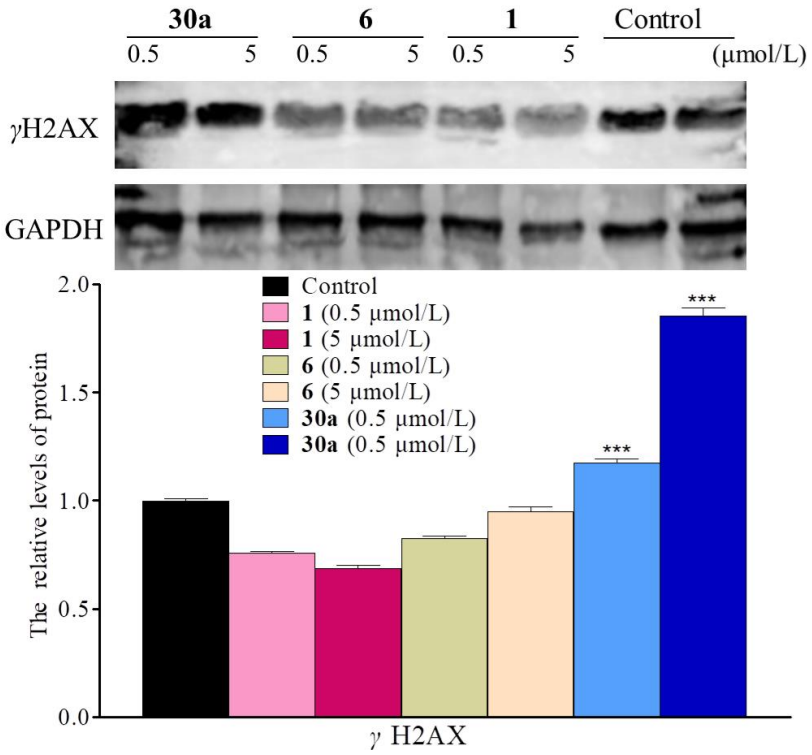


Figure S4 Western blot probing for γH2AX in HCT116 cells after 24 h treatment with compounds **1**, **6** and **30a**. Data were expressed as mean ± SD (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs.* the control group, determined with student's *t* test.

