Supporting Information

Small Molecules Simultaneously Inhibiting p53-Murine Double Minute 2 (MDM2) Interaction and Histone Deacetylases (HDACs): Discovery of Novel Multi-targeting Antitumor Agents

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Table S1. Water Solubility of the Selected Compounds (μM).

Compound	**11c**	**11d**	**12c**	**12d**	**13**	**14c**	**14d**	**15a**	**16b**	**1**	**2**	**3**
Solubility	10	10	26	20	73	90	93	91	18	>100	94	83

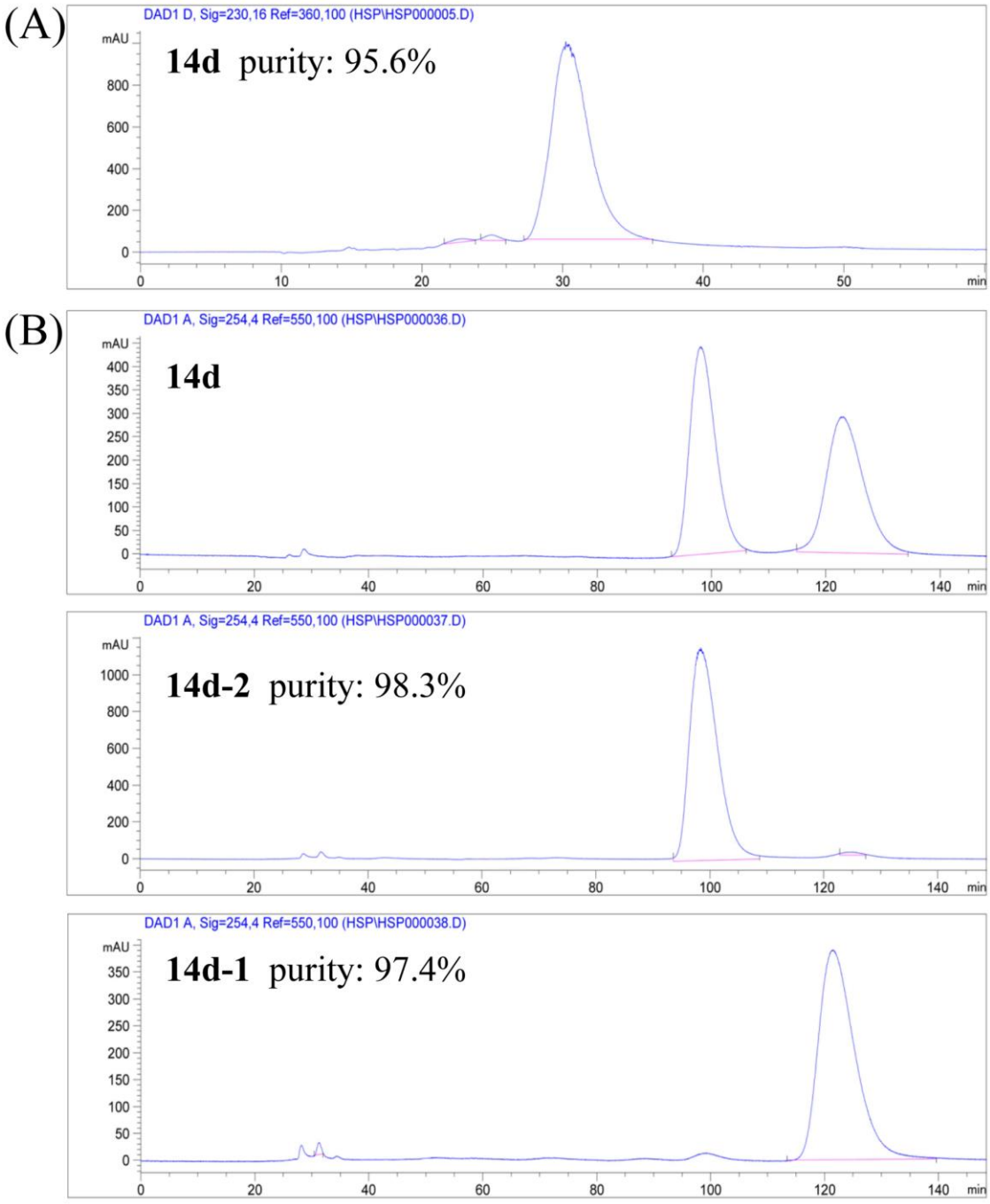
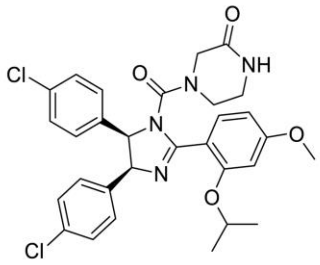


Figure S1. The chiral separation of racemic **14d**. (A) The purity result of compound **14d**. (B) The enantiomers were separated by high performance liquid chromatography

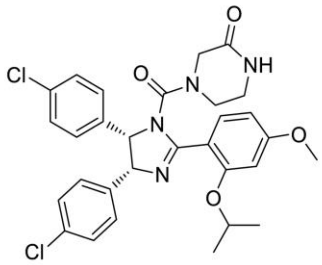
(HPLC) using hexane/2-propanol (hexane: 2-propanol = 85: 15) as the mobile phase with a flow rate of 0.8 mL/min on a Daicel Chiralpak AD–H column.

Compound	Specific rotation
Nutlin-3a	$[\alpha]_D^{25} = -172$ (c = 0.20 in CH_2Cl_2)
Nutlin-3b	$[\alpha]_D^{25} = 164$ (c = 0.20 in CH_2Cl_2)

Specific rotation	Compound
$[\alpha]_D^{25} = -328$ (c = 0.10 in CH_2Cl_2)	**14d-2**
$[\alpha]_D^{25} = 354$ (c = 0.10 in CH_2Cl_2)	**14d-1**



Nutlin-3a **(-)-Nutlin-3**



Nutlin-3b **(+)-Nutlin-3**

Figure S2. The configuration of enantiomers of **14d** could be identified by the comparison of optical rotation to that of **14d-1** and **14d-2**.

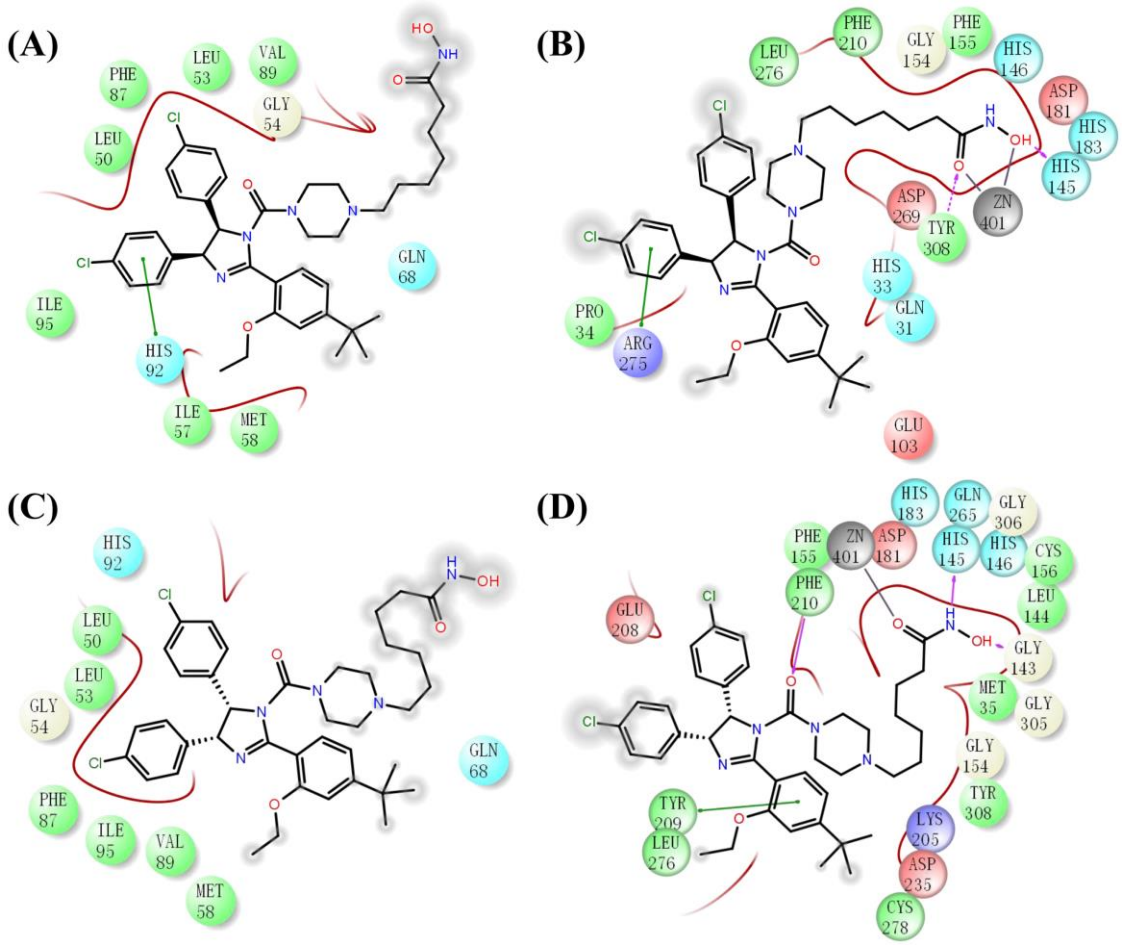
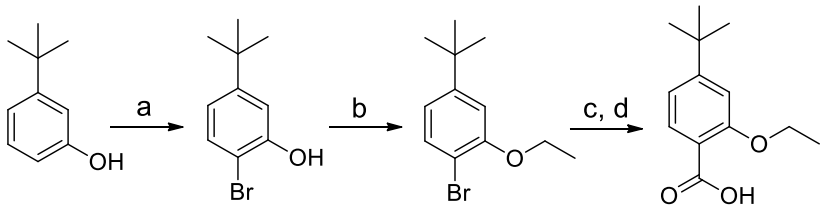


