Supporting Information

Discovery of Novel Indoleamine 2,3-dioxygenase 1 (IDO1) and Histone Deacetylase 1 (HDAC) Dual Inhibitors

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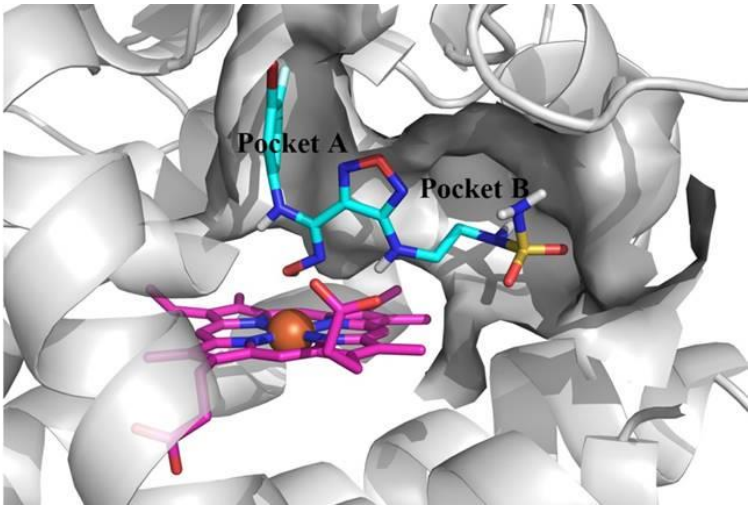
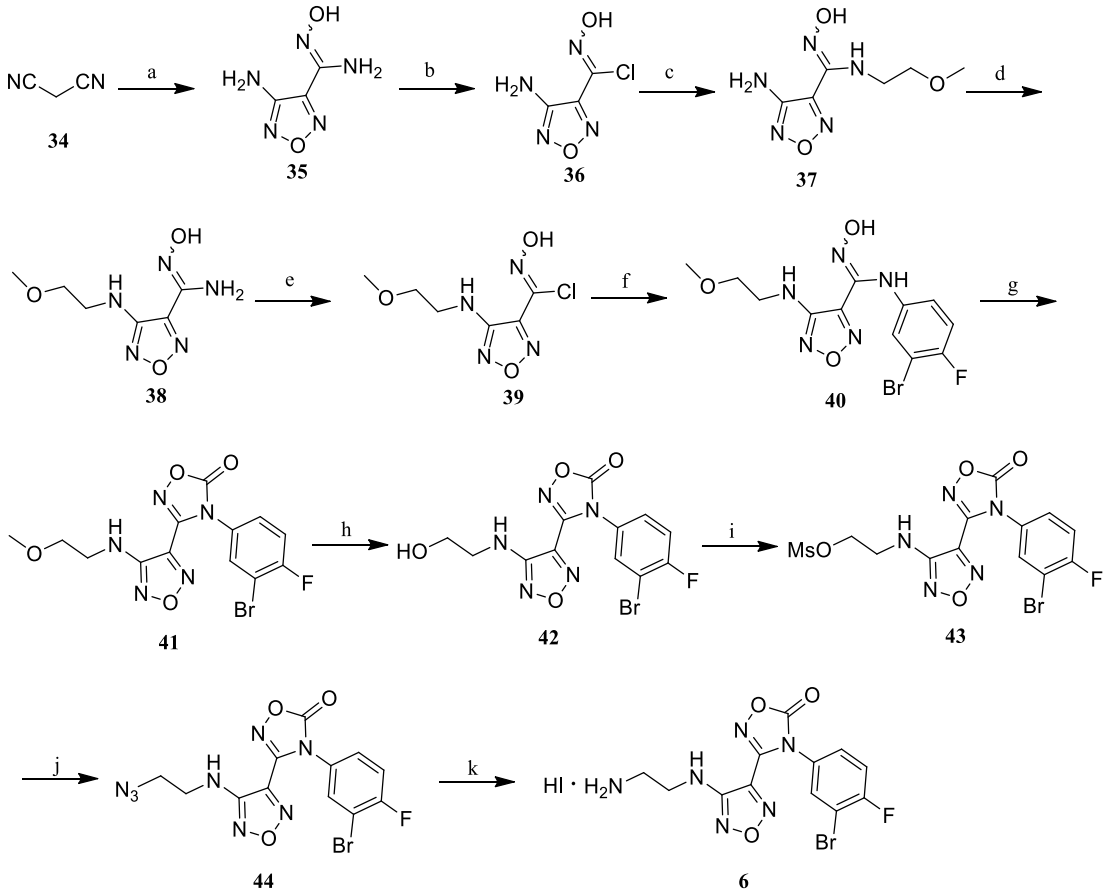


Figure S1. Model of compound **2** bound to IDO1 (PDB ID: 4PK5).