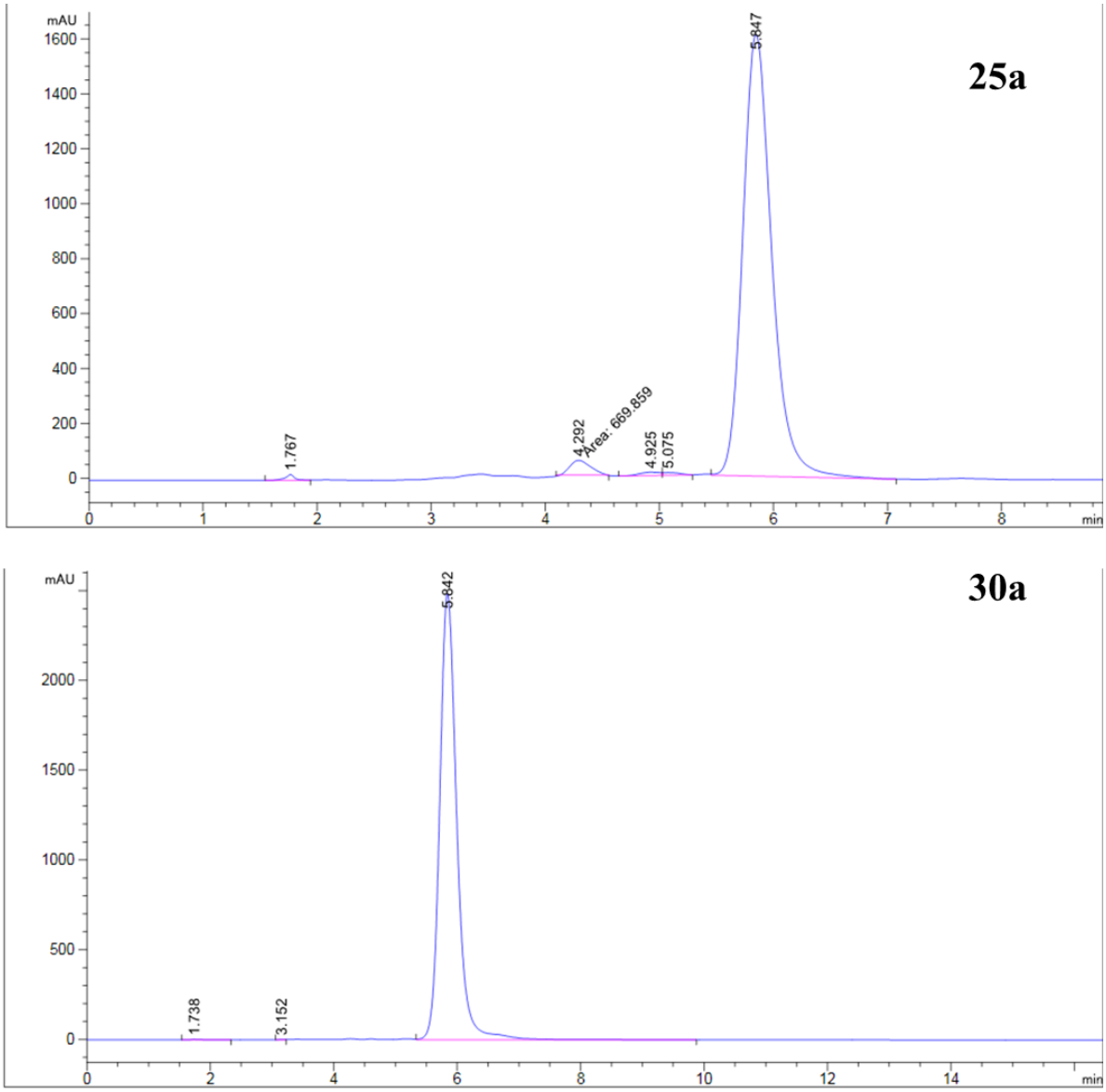


Figure S5 HPLC spectra of compounds **25a** and **30a**.