Figure S3. Schematic depiction of the interactions between compound 14d

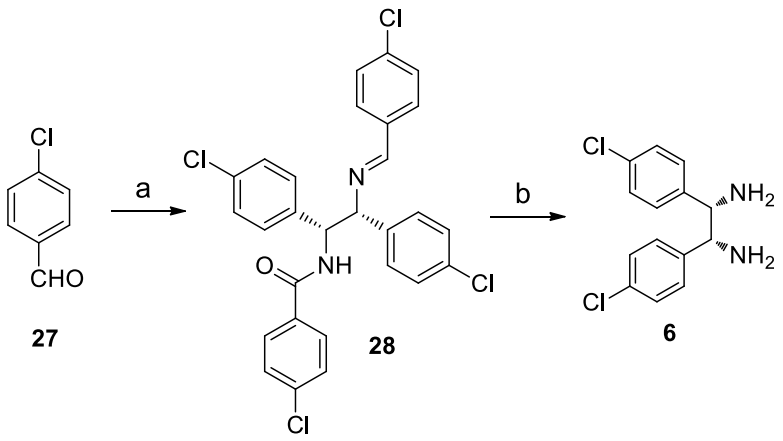
enantiomers with MDM2 and HDAC1. (A) The interactions between compound 14d-2 and MDM2; (B) The interactions between compound 14d-2 and HDAC1; (C) The interactions between compound 14d-1 and MDM2; (D) The interactions between compound 14d-2 and HDAC1.

Scheme S1. Synthesis of Intermediate 5[α]



[α]**Scheme S1.** Reagents and Conditions: (a) Br_2 (1.0 eq), CH_2Cl_2, rt, 0.5 h, yield 95%; (b) CH_3CH_2I (1.0 eq), KTB, THF, 70 °C, 3 h, 95%; (c) Mg (1.1 eq), I_2, CO_2, THF, 78 °C, 5 h; (d) HCl (3.0 eq), 2 h.

Scheme S2. Synthesis of Intermediate 6[α]



[α]**Scheme S2.** Reagents and Conditions: (a) CH_3COONH_4 (7.0 eq), 200 °C, 5 h, yield 86%; (b) con.H_2SO_4, 160 °C, 3 h, 34%;

Chemical Synthesis and Structural Characterization of Compounds 5-6 and Intermediates.

General. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE300 and AVANCE600 spectrometer (Bruker Company, Germany), using TMS as an internal standard and DMSO-d_6 as solvents. Chemical shift are given in ppm (δ). The mass spectra were recorded on an Esquire 3000 LC-MS mass spectrometer. TLC analysis was carried out on silica gel plates GF254 (Qingdao Haiyang Chemical, China). Silica gel column chromatography was performed with Silica gel 60 G (Qingdao Haiyang Chemical, China). Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Chemical names were created using ChemDraw Ultra 10.0 software.

2-Bromo-5-*tert*-butylphenol (25). To a 500 mL round bottom three necked flask, dichloromethane (100 mL) was added followed by 3-tert-butylphenol **24** (9.0 g, 60.0 mmol), then a solution of bromine (3.13 mL, 61.0 mmol) in dichloromethane (50 mL) was added over 30 min at 0 °C. After the addition was complete, TLC analysis indicated a complete reaction. The reaction was then quenched with 50 mL of 1% $NaHSO_3$ aqueous solution while stirring. After 10 min, the organic layer became clear and was separated from the aqueous layer, the organic layer was washed once with water (200 mL), dried over anhydrous Na_2SO_4, and concentrated at 30 °C to afford 2-bromo-5-tert-butylphenol **25** (13.5 g, 98% yield) as a colorless oil, which was used directly in the next step.

1-Bromo-2-ethoxy-4-*tert*-butylbenzene (26). To a solution of 3-tert-butylphenol **25**

(10.3 g, 44.9 mmol) in THF (50 mL) was added potassium tert-butoxide (5.04 g, 45.0 mmol) followed by iodoethane (7.3 g, 46.0 mmol). The mixture was heated to reflux for 3 h. TLC analysis indicated complete reaction. The resulting solution was cooled to room temperature and solvent was removed by rotary evaporation at 40 °C. The residues was dissolvent in EtOAc (150 mL) and washed with water (50 mL ×2) and saturated NaCl solution (50 mL), then the organic phase was concentrated at 30 °C to afford 1-bromo-2-ethoxy-4-tert-butylbenzene **26** (11.2 g, 97% yield) as a colorless oil, which was directly used in the next step. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.29 (s, 9H), 1.37 (t, J = 7.8 Hz, 3H), 4.14 (dd, J = 7.8, 3.06 Hz, 2H), 6.90 (d, J = 7.98, 1H), 7.06 (s, 1H), 7.46 (d, J = 8.02 Hz, 1H).

4-*tert*-Butyl-2-ethoxy-benzoic Acid (5). A dry, 500 mL, three necked, round bottomed flask, equipped with a magnetic stirrer, thermometer, and a reflux condenser, was charged under nitrogen with magnesium (2.16 g, 88.8 mmol) and THF (160 mL). Compound **26** (2.0 g, 7.8 mmol) was added, followed by a few crystals of iodine. Upon heating to 40 °C, the reaction initiated, and then additional **26** (18.0 g, 70.0 mmol) was added dropwise to the solution with a gentle stirring. The resulting mixture was heated to reflux for 4 h. After the reaction was complete, the reaction mixture was cooled to -20 °C and the carbon dioxide was bubbled into the reaction mixture until the absorption of gas was complete. TLC analysis indicated complete reaction. The reaction mixture was allowed to warm to room temperature and was stirred overnight. Then 1 M HCl (160 mL, 160 mmol) was added, and the mixture was extracted with EtOAc (100 mL × 3). The combined organic layer was washed with brine, dried over

anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give crude product as an orange solid. This crude material was purified by silica gel column to give compound **5** (1.48 g, 85.6% yield) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.31 (s, 9H), 1.33 (t, J = 7.0 Hz, 3H), 4.12 (dd, J = 7.0, 3.8 Hz, 2H), 7.02 (dd, J = 7.92, 1.53 Hz, 1H), 7.58 (d, J = 7.92 Hz, 1H), 12.34 (s, 1H).

(1*R*,2*S*)-1,2-bis(4-chlorophenyl)ethane-1,2-diamine (6). A mixture of **27** (14.0 g, 100 mmol) and CH_3COONH_4 (56 g, 714 mmol) was heated to 200 °C in 500 mL round bottom flask, then the reaction mixture was stirred at this temperature for 5 h. After reaction was complete, the cooled white residue was washed with water. The suspension was filtered by suction and the residue cake was washed with 10% n-hexane in ethyl acetate, then filtered again and dried to afford **28** (43 g, 80%) as a white solid. Compound **28** (20 g, 37 mmol) was added slowly to the 70% H_2SO_4 (20 mL) under stirring in the 100 mL round bottom flask, the reaction mixture was then heated to 160 °C and the temperature was maintained for 4 h. then, the reaction mixture was poured into a large quantity of ice/water and the resulting slurry was extracted with EtOAc (20 mL × 3) to remove the impurities and separated, the aqueous layer was adjusted pH = 11 under stirring by NaOH solution (2 N). The aqueous phase was further extracted with EtOAc (50 mL × 3), and the combined organic extract was dried and evaporated to give **6** (3.8 g, 36.6%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.28 (d, J = 8.3 Hz, 2H), 7.17 (d, J = 8.3 Hz, 2H), 3.93 (s, 2H), 1.74 (s, 4H).

Methyl-6-(2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro

-1*H*-imidazole-1-carboxamido)hexanoate (10b). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.10-1.25 (m, 7H), 1.33 (s, 9H), 1.33-1.40 (m, 2H), 2.22 (m, 2H), 2.65-2.78 (m, 2H), 3.58 (s, 3H), 4.05-4.09 (m, 1H), 4.09-4.15 (m, 1H), 5.70 (s, 1H), 6.01 (s, 1H), 6.23 (s, 1H), 7.02-7.11 (m, 4H), 7.13-7.26 (m, 6H), 7.43 (d, J = 6.28 Hz, 1H).

Methyl-7-(2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro -1*H*-imidazole-1-carboxamido)heptanoate (10c). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.88-0.98 (m, 4H), 1.03-1.28 (m, 7H), 1.34 (s, 9H), 1.45-1.52 (m, 2H), 2.16 (t, J = 7.27 Hz, 2H), 3.58 (s, 3H), 4.06-4.10 (m, 1H), 4.22-4.31 (m, 1H), 5.78 (s, 1H), 5.98 (s, 1H), 6.02 (s, 1H), 7.01-7.32 (m, 10H), 7.42 (d, J = 7.77 Hz, 1H).