Synthetic Schemes

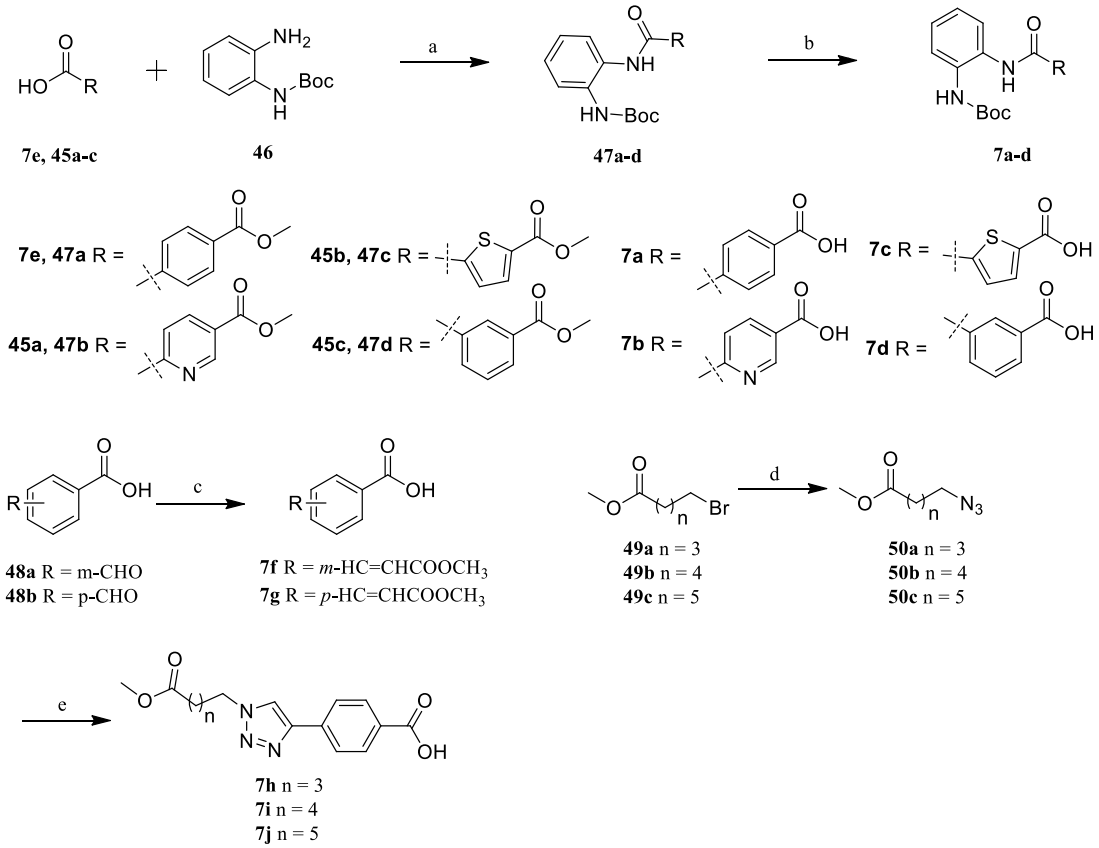
Scheme S1. Synthesis of intermediate 6.[a]



[a]**Scheme S1.** Reagents and Conditions: (a) 1.1. HCl, $NaNO_2$, H_2O, rt, 2 h; 1.2. H_2NOH-HCl, rt, 1

h → reflux, 2 h, yield 76%; (b) HCl, $NaNO_2$, H_2O, 0 ^oC, 3 h, 44%; (c) 2-Methoxyethylamine, Et_3N, 0 ^oC, 1 h, 98%; (d) KOH, H_2O, reflux, 24 h, 76%; (e) 6M aq. HCl solution, $NaNO_2$, NaCl, rt, 3 d, 96%; (f) 3-Bromo-4-fluoroaniline, $NaHCO_3$, H_2O, 60 ^oC, 2 h, 86%; (g) N, N'-Carbonyldiimidazole, ethyl acetate, 60 ^oC, 2 h, 96%; (h) BBr_3, DCM, -67 ~ 0 ^oC, 1 h, 75%; (i) Methanesulfonyl chloride, Et_3N, ethyl acetate, rt, 2 h, 93%; (j) NaN_3, DMF, 50 ^oC, 6 h, 78%; (k) NaI, Chlorotrimethylsilane, MeOH, rt, 1 h, 87%.