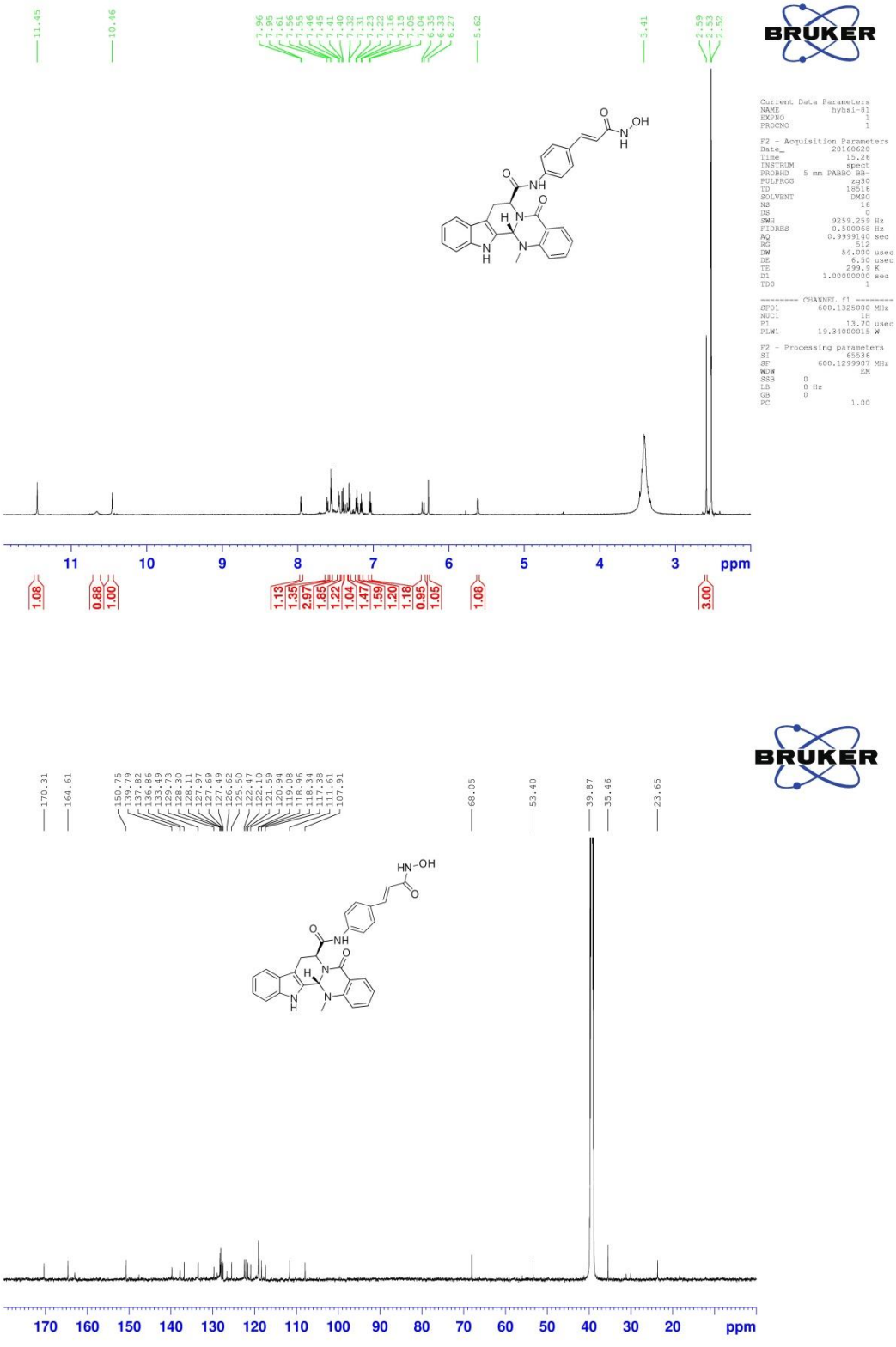


Figure S6 ^{1}H NMR and ^{13}C NMR spectra of compound **25a**.