Methyl-8-(2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro -1*H*-imidazole-1-carboxamido)octanoate (10d). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.90-1.33 (m, 9H), 1.34 (s, 9H), 1.42-1.54 (m, 4H), 2.13 (m, 1H), 2.32 (t, J = 7.70 Hz, 2H), 3.57 (s, 3H), 4.08-4.12 (m, 1H), 4.19-4.27 (m, 1H), 5.68 (s, 1H), 5.96 (s, 1H), 6.01 (s, 1H), 7.01-7.16 (m, 6H), 7.16-7.34 (m, 4H), 7.41 (d, J = 6.68 Hz, 1H).

Methyl-4-((2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydr o-1*H*-imidazole-1-carboxamido)methyl)benzoate (18). ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.28 (t, J = 6.69 Hz, 3H), 1.33 (s, 9H), 3.67 (s, 3H), 3.92-4.08 (m, 2H), 4.16 (dd, J = 9.68 Hz, 6.56Hz, 2H), 5.67 (d, J = 10.08 Hz, 1H), 5.74 (d, J = 10.08 Hz, 1H), 6.81 (s, 1H), 6.91 (d, J = 7.53 Hz, 2H), 6.94-7.08(m, 4H), 7.10-7.19 (m, 6H), 7.36 (d, J = 8.21 Hz, 1H), 7.49 (d, J = 7.72 Hz, 2H).

Ethyl2-(4-(2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro -1*H*-imidazole-1-carbonyl)piperazin-1-yl)acetate (20a). ^{1}H NMR (DMSO-d_6, 300

MHz) δ: 1.23 (t, J = 8.32 Hz, 3H), 1.31 (t, J = 6.74 Hz, 3H), 1.35 (s, 9H), 1.91 (s, 4H), 2.95 (s, 1H), 3.00 (s, 4H), 3.19 (s, 1H), 3.95-4.14 (m, 4H), 5.53 (d, J = 9.78 Hz, 1H), 5.67 (d, J = 9.78 Hz, 1H), 6.96 (d, J = 7.93 Hz, 2H), 7.02 (d, J = 8.19 Hz, 2H), 7.05-7.14 (m, 4H), 7.16 (d, J = 8.19 Hz, 2H), 7.51 (d, J = 7.93 Hz, 1H).

Methyl-5-(4-(2-(4-(*Tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)pentanoate (20b). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.10-1.25 (m, 4H), 1.31 (t, J = 6.68 Hz, 3H), 1.33 (s, 9H), 1.62-1.89 (m, 4H), 1.92 (t, J = 8.12 Hz, 2H), 2.11 (t, J = 7.74Hz, 2H), 2.92-3.12 (m, 4H), 3.61(s, 3H), 4.01-4.16 (m, 2H), 5.52 (d, J = 9.98 Hz, 1H), 5.70 (d, J = 9.98 Hz, 1H), 6.95 (t, J = 7.92 Hz, 2H), 7.01 (t, J = 8.22 Hz, 2H), 7.04-7.13 (m, 4H), 7.15 (t, J = 8.22 Hz, 2H), 7.57 (d, J = 7.72 Hz, 1H).

Methyl-6-(4-(2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)hexanoate (20c). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.12-1.26 (m, 4H), 1.30 (t, J = 6.98 Hz, 3H), 1.33 (s, 9H), 1.41 (t, J = 6.86 Hz, 2H), 1.50-1.60 (m, 4H), 1.87 (t, J = 7.72 Hz, 2H), 1.97 (t, J = 7.16 Hz, 2H), 2.86 (s, 4H), 3.58(s, 3H), 4.01-4.20 (m, 2H), 5.52 (d, J = 9.98 Hz, 1H), 5.71 (d, J = 9.92 Hz, 1H), 6.89-7.04 (m, 6H), 7.05-7.20 (m, 4H), 7.52 (d, J = 7.55 Hz, 1H).

Ethyl-7-(4-(2-(4-(*Tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)heptanoate (20d). ^{1}H NMR (DMSO-d6, 300 MHz) δ: 1.10-1.18 (m, 6H), 1.21(t, J = 4.12 Hz, 3H), 1.28 (t, J = 5.69 Hz, 3H), 1.31 (s, 9H), 1.38-1.46(m, 2H), 1.58-1.80(m, 4H), 1.97 (t, J = 6.92 Hz, 2H), 2.20 (t, J = 7.26 Hz, 2H), 2.96 (s, 4H), 3.95-4.10 (m, 4H), 5.49 (d, J = 10.12 Hz, 1H), 5.64 (d, J =

10.12 Hz, 1H), 6.93 (d, *J* = 8.04 Hz, 2H), 6.98 (d, *J* = 8.25 Hz, 2H), 7.06 (t, *J* = 7.78 Hz, 4H), 7.13 (d, *J* = 8.36 Hz, 2H), 7.47 (d, *J* = 7.93 Hz, 1H).

Methyl-8-(4-(2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)octanoate (20e). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.18-1.35 (m, 6H), 1.41 (s, 9H), 1.48 (t, *J* = 6.53 Hz, 3H), 1.48-1.64(m, 2H), 1.73-1.93 (m, 4H), 2.09 (t, *J* = 6.98 Hz, 2H), 2.28 (t, *J* = 7.51 Hz, 2H), 2.79 (s, 2H), 3.11 (m, 4H), 3.62(s, 3H), 4.14-4.22 (m, 2H), 5.57 (d, *J* = 9.97 Hz, 1H), 5.75 (d, *J* = 9.97 Hz, 1H), 7.04-7.12 (m, 4H), 7.12-7.20 (m, 6H), 7.59 (d, *J* = 7.86 Hz, 1H).

(*E*)-Methyl-3-(4-(1-((4S,5R)-2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperidine-4-carboxamido)phenyl)acrylate (21a). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.81-0.96 (m, 2H), 1.31-1.35 (m, 12H), 1.51 (d, *J* = 11.84 Hz, 2H), 2.19-2.28 (m, 1H), 2.41 (t, *J* = 5.92 Hz, 1H) 2.52 (t, *J* = 12.92 Hz, 1H), 3.61 (d, *J* = 13.46 Hz, 1H), 3.69 (d, *J* = 12.92 Hz, 1H), 3.71 (s, 3H), 4.06-4.15 (m, 2H), 5.54 (d, *J* = 9.69 Hz, 1H), 5.68 (d, *J* = 9.69 Hz, 1H), 6.50 (d, *J* = 16.16 Hz, 1H), 6.98 (d, *J* = 7.80 Hz, 2H), 7.04 (t, *J* = 9.76 Hz, 3H), 7.08 (d, *J* = 7.80 Hz, 1H), 7.11 (d, *J* = 7.80 Hz, 2H), 7.16 (d, *J* = 7.56 Hz, 2H), 7.51 (d, *J* = 7.80 Hz, 1H), 7.56 (d, *J* = 5.61 Hz, 1H), 7.58 (d, *J* = 8.53 Hz, 2H), 7.63 (d, *J* = 8.53 Hz, 2H), 9.92 (s, 1H).

Ethyl-2-(4-(2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)pyrimidine-5-carboxylate (21b). ^{1}H NMR (DMSO-d_6, 300 MHz) δ:1.11-1.23 (m, 12H), 1.23-1.35 (m, 3H), 1.28-1.36 (m, 4H), 3.09 (s, 4H), 4.11 (dd, *J* = 14.08 Hz, 7.04 Hz, 2H), 4.26 (dd, *J* = 14.08 Hz, 7.04

Hz, 2H), 5.56 (d, *J* = 9.36 Hz, 1H), 5.70 (d, *J* = 9.36 Hz, 1H), 6.95-7.03 (m, 3H), 7.02-7.13 (m, 5H), 7.18 (d, *J* = 7.83 Hz, 2H), 7.59 (d, *J* = 7.83 Hz, 1H), 8.75 (s, 2H).

Ethyl-2-((1-(2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperidin-4-yl)amino)pyrimidine-5-carboxylate (22a). ^{1}HNMR (DMSO-d_6, 300 MHz) δ: 0.83-0.92 (m, 2H), 1.26-1.38 (m, 15H), 1.56-1.60 (m, 2H), 2.49 (t, *J* = 10.38 Hz, 2H), 2.60 (m, 1H), 3.50-3.64 (m, 3H), 4.11 (dd, *J* = 13.98 Hz, 6.66 Hz, 2H), 4.13 (m, 2H), 5.54 (d, *J* = 9.99 Hz, 1H), 5.68 (d, *J* = 9.99 Hz, 1H), 6.97(d, *J* = 7.90 Hz, 2H), 7.02-7.09 (m, 4H), 7.11 (d, *J* = 7.90 Hz, 2H), 7.16 (d, *J* = 7.90 Hz, 2H), 7.51 (d, *J* = 8.11 Hz, 1H), 8.56 (s, 2H).

Methyl-4-((4-(2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)methyl)benzoate (22b). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.29 (t, *J* = 6.67 Hz, 3H), 1.40 (s, 9H), 1.77 (s, 4H), 3.02 (s, 4H), 3.18 (dd, *J* = 9.05 Hz 2H), 3.83 (m, 3H), 4.07 (dd, *J* = 13.74 Hz, 6.04 Hz, 2H), 5.51 (d, *J* = 9.99 Hz, 1H), 5.67 (d, *J* = 9.99 Hz, 1H), 6.90-7.05 (m, 4H), 7.06-7.18 (m, 6H), 7.30 (d, *J* = 7.91 Hz, 2H), 7.49 (d, *J* = 8.33 Hz, 1H), 7.89 (d, *J* = 7.91 Hz, 2H).