Scheme S2. Synthetic Approaches to Intermediate 7a-j.[a]



[a]**Scheme S2.** Reagents and Conditions: (a) HATU, DIPEA, DMF, rt, overnight, yield (49-72%); (b) LiOH, MeOH, H_2O, rt, overnight, yield (77-86%); (c) Trimethyl phosphonoacetate, K_2CO_3, H_2O, rt, overnight, yield (93-95%); (d) NaN_3, DMF, 80 ^oC, 8 h, yield (98-99%); (e) 4-Ethynylbenzoic acid, NaVc, $CuSO_4$, THF, H_2O, rt, overnight, yield (63-74%).

Table S1. Docking Scores for 1,2,3-triazol derivatives 17-19 with HDAC1 (PDB: 4BKX)

Compounds	GlideScore
17	-6.658
18	-8.153

19	-9.681

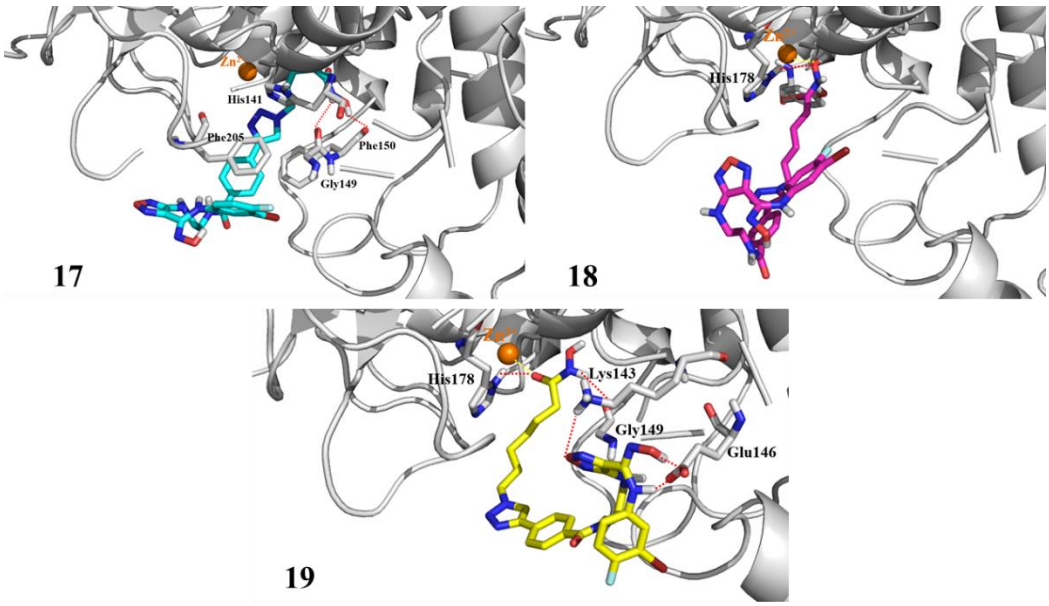


Figure S2. Binding mode of compounds 17-19 with HDAC1.

Table S2. HDACs inhibitory activities of compound 10[a].

Compds	HDAC2 (IC_{50} nM)	HDAC3 (IC_{50} nM)	HDAC4 (IC_{50} μM)	HDAC6 (IC_{50} nM)	HDAC8 (IC_{50} μM)
10	179 ± 1	45 ± 1	60 ± 4	70 ± 12	2.1 ± 0.1
SAHA	180 ± 4	29 ± 5	251 ± 17	24 ± 1	6.3 ± 0.3

[a]Data are presented as mean ± SD.

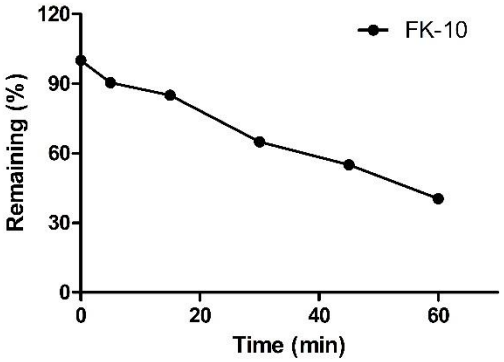


Figure S2. Mouse microsomal stability data for **10**. Plot of parent remaining against time for mouse microsome.