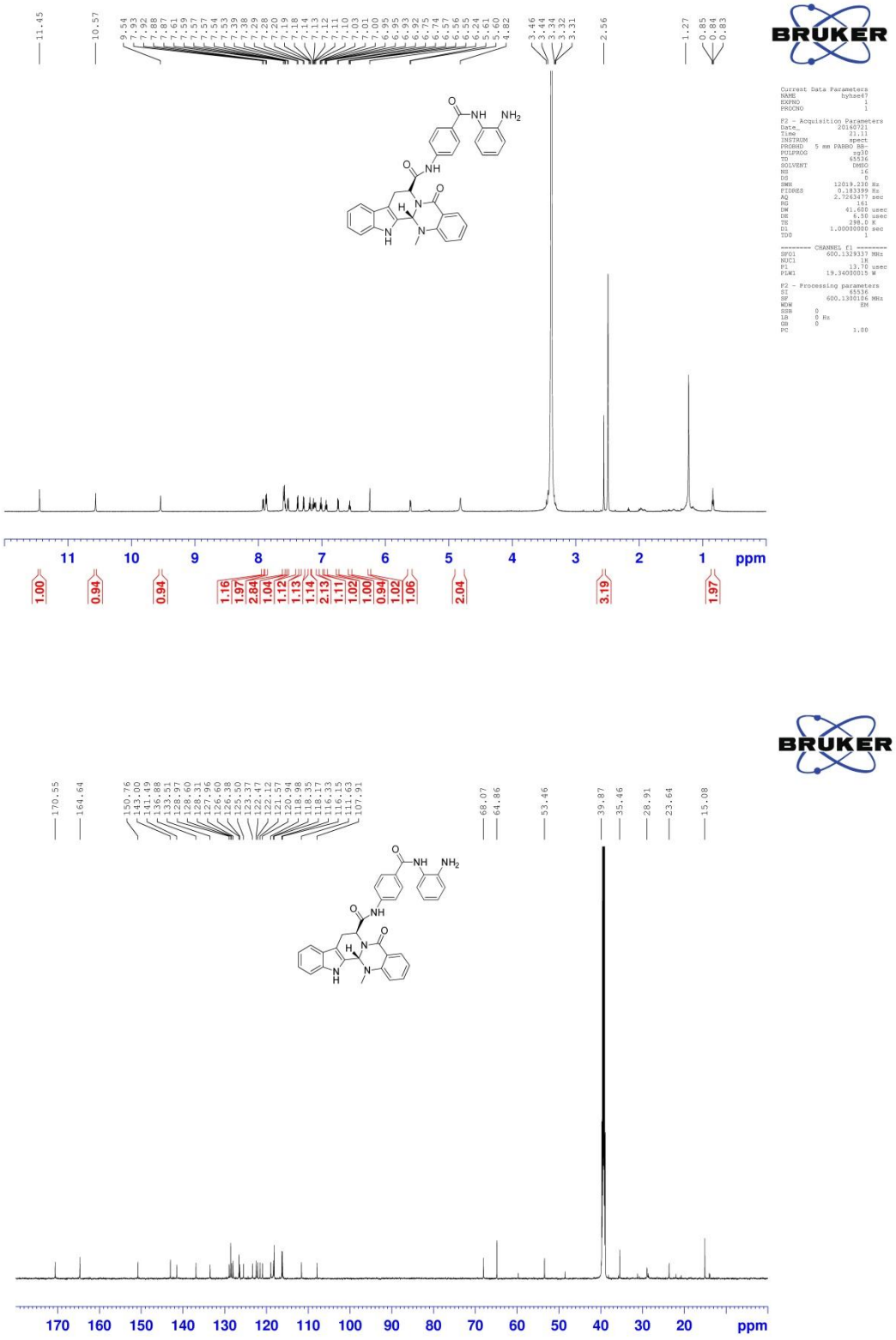


Figure S7 ^{1}H NMR and ^{13}C NMR spectra of compound **30a**.

Structural characterization of compounds 37 and 46

Compounds **37** and **46** were obtained from a similar protocol described for compound **20a**.

*3-(((3-Fluoro-14-methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2′,3′:3,4]pyrido[2,1-b]quinazolin-10-yl)oxy)methyl)-N-hydroxybenzamide (**37**).* Light yellow solid (Yield: 74%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.05 (s, 1H), 7.88 (s, 1H), 7.70 (d, J = 7.6 Hz, 1H), 7.57 (d, J = 6.0 Hz, 1H), 7.53 (dd, J = 2.8, 8.5 Hz, 1H), 7.43 (t, J = 7.1 Hz, 1H), 7.40–7.35 (m, 1H), 7.27 (d, J = 8.9 Hz, 1H), 7.18 (q, J = 4.5 Hz, 1H), 7.12 (d, J = 2.5 Hz, 1H), 6.87 (dd, J = 2.5, 8.6 Hz, 1H), 6.06 (s, 1H), 5.13 (s, 2H), 4.63–4.60 (m, 1H), 3.22–3.17 (m, 1H), 2.83–2.79 (m, 2H), 2.68 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6, TMS) δ 163.51, 158.35, 156.77, 152.80, 146.46, 138.41, 133.93, 132.41, 130.84, 130.26, 128.77, 126.59, 126.46, 126.33, 122.46, 122.41, 122.06, 122.01, 121.09, 120.94, 113.93, 113.77, 113.12, 112.83, 111.89, 102.37, 102.26, 70.07, 69.76, 40.85, 37.06, 20.11. MS (ESI positive): m/z $[M+H]^+$: 487.18.