Methyl-1-(2-(4-(*tert*-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperidine-4-carboxylate (23). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.81-0.87 (m, 2H), 1.20-1.42 (m, 5H), 1.32 (s, 9H), 1.51-1.82 (m, 1H), 2.22-2.36 (m, 2H), 3.59 (d, *J* = 10.11 Hz, 1H), 3.60 (s, 3H), 3.67 (d, *J* = 10.11 Hz, 1H), 4.05-4.16 (m, 2H), 5.57 (d, *J* = 7.68 Hz, 1H), 5.81 (d, *J* = 7.68 Hz, 1H), 6.90-7.20 (m, 10H), 7.59 (s, 1H).

Molecular Docking. The crystal structure of MDM2 was obtained from protein database bank (PDB ID: 4IPF[1]) and prepared for docking using the protein preparation tool in Discovery Studio 3.0.[2] 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 analyse the ligand–protein interaction. The image representing the best pose was prepared using PyMol. Docking analysis of HDAC1 (4BKX)[3] with compounds was performed as described above.

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 administered a single 2 mg/kg dose as an IV bolus via the tail vein or a single 20 mg/kg PO dose by gavage. Both the IV and PO dose was administered as a solution in 5% DMSO + 10% Solutol + 85% Saline. After administration of compound **14d** (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 hours post-dose. Blood samples were transferred to microcentrifuge tubes and centrifuged (8000 r/min, 6 min at 2-8 ^{o}C). The separated plasma was stored at approximately -80 ^{o}C until thawed for LC-MS/MS. Data acquisition: Analyst version 1.5.1. Pharmacokinetic data analysis was performed on mean plasma

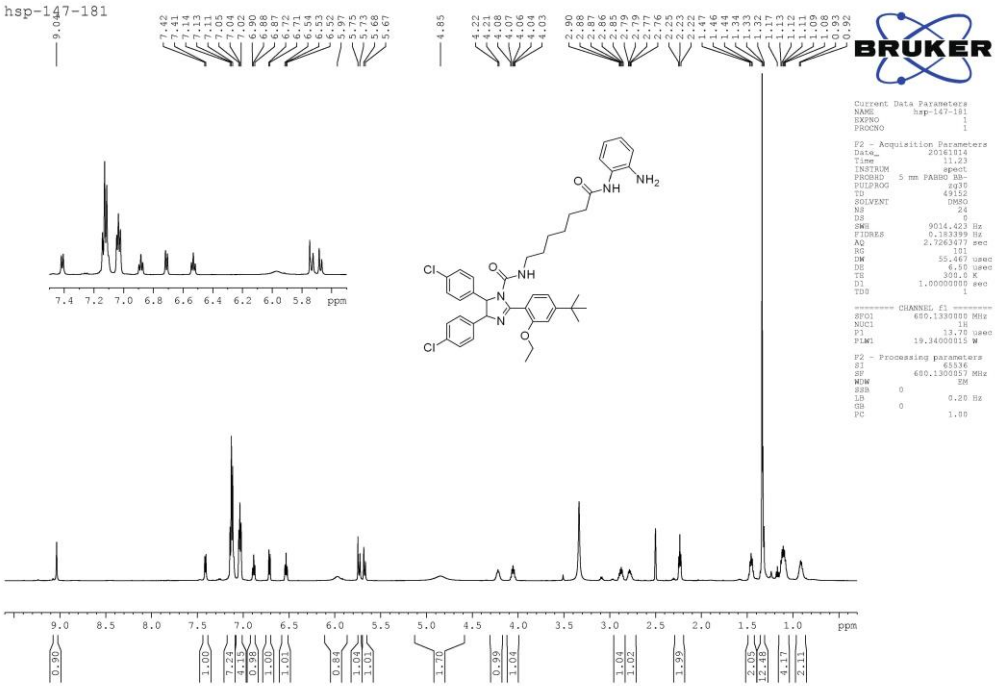
concentration-time data and pharmacokinetic parameters were calculated using WinNonlin version 5.2.

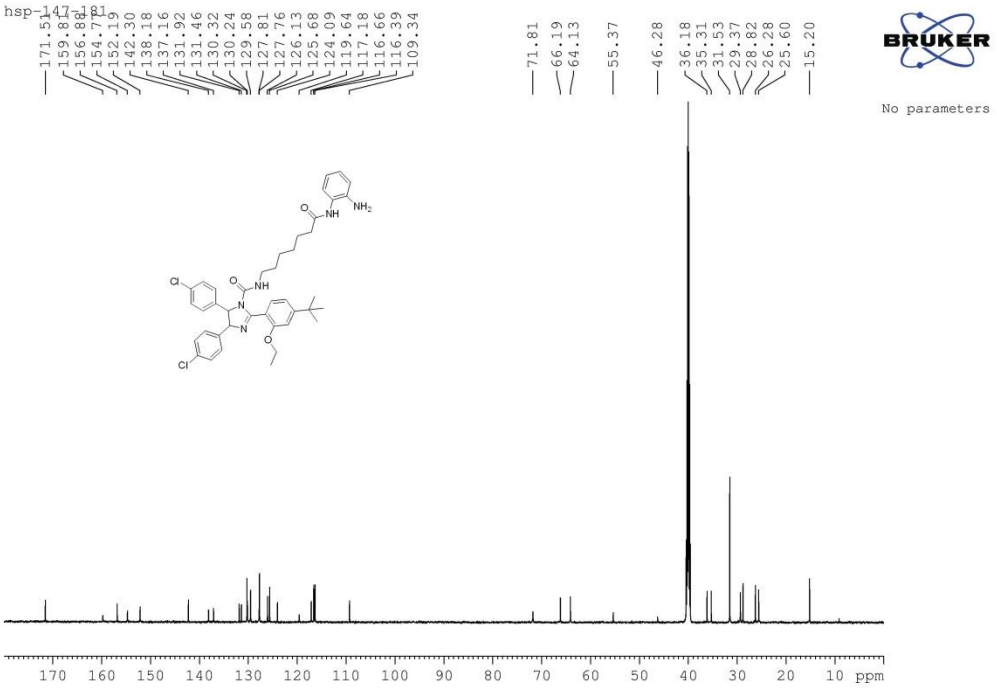
Solubility Assay. A 10 mM stocks solution of drug in DMSO were diluted into universal phosphate buffer to a maximum concentration of 100 μM. The 1% DMSO buffered drug solutions were incubated with gentle shaking over a period of 24 h and then were filtered through a 96 well Millipore Multi-Screen plate. Standards were prepared at 100 μM in DMSO. Compound solubility was determined by the ratio of the UV signal at the maximum absorption peak versus to that of the corresponding 100 μM standard.[4]

Spectral data

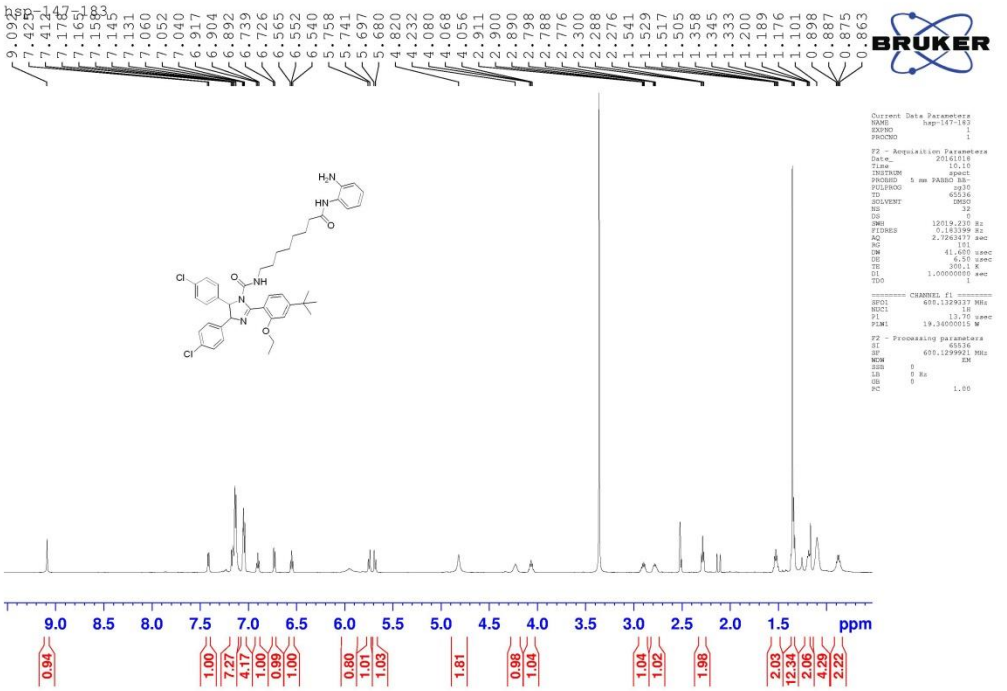
Copies of the NMRs for representative compounds