Chemical synthesis and Structural Characterization of the 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 CD_3OD or DMSO-d_6 as solvents. Chemical shift are given in ppm (δ). Elemental analyses were performed with a MOD-1106 instrument and were consistent with theoretical values within 0.4%. 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). Purity of the compounds was analyzed by HPLC (Agilent Technologies 1260 Infinity) using a gradient elution starting from a 65% solution of MeOH and a 35% solution of water and 0.1% TFA to a 70% solution of MeOH and a 30% solution of water and 0.1% TFA at 0.5 mL/min on a C18 column (Aglilent 20RBA × SB-C18, 5 μm, 4.6 mm × 150 mm). All compounds exhibited greater than 90% purity. 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.

Intermediate **6** were prepared according to a modification of the procedure reported by *Yue, E. W. et al*[1] as follows:

4-Amino-*N'*-hydroxy-1,2,5-oxadiazole-3-carboximidamide (35). Malonoitrile (20.0 g, 0.3 mol) was added in 2 N hydrochloric acid (300 mL) and stirred for 5 min. The resulting solution was cooled in an ice bath and sodium nitrite (22.8 g, 0.3 mol) in water (50 mL) was added. After 15 min the cold bath was removed and the reaction mixture was stirred for 4 h at room temperature. The reaction mixture was cooled to

10 ^{o}C and hydroxylamine hydrochloride (62.5 g, 0.9 mol) was added all at once. The pH of the solution was adjusted to 7 by 50% aqueous NaOH and stirring was continued for 1h at 25 ^{o}C. Reflux was maintained for 2 h and the reaction mixture was allowed to cool overnight. The reaction mixture was stirred in an ice bath and 6 M hydrochloric acid aqueous solution (40 mL) was added in portions over 40 min to pH 7. The precipitate was collected by filtration, washed well with water and dried to give the desired product (32.6 g, 76 %). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 6.17 (s, 2H), 6.26 (s, 2H), 10.45 (s, 1H).

4-Amino-*N*-hydroxy-1,2,5-oxadiazole-3-carbimidoyl chloride (36). Intermediate **35** (10.0 g, 0.07 mol) was added to a mixture of water (80 mL) and hydrochloric acid (60 mL) and this suspension was stirred until complete solution was achieved. A solution of sodium nitrite (4.83 g, 0.07 mol) in water (20 mL) was added over 2.5 h while maintaining the temperature below 0 ^{o}C. After complete addition stirring was continued in the ice bath for 1 h and then the precipitate was collected by filtration, washed well with water and dried to give the desired product (10.0 g, 44%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 5.95 (s, 2H), 6.26 (s, 2H), 7.85 (s, 1H).

4-Amino-*N'*-hydroxy-*N*-(2-methoxyethyl)-1,2,5-oxadiazole-3-carboximidamide (37). Intermediate **36** (10.0 g, 61.5 mmol) was mixed with ethyl acetate (150 mL). At 0-5 ^{o}C, 2-methoxyethylamine (5.8 mL, 67.5 mmol) was added in one portion while stirring. Then triethylamine (12.8 mL, 92 mmol) was added and the reaction mixture was stirred for 1 h at room temperature. After reaction, the solvent was washed with water and brine, dried over sodium sulfate, and concentrated to give the desired

product (12.1 g, 98%) as a crude dark oil. ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 10.65 (s, 1H), 6.27 (s, 2H), 6.10 (t, J = 6.5 Hz, 1H), 3.50 (m, 2H), 3.35 (d, J = 5.8 Hz, 2H), 3.08 (s, 3H).

***N'*-Hydroxy-4-((2-methoxyethyl)amino)-1,2,5-oxadiazole-3-carboximidamide (38).** Intermediate **37** (10 g, 50 mmol) was mixed with water (100 mL). Potassium hydroxide was added. The reaction was refluxed for 24 h. TCL with 50% ethyl acetate (containing 1% ammonium hydroxide) in hexane indicated reaction completed. The reaction was cooled to room temperature and extracted with ethyl acetate (3 × 150 mL). The combined ethyl acetate solution was dried over sodium sulfate and concentrated to give the desired product (7.6 g, 76%) as a crude off-white solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 10.54 (s, 1H), 6.22 (s, 2H), 6.15 (t, J = 5.8 Hz, 1H), 3.45 (t, J = 5.3 Hz, 2H), 3.35 (m, 2H), 3.22 (s, 3H).