*N-(4-(Hydroxyamino)-4-oxobutyl)-4-((4-methoxy-5-oxo-7,8-dihydroindolo[2′,3′:3,4]pyrido[2,1-b]quinazolin-14(5H,13H,13bH)-yl)methyl)benzamide (**46**).* Off-white solid (Yield: 43%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.40 (s, 1H), 8.53 (s, 1H), 7.68 (d, J = 7.76 Hz, 2H), 7.49 (d, J = 7.88 Hz, 1H), 7.37 (d, J = 8.18 Hz, 1H), 7.23 (t, J = 8.19 Hz, 1H), 7.17 (d, J = 7.74 Hz, 2H), 7.11 (t, J = 7.51 Hz, 1H), 7.01 (t, J = 7.51 Hz, 1H), 6.59 (d, J = 8.65 Hz, 1H), 6.46 (d, J = 8.42 Hz, 1H), 6.03 (s, 1H), 4.48 (d, J = 15.93 Hz, 1H), 4.36–4.38 (m, 1H), 4.18 (d, J = 15.93 Hz, 1H), 3.74 (s, 3H), 3.39–3.41 (m, 3H), 3.18–3.24 (m, 2H), 2.78–2.82 (m, 1H), 2.69–2.73 (m, 1H). ^{13}C NMR (DMSO-d_6, 600 MHz) δ 167.47, 166.24, 162.74, 161.00, 150.40, 142.32, 137.09, 133.53, 133.30, 129.56, 127.90, 127.50, 126.21, 122.43, 119.33, 118.83, 112.49, 112.10, 111.64, 111.13, 105.60, 68.62, 56.09, 52.12, 36.51, 32.85, 25.41, 19.97.

Molecular docking

The crystal structure of HDAC1 was obtained from protein database bank (PDB ID: 4BKX)[1] and prepared for docking using the protein preparation tool in Discovery Studio 3.0. During this process, the ligands and waters were removed and hydrogens were added to the structure. Staged minimization was performed with default setting. The docking studies were carried out using GOLD 5.0. Binding site was defined as whole residues within a 10 Å radius subset encompassing the ligand. Conformations were generated by genetic algorithm and scored using GoldScore as fitness function. The best conformation was chosen to analyze the ligand–protein interaction. Docking analysis of TOP2α (PDB ID: 5GWK)[2] with compound

30a was performed using a similar procedure.

Determination of *in vitro* metabolic stability in female ICR mice liver microsomes

Liver microsomal incubations were conducted in triplicate. Incubation mixtures (0.5 mg/mL microsome protein, pH 7.4 of 100 mmol/L phosphate buffer, 1 μmol/L compounds in acetonitrile) were first shaken for pre-incubation at 37 °C. The reaction was initiated by adding NAPDH to obtain concentration of 1 mmol/L NAPDH in the mixture. For metabolic stability studies, aliquots of the incubation sample mixture were collected at 0, 5, 15, 30 and 45 min. After collection of samples, the reaction was terminated with acetonitrile containing the internal standard (200 ng/mL tolbutamide). The mixture was then centrifuged to remove the protein and the supernatant was subsequently applied to LC–MS/MS analysis. Positive control samples were prepared as described above, except the test compound was replaced with the known P450 substrate (1 μmol/L midazolam).

Pharmacokinetic studies

Male SD rats (6–8 weeks old, body weight 180–220 g) were obtained from Shanghai Sippr-BK laboratory animal Co., Ltd., China. Three animals were given *p.o*. (i.p.) at a dose of 20 mg/kg (20 mg/kg) of compound **30a**. The dose solution was prepared in 5% DMSO + 10% solutol + 85% saline. After administration of compound **30a** (*n* = 3 per time point), 0.25 mL of blood was collected *via* Jugular vein puncture and put on the ice (heparin prevents clotting). Pharmacokinetic time points were 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post-dose. Blood samples were transferred to microcentrifuge tubes and centrifuged (8000 rpm, Eppendorf, Hamburg, SN, Germany, 6 min at 2–8 °C). The separated plasma was stored at approximately –80 °C until thawed for LC–MS/MS. Data acquisition: Analyst version 1.5.1. Pharmacokinetic data analysis was performed on mean plasma concentration-time data and pharmacokinetic parameters were calculated using WinNonlin version 5.2.

References

1. Millard CJ, Watson PJ, Celardo I, Gordiyenko Y, Cowley SM, Robinson CV, et al. Class I HDACs share a common mechanism of regulation by inositol phosphates. *Mol Cell* 2013;**51**:57-67.
2. Wang Y, Chen S, Wu C, Liao Y, Lin T, Liu K, et al. Producing irreversible topoisomerase II-mediated DNA breaks by site-specific Pt(II)-methionine coordination chemistry. *Nucleic Acids Res* 2017;**45**:10920.