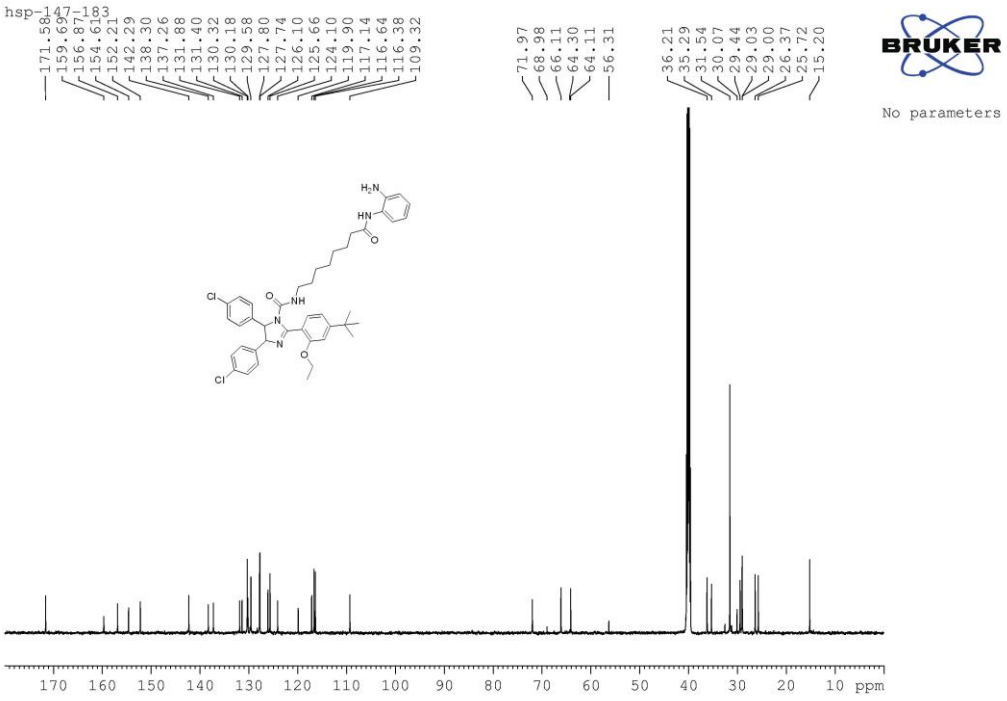
Compound **11c**



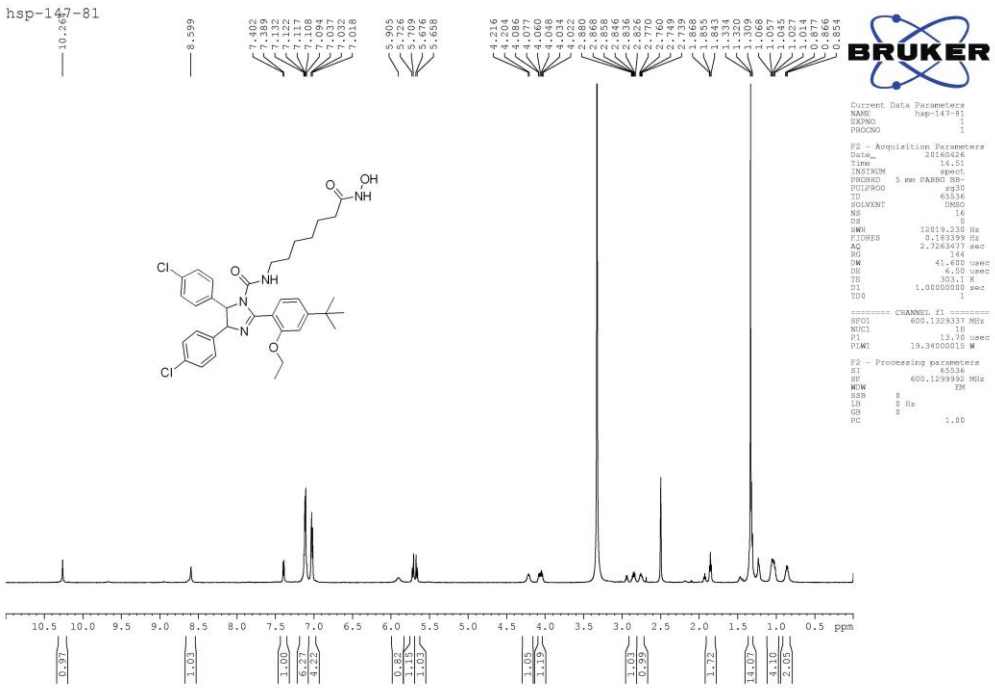


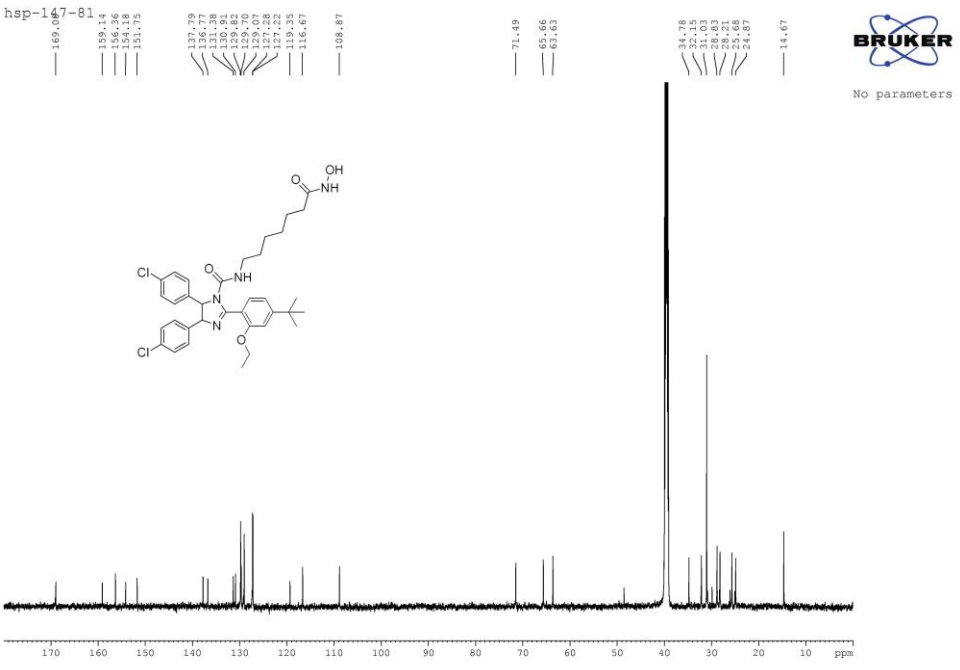
Compound **11d**





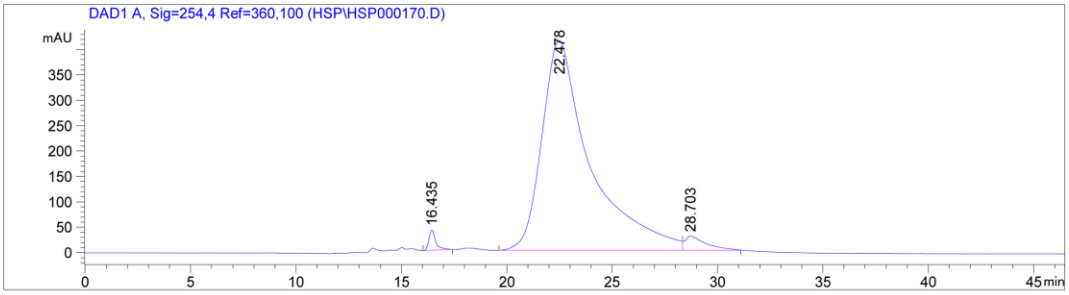
Compound **12c**





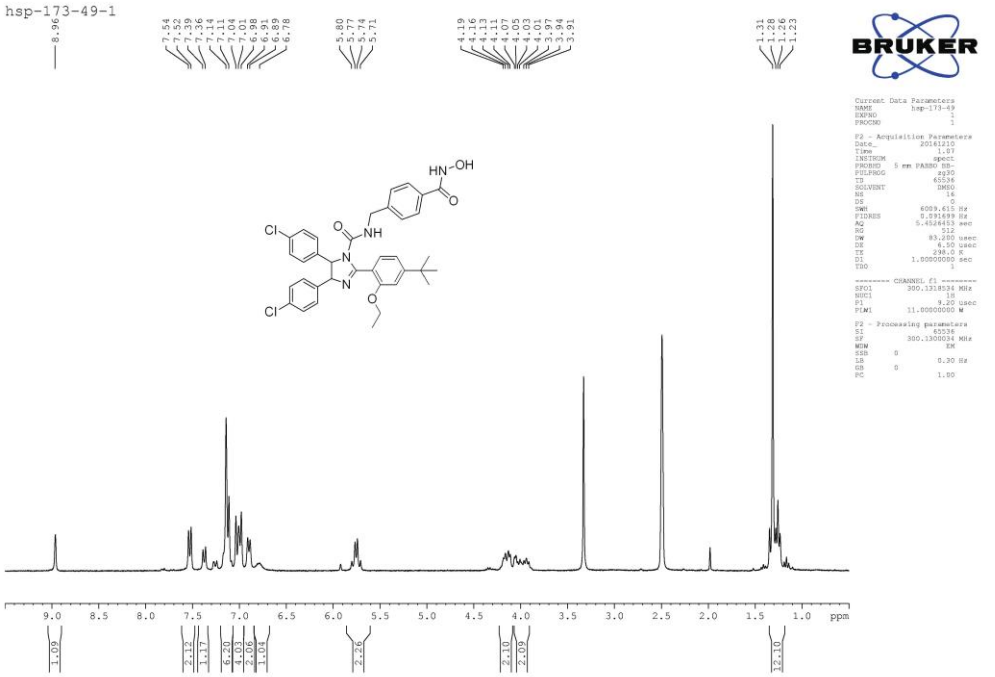
数据文件：C:\CHEM32\1\DATA\HSP\HSP000170.D
样品名称：hsp-42