***N*-Hydroxy-4-((2-methoxyethyl)amino)-1,2,5-oxadiazole-3-carbimidoyl chloride (39).** Intermediate **38** (6.0 g, 29.8 mmol) was dissolved in 6 M hydrochloric acid aqueous solution (30 mL). Sodium chloride (5.2 g, 89.4 mmol) was added followed by water (30 mL) and ethyl acetate (30 mL). A solution of sodium nitrite (2.0 g, 29.8 mmol) in water (10 mL) was added over 0.5 h while maintaining the temperature below 0 ^{o}C. The reaction was stirred at 3-8 ^{o}C for 2 h and then room temperature over 3 days. After reaction, the solvent was extracted with ethyl acetate (2 × 150 mL). The combined ethyl acetate solution was dried over sodium sulfate and concentrated to give the desired product (6.6 g, 96%) as a crude white solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 13.43 (s, 1H), 5.85 (t, J = 5.6 Hz, 1H), 3.50 (t, J = 5.6 Hz, 2H), 3.37 (dd, J =

10.8, 5.6 Hz, 2H), 3.25 (s, 3H).

***N*-(3-Bromo-4-fluorophenyl)-*N'*-hydroxy-4-((2-methoxyethyl)amino)-1,2,5-oxadiazole-3-carboximidamide (40).** Intermediate **39** (6.5 g, 29.5 mmol) was mixed with water (100 mL). The mixture was heated to 60 ^{o}C. 3-Bromo-4-fluoroaniline (6.17 g, 32.5 mmol) was added and stirred for 10 min. A sodium bicarbonate (3.7 g, 44.4 mmol) solution (50 mL water) was added over 15 min. The reaction was stirred at 60 ^{o}C for 1 h. The reaction was solution was cooled to room temperature and extracted with ethyl acetate (3 × 100 mL). The combined ethyl acetate solution was dried over sodium sulfate and concentrated to give the desired product (11.0 g, 86%) as a crude brown solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 11.56 (s, 1H), 8.84 (s, 1H), 7.13 (t, J = 8.8 Hz, 1H), 7.08 (dd, J = 6.1, 2.7 Hz, 1H), 6.75 (m, 1H), 6.14 (t, J = 5.8 Hz, 1H), 3.48 (t, J = 5.2 Hz, 2H), 3.35 (dd, J = 10.8, 5.6 Hz, 2H), 3.22 (s, 3H).

4-(3-Bromo-4-fluorophenyl)-3-(4-((2-methoxyethyl)amino)-1,2,5-oxadiazol-3-yl)-1,2,4-oxadiazol-5(4*H*)-one (41). A mixture of intermediate **40** (7.0 g, 18.7 mmol), *N*, *N*'-carbonyldiimidazole and ethyl acetate (150 mL) was heated to 60 ^{o}C and stirred for 1 h. The reaction was cooled to room temperature, washed with 1 N HCl (2 × 100 mL), dried over sodium sulfate, and concentrated to give the desired product (7.5 g, 96%) as a crude brown solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 7.94 (t, J = 8.2 Hz, 1H), 7.72 (dd, J = 9.1, 2.3 Hz, 1H), 7.42 (m, 1H), 6.42 (t, J = 5.7 Hz, 1H), 3.46 (t, J = 5.4 Hz, 2H), 3.36 (t, J = 5.8 Hz, 2H), 3.26 (s, 3H).

4-(3-Bromo-4-fluorophenyl)-3-(4-((2-hydroxyethyl)amino)-1,2,5-oxadiazol-3-yl)-1,2,4-oxadiazol-5(4*H*)-one (42). Intermediate **41** (6.0 g, 15 mmol) was dissolved in

dichloromethane (80 mL). At -67 °C boron tribromide (2.8 mL, 30 mmol) was added over 15 min under nitrogen. The mixture was further stirred for 2 h at room temperature. At 0-5 °C the reaction was slowly quenched with saturated sodium bicarbonate solution (100 mL) over 30 min. The reaction was extracted with ethyl acetate (2 × 150 mL). The combined organic layers were dried over sodium sulfate and concentrated to give the desired product (4.3 g, 75%) as a crude brown solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 8.08 (dd, J = 6.2, 2.5 Hz, 1H), 7.70 (m, 1H), 7.68 (t, J = 8.7 Hz, 1H), 6.33 (t, J = 5.6 Hz, 1H), 4.85 (t, J = 5.0 Hz, 1H), 3.56 (dd, J = 10.6, 5.6 Hz, 2H), 3.29 (dd, J = 11.5, 5.9 Hz, 2H).

2-((4-(4-(3-Bromo-4-fluorophenyl)-5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1,2,5-oxadiazol-3-yl)amino)ethyl methanesulfonate (43). To a solution of intermediate **42** (4.0 g, 10.4 mmol) in ethyl acetate (80 mL) was added methanesulfonyl chloride (1.6 mL, 20.8 mmol) dropwise over 15 min at room temperature. Triethylamine (2.9 mL, 20.8 mmol) was added dropwise over 10 min. After 2 h, the reaction mixture was washed with water, brine, dried over sodium sulfate, and concentrated to give the desired product (4.8 g, 93%) as a tan solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 8.08 (dd, J = 6.2, 2.5 Hz, 1H), 7.72 (m, 1H), 7.58 (t, J = 8.7 Hz, 1H), 6.75 (t, J = 5.9 Hz, 1H), 4.36 (t, J = 5.3 Hz, 2H), 3.58 (dd, J = 11.2, 5.6 Hz, 2H), 3.18 (s, 3H).

3-(4-((2-Azidoethyl)amino)-1,2,5-oxadiazol-3-yl)-4-(3-bromo-4-fluorophenyl)-1,2,4-oxadiazol-5(4*H*)-one (44). To a solution of intermediate **43** (4.0 g, 8.6 mmol) in dimethylformamide (20 mL) was added sodium azide (0.7 g, 10.9 mmol). The reaction was heated at 50 °C for 6 h, poured into ice/water (150 mL), and extracted

with ethyl acetate (2 × 150 mL). The combined organic layers was washed with water, brine, dried over sodium sulfate, and concentrated to give the desired product (3.5 g, 78%) as a tan solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 8.08 (dd, J = 6.2, 2.5 Hz, 1H), 7.72 (m, 1H), 7.58 (t, J = 8.7 Hz, 1H), 6.75 (t, J = 5.9 Hz, 1H), 3.54 (t, J = 5.3 Hz, 2H), 3.45 (dd, J = 11.1, 5.2 Hz, 2H).

3-(4-((2-Aminoethyl)amino)-1,2,5-oxadiazol-3-yl)-4-(3-bromo-4-fluorophenyl)-1,2,4-oxadiazol-5(4*H*)-one hydroiodide (6). A solution of intermediate **44** (3.0 g, 7.1 mmol) in methanol (54 mL) was treated with sodium iodide (6.3 g, 42 mmol) and stirred at 25 ^{o}C for 5 min. The reaction mixture was treated with a solution of chlorotrimethylsilane (5.4 mL, 42 mmol) in methanol (11 mL) dropwise and stirred at 25 ^{o}C for 1 h. The mixture was slowly poured into a solution of sodium thiosulfate (7.5 g, 48 mmol) in water (300 mL) that was cooled at 0 ^{o}C. The solid that precipitated was filtered, washed with water, and dried to give the desired product (3.2 g, 87%) as a white solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 8.12 (m, 4H), 7.76 (m, 1H), 7.58 (t, J = 8.7 Hz, 1H), 6.76 (t, J = 6.1 Hz, 1H), 3.51 (dd, J = 11.8, 6.1 Hz, 2H), 3.02 (m, 2H).

General procedure for the synthesis of protected and substituted carboxylic acids 7a-d.

The respective methoxycarbonyl aroylic acids (10.0 mmol) (**7e**, **45a**, **45b** and **45c**) were dissolved in dry DMF (20 mL), and 1.1 equiv of HATU, 3.0 equiv of DIPEA, and 1.1 equiv of *tert*-butyl (2-aminophenyl)carbamate (**46**) were added. After stirring for 24 h at room temperature, the reaction was poured into ice/water and extracted

with ethyl acetate. The combined organic layers was washed with water, brine, dried over sodium sulfate, purified by column chromatography to give carboxylic acid methyl esters **47a-d**.