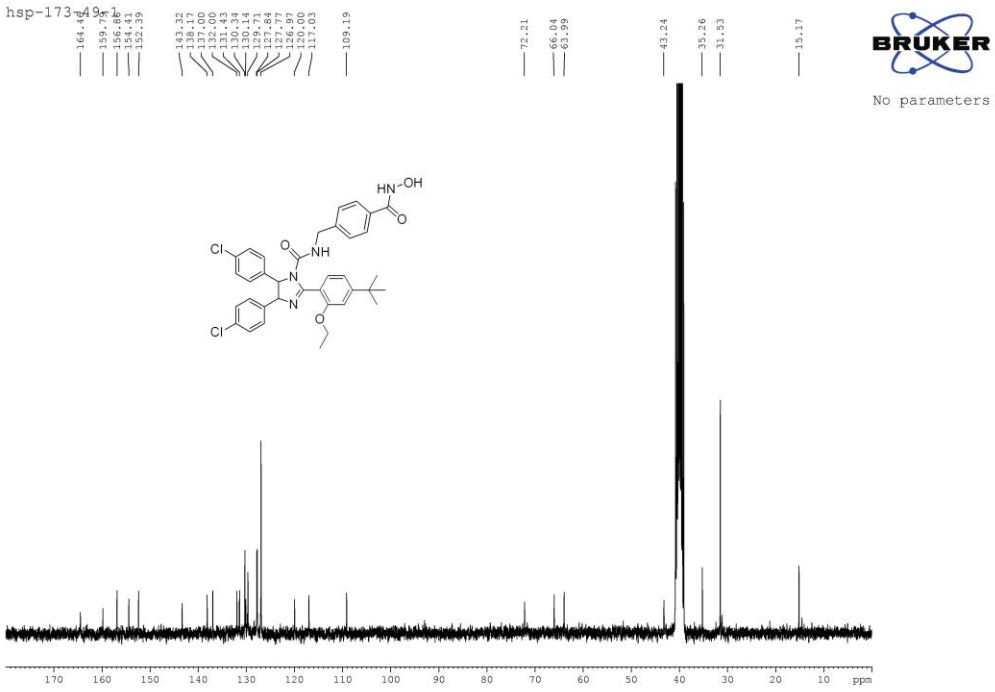
```
======================================================================
操作者            : hsp
仪器              : 仪器 1                              位置 :    -
进样日期          : 2018-5-26 7:56:52 下午
采集方法          : C:\CHEM32\1\METHODS\AD.M
最后修改          : 2018-5-26 7:54:04 下午 :  hsp
                    (调用后修改)
分析方法          : C:\CHEM32\1\METHODS\AD.M
最后修改          : 2018-5-28 7:25:09 下午 :  hsp
方法信息          : hsp

样品信息          : 90:10  i-Pr/Hexane  1.0ml/min  AD-H  254nm
```



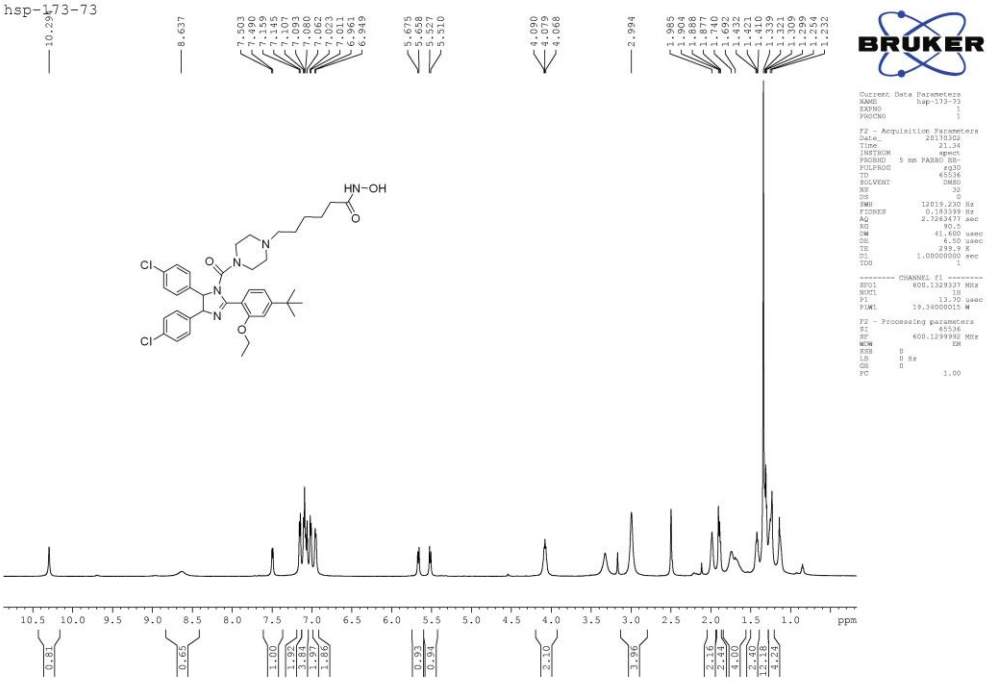

```
======================================================================
                          面积百分比报告
======================================================================

排序                :      信号
乘积因子            :      1.0000
稀释因子            :      1.0000
样品量              :      1.00000  [ng/ul]   (校正中没有使用)
内标使用乘积因子和稀释因子


信号 1: DAD1 A, Sig=254,4 Ref=360,100

 峰  保留时间 类型  峰宽      峰面积       峰高      峰面积
  #    [min]        [min]    [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  16.435 VB    0.3467  935.36615   40.30850   1.3604
   2  22.478 BB    1.8891 6.58496e4   413.23520  95.7696
   3  28.703 BB    0.8650 1973.42285   28.11074   2.8701

总量 :                   6.87584e4   481.65443


======================================================================
                        *** 报告结束 ***
```

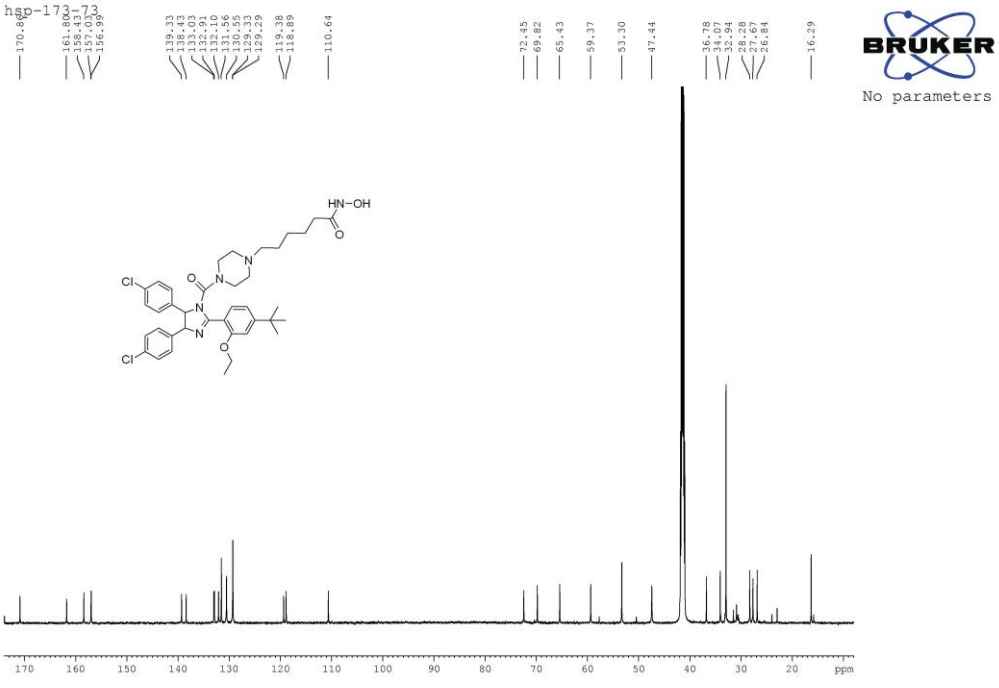
Compound **13**


hsp-173-49-1
BRUKER
HN-OH
O
NH
Cl
N
O
Cl
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm

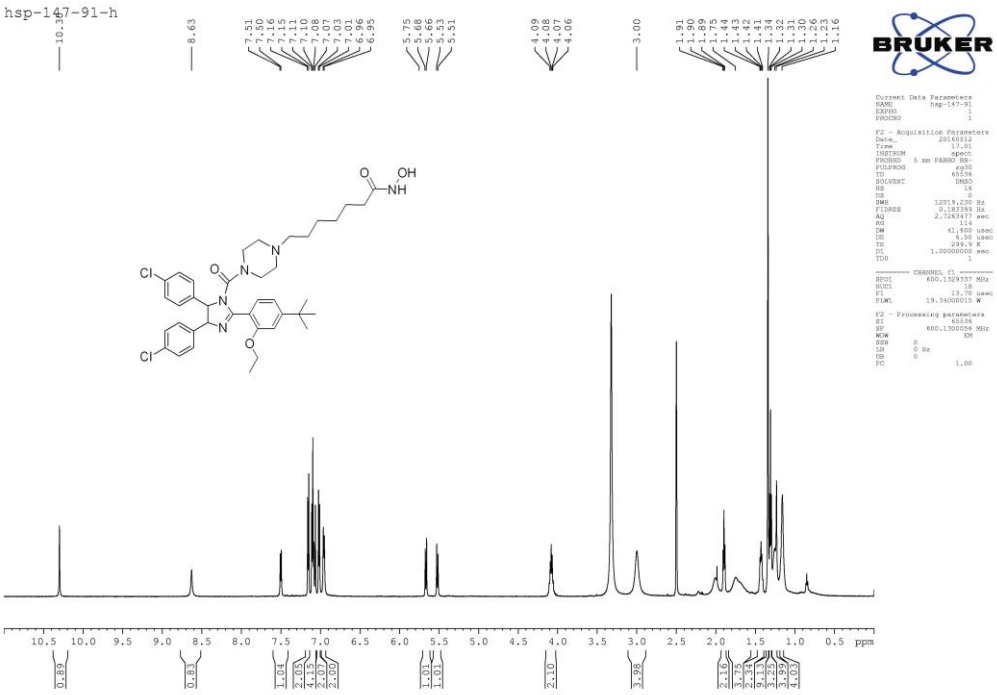

hsp-173-49-1
BRUKER
No parameters
HN-OH
O
NH
Cl
N
O
Cl
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

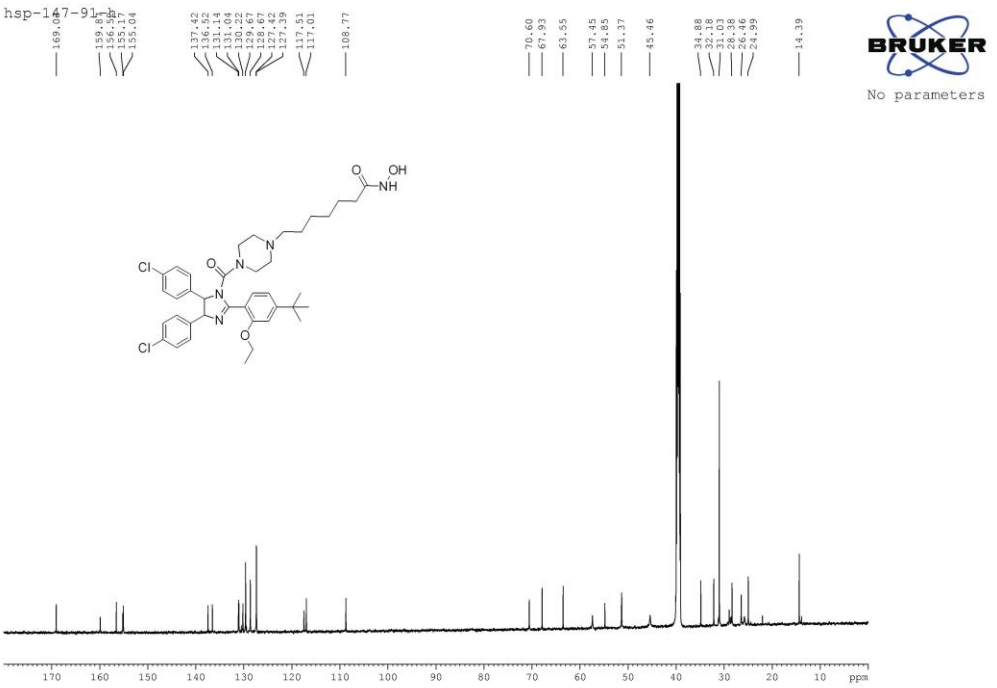
Compound **14c**





Compound **14d**





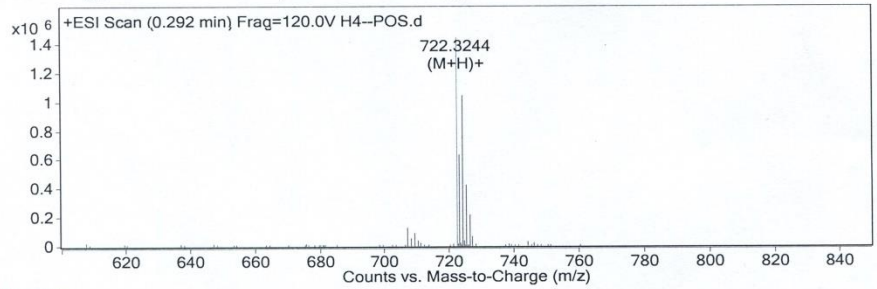
Qualitative Analysis Report

Data Filename	H4--POS.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-D3
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	11/2/2017 10:39:35 AM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
338.3417		669853.9		
361.666	2	2044955.4		
362.1675	2	950832.1		
362.6652	2	1549624.1		
363.1662	2	641174.1		
363.6655	2	349502.9		
722.3244	1	1444457.6	C39 H50 Cl2 N5 O4	(M+H)+
723.3274	1	629625.4	C39 H50 Cl2 N5 O4	(M+H)+
724.3229	1	1042737.1	C39 H50 Cl2 N5 O4	(M+H)+
725.3253	1	421272.3	C39 H50 Cl2 N5 O4	(M+H)+

Formula Calculator Element Limits

Element	Min	Max
C	3	200
H	0	400
O	4	18
N	5	18
Cl	0	2

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C39 H49 Cl2 N5 O4	TRUE	721.3172	721.3162	-1.41	C39 H50 Cl2 N5 O4	98.38

--- End Of Report ---

数据文件：C:\CHEM32\1\DATA\HSP\HSP000005.D
样品名称：hsp-4

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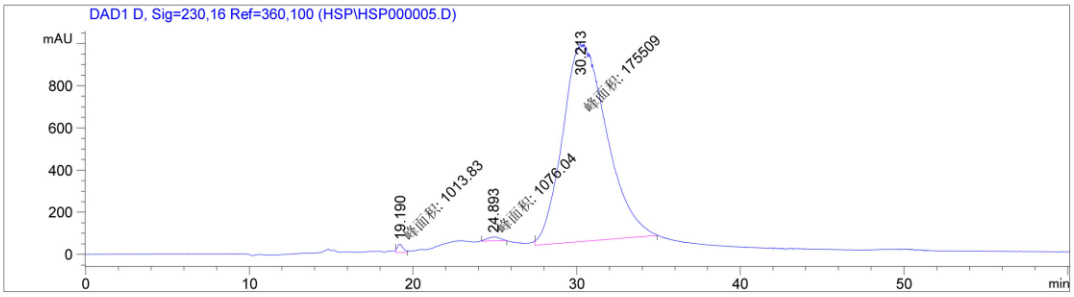
操作者 : hsp
仪器 : 仪器 1 位置 : -
进样日期 : 2017-6-9 4:34:16 下午
采集方法 : C:\CHEM32\1\METHODS\AD.M
最后修改 : 2017-6-9 4:26:27 下午 : hsp
(调用后修改)
分析方法 : C:\CHEM32\1\METHODS\AD.M
最后修改 : 2017-6-10 5:03:19 下午 : hsp
(调用后修改)
方法信息 : ad

样品信息 : 5:95 Water/MeOH 0.8 ml/min C18 254nm



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面积百分比报告

==========

排序 : 信号
乘积因子 : 1.0000
稀释因子 : 1.0000
样品量 : 1.00000 [ng/ul] (校正中没有使用)
内标使用乘积因子和稀释因子

信号 1: DAD1 D, Sig=230,16 Ref=360,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	19.190	MM	0.4099	1013.83295	41.22291	0.5709
2	24.893	MM	0.9128	1076.04395	19.64720	0.6059
3	30.213	MM	3.0818	1.75509e5	949.16327	98.8233