The respective methyl esters (4.0 mmol) (**47a**, **47b**, **47c** and **47d**) were dissolved in MeOH (25 mL), and 2 equip of LiOH in H_2O (25 mL) was added. After stirring at room temperature overnight, MeOH was removed under reduced pressure, and the aqueous layer was cooled to 0 °C, and acidified with 2M HCl to pH = 5-6. The resulting precipitate was removed by filtration and dried in vacuo to afford the carboxylic acids **7a-d**.

4-((2-((*Tert*-butoxycarbonyl)amino)phenyl)carbamoyl)benzoic acid (7a). Light yellow solid (1.18 g, 83%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 13.29 (s, 1H), 9.95 (s, 1H), 8.71 (s, 1H), 8.04-8.08 (m, 4H), 7.54 (dd, J = 14.3, 7.9 Hz, 2H), 7.21 (td, J = 7.8, 1.4 Hz, 1H), 7.14 (td, J = 7.9, 1.0 Hz, 1H), 1.43 (s, 9H); Molecular formula $C_{19}H_{20}N_2O_5$; Mass required 355.13 [M – H]; Mass found ESI-MS (m/s): 355.26 [M – H].

6-((2-((*Tert*-butoxycarbonyl)amino)phenyl)carbamoyl)nicotinic acid (7b). White solid (1.16 g, 81%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 13.33 (s, 1H), 10.52 (s, 1H), 9.13 (s, 1H), 9.05 (d, J = 1.4 Hz, 1H), 8.50 (dd, J = 8.1, 2.1 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 7.6 Hz, 1H), 7.22-7.28 (m, 2H), 7.17 (td, J = 7.7, 1.4 Hz, 1H), 1.48 (s, 9H); Molecular formula $C_{18}H_{19}N_3O_5$; Mass required 356.13[M – H]; Mass found ESI-MS (m/s): 356.32 [M – H].

5-((2-((*Tert*-butoxycarbonyl)amino)phenyl)carbamoyl)thiophene-2-carboxylic

acid (7c). Light yellow solid (1.12 g, 77%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 13.31 (s, 1H), 9.97 (s, 1H), 8.69 (s, 1H), 7.91 (d, J = 3.9 Hz, 1H), 7.78 (d, J = 3.9 Hz, 1H), 7.57 (d, J = 7.9 Hz, 1H), 7.45 (dd, J = 7.9, 1.2 Hz, 1H), 7.21 (td, J = 7.7, 1.4 Hz, 1H), 7.12 (td, J = 7.8, 1.4 Hz, 1H), 1.43 (s, 9H); Molecular formula $C_{17}H_{18}N_2O_5S$; Mass required 361.09 [M – H]; Mass found ESI-MS (m/s): 361.28 [M – H].

3-((2-((*Tert*-butoxycarbonyl)amino)phenyl)carbamoyl)benzoic acid (7d). White solid (1.26 g, 86%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 13.28 (s, 1H), 10.01 (s, 1H), 8.70 (s, 1H), 8.54 (s, 1H), 8.20 (d, J = 7.7 Hz, 1H), 8.15 (d, J = 7.8 Hz, 1H), 7.67 (t, J = 7.7 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 7.6 Hz, 1H), 7.21 (td, J = 7.7, 1.3 Hz, 1H), 7.14 (td, J = 7.8, 1.2 Hz, 1H), 1.43 (s, 9H); Molecular formula $C_{19}H_{20}N_2O_5$; Mass required 355.13 [M – H]; Mass found ESI-MS (m/s): 355.26 [M – H].

General procedure for the synthesis of benzoic acids 7f, 7g.

The respective formylbenzoic acids (10 mmol) (**48a**, **48b**) and K_2CO_3 (30 mmol) was added to water (40 mL) and cooled to 0-5 °C. Trimethyl phosphonoacetate (12 mmol) was added dropwise and the reaction was then stirred at room temperature before acidifying to pH ~ 2. The resulting precipitate was filtered and and dried in vacuo to afford the benzoic acids **7f**, **7g**.

(*E*)-3-(3-Methoxy-3-oxoprop-1-en-1-yl)benzoic acid (7f). White solid (1.91 g, 93%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 13.15 (s, 1H), 8.19 (s, 1H), 7.98 (dd, J = 16.8, 7.8 Hz, 2H), 7.73 (d, J = 16.2 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 6.78 (d, J = 16.1 Hz, 1H), 3.73 (s, 3H); Molecular formula $C_{11}H_{10}O_4$; Mass required 207.07 [M + H]; Mass found ESI-MS (m/s): 207.23 [M + H].

(*E*)-4-(3-Methoxy-3-oxoprop-1-en-1-yl)benzoic acid (7g). White solid (1.96 g, 95%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 13.17 (s, 1H), 7.97 (d, J = 8.2 Hz, 2H), 7.86 (d, J = 8.3 Hz, 2H), 7.74 (d, J = 16.0 Hz, 1H), 6.71 (d, J = 16.2 Hz, 1H), 3.77 (s, 3H); Molecular formula $C_{11}H_{10}O_4$; Mass required 207.07 [M + H]; Mass found ESI-MS (m/s): 207. 15 [M + H].

General procedure for the synthesis of 1,2,3-triazol benzoic acids 7h-j.

The aliphatic ester (10 mmol) (**49a**, **49b** and **49c**) and sodium azide (30 mmol) were stirred in DMF (20 mL). The reaction mixture was heated to 80 °C overnight, and salts were removed by filtration. The filtrate was poured into ethyl acetate (100 mL), and washed with water (3 × 100 mL). The organic phase was dried over $MgSO_4$ and concentrated to give the azido compounds **50a-c**.

The respective the azido compounds (5 mmol) (**50a**, **50b** and **50c**) and 4-ethynylbenzoic acid (5 mmol) were suspended in a solution of water/tert-butanol 1:1. Sodium ascorbate (0.5 mmol) of a freshly prepared 1 M solution in water was added, followed by the addition of copper (II) sulfate pentahydrate (0.05 mmol). After stirring for 24 h at room temperature, the reaction was poured into ice/water and the precipitate was collected by filtration.

4-(1-(5-Methoxy-5-oxopentyl)-1*H*-1,2,3-triazol-4-yl)benzoic acid (7h). White solid (0.96 g, 63%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 12.98 (s, 1H), 8.71 (s, 1H), 7.99 (s, 4H), 4.42 (t, J = 6.8 Hz, 2H), 3.57 (s, 3H), 2.36 (t, J = 7.5 Hz, 2H), 1.88 (q, J = 6.7 Hz, 2H), 1.51 (q, J = 7.4 Hz, 2H); Molecular formula $C_{15}H_{17}N_3O_4$; Mass required 304.12 [M + H]; Mass found ESI-MS (m/s): 304.43 [M + H].

4-(1-(6-Methoxy-6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)benzoic acid (7i). White solid (1.17 g, 74%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 12.98 (s, 1H), 8.73 (s, 1H), 8.01 (s, 4H), 4.43 (t, J = 7.0 Hz, 2H), 3.59 (s, 3H), 2.33 (t, J = 7.4 Hz, 2H), 1.90 (q, J = 7.4 Hz, 2H), 1.59 (q, J = 7.6 Hz, 2H), 1.30 (q, J = 7.8 Hz, 2H); Molecular formula $C_{16}H_{19}N_3O_4$; Mass required 318.15 [M + H]; Mass found ESI-MS (m/s): 318.61 [M + H].

4-(1-(7-Ethoxy-7-oxoheptyl)-1*H*-1,2,3-triazol-4-yl)benzoic acid (7j). White solid (1.16 g, 70%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 12.97 (s, 1H), 8.71 (s, 1H), 7.99 (s, 4H), 4.39 (t, J = 6.9 Hz, 2H), 4.02 (q, J = 7.0 Hz, 2H), 2.25 (t, J = 7.3 Hz, 2H), 1.85 (q, J = 6.9 Hz, 2H), 1.50 (q, J = 7.2 Hz, 2H), 1.25-1.30 (m, 4H), 1.15 (t, J = 7.0 Hz, 3H); Molecular formula $C_{18}H_{23}N_3O_4$; Mass required 346.17 [M + H]; Mass found ESI-MS (m/s): 346.53 [M + H].

***Tert*-butyl(2-(4-((2-((4-(4-(3-bromo-4-fluorophenyl)-5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1,2,5-oxadiazol-3-yl)amino)ethyl)carbamoyl)benzamido)phenyl)carbamate (8a).** A mixture of carboxylic acid **7a** (0.14 g, 0.4 mmol), intermediate **6** (0.20 g, 0.4 mmol), HATU