总量 : 1.77598e5 1010.03338

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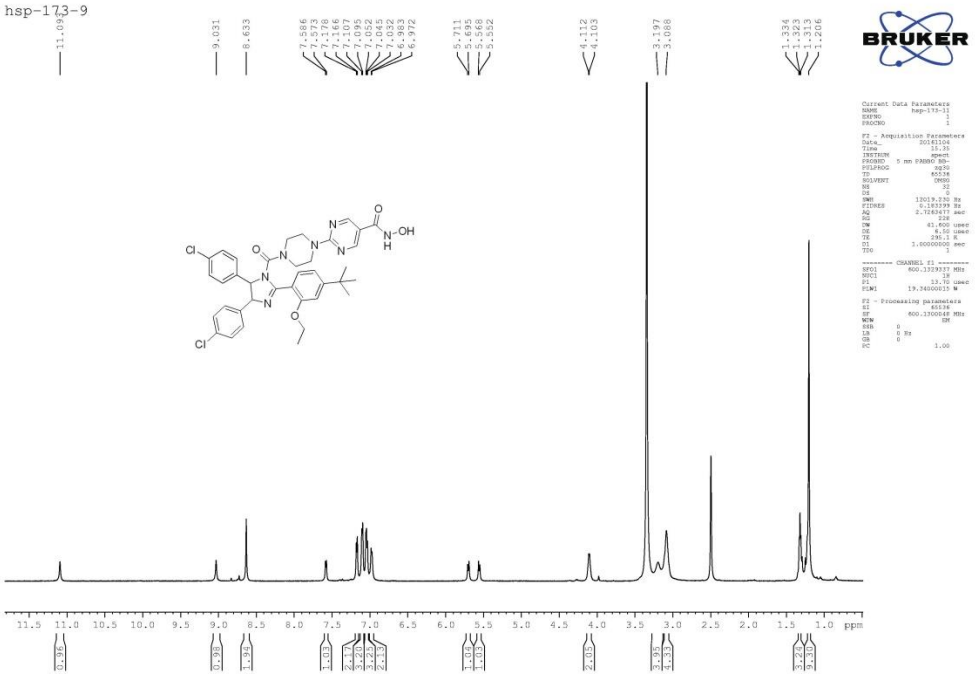
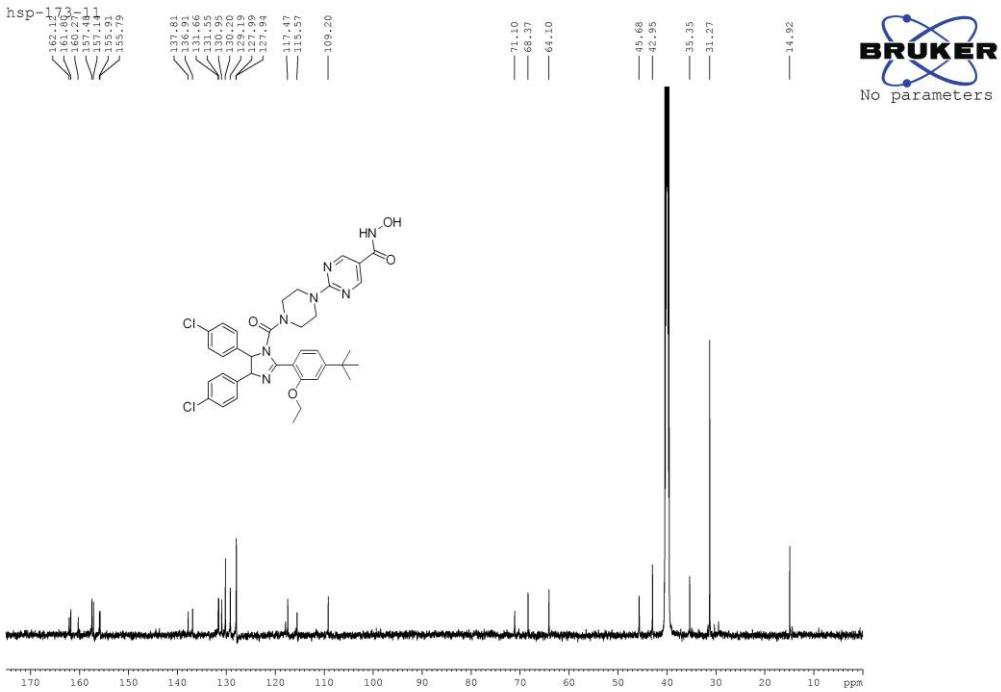
*** 报告结束 ***

Compound **15b**





Qualitative Analysis Report

Data Filename	H-13.d	**Sample Name**	
Sample Type	Sample	**Position**	P2-B7
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	12/26/2017 10:58:24 AM
IRM Calibration Status	Success	**DA Method**	E.m
Comment			

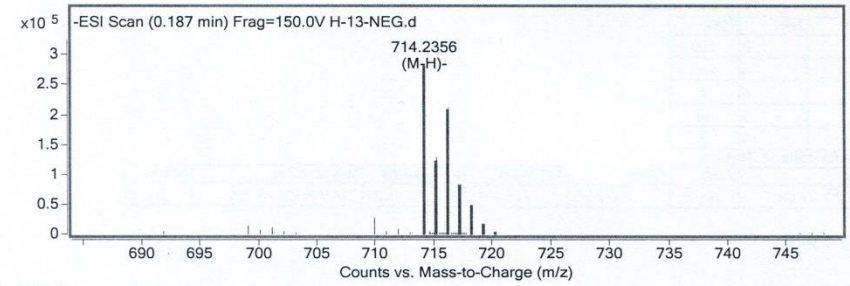
Sample Group **Info.**

Data Filename	H-13-NEG.d	**Sample Name**	
Sample Type	Sample	**Position**	P2-B7
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-NEG-WL.m	**Acquired Time**	12/26/2017 11:32:41 AM
IRM Calibration Status	Some Ions Missed	**DA Method**	E.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
150	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
714.2356	1	280576.3	C37 H38 Cl2 N7 O4	(M-H)-
715.2374	1	129080.4	C37 H38 Cl2 N7 O4	(M-H)-
716.2335	1	213284.5	C37 H38 Cl2 N7 O4	(M-H)-
717.2355	1	82128.7	C37 H38 Cl2 N7 O4	(M-H)-
752.2083		54709.4		
805.9823		76075.2		
955.9689		118290.2		
966.0007	1	381029.3		
966.9999	1	74306.5		
982.9871		55145.1		

Formula Calculator Element Limits

Element	Min	Max
C	0	100
H	0	200
O	4	4
N	0	10
Cl	0	2

Formula Calculator Results

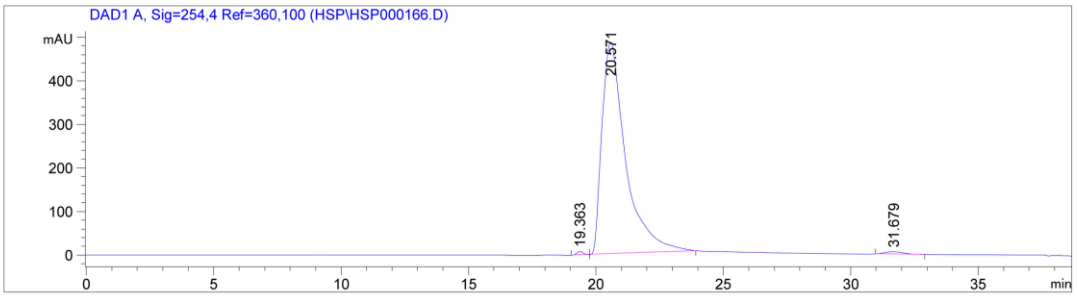
Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C37 H39 Cl2 N7 O4	TRUE	715.2427	715.2441	1.87	C37 H38 Cl2 N7 O4	93.51

Fragmentor Voltage	Collision Energy	Ionization Mode

数据文件：C:\CHEM32\1\DATA\HSP\HSP000166.D
样品名称：hsp-31

```
==========================================================================
操作者              : hsp
仪器                : 仪器 1                                  位置 :    -
进样日期            : 2018-5-26 3:02:10 下午
采集方法            : C:\CHEM32\1\METHODS\AD.M
最后修改            : 2018-5-26 3:26:48 下午 :  hsp
                      (调用后修改)
分析方法            : C:\CHEM32\1\METHODS\AD.M
最后修改            : 2018-5-28 7:25:09 下午 :  hsp
方法信息            : hsp

样品信息            : 85:15  i-Pr/Hexane  0.8ml/min  AD-H  254nm
```




```
==========================================================================
                              面积百分比报告
==========================================================================

排序                :      信号
乘积因子            :      1.0000
稀释因子            :      1.0000
样品量              :      1.00000  [ng/ul]   (校正中没有使用)
内标使用乘积因子和稀释因子


信号 1: DAD1 A, Sig=254,4 Ref=360,100

 峰  保留时间 类型  峰宽      峰面积       峰高      峰面积
  #    [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  19.363 BV    0.2593  145.27344    8.17676   0.4549
   2  20.571 VB    0.9693 3.14860e4   486.36862  98.5867
   3  31.679 BB    0.6167  306.11264    5.90583   0.9585

总量 :                   3.19374e4   500.45121


==========================================================================
                            *** 报告结束 ***
```

References

1. Tovar, C.; Graves, B.; Packman, K.; Filipovic, Z.; Higgins, B.; Xia, M.; Tardell, C.; Garrido, R.; Lee, E.; Kolinsky, K.; To, K.-H.; Linn, M.; Podlaski, F.; Wovkulich, P.; Vu, B.; Vassilev, L. T. MDM2 small-molecule antagonist RG7112 activates p53 signaling and regresses human tumors in preclinical cancer models. *Cancer Res.* **2013**, *73*, 2587-2597.

2. Colombano, G.; Travelli, C.; Galli, U.; Caldarelli, A.; Chini, M. G.; Canonico, P. L.; Sorba, G.; Bifulco, G.; Tron, G. C.; Genazzani, A. A. A novel potent nicotinamide phosphoribosyltransferase inhibitor synthesized via click chemistry. *J. Med. Chem.* **2010**, *53*, 616-623.

3. Millard, C. J.; Watson, P. J.; Celardo, I.; Gordiyenko, Y.; Cowley, S. M.; Robinson, C. V.; Fairall, L.; Schwabe, J. W. R. Class I HDACs share a common mechanism of regulation by inositol phosphates. *Mol. Cell* **2013**, *51*, 57-67.

4. Zheng, X.; Bauer, P.; Baumeister, T.; Buckmelter, A. J.; Caligiuri, M.; Clodfelter, K. H.; Han, B.; Ho, Y.-C.; Kley, N.; Lin, J.; Reynolds, D. J.; Sharma, G.; Smith, C. C.; Wang, Z.; Dragovich, P. S.; Oh, A.; Wang, W.; Zak, M.; Gunzner-Toste, J.; Zhao, G.; Yuen, P.-w.; Bair, K. W. Structure-Based identification of ureas as novel nicotinamide phosphoribosyltransferase (nampt) inhibitors. *J. Med. Chem.* **2013**, *56*, 4921-4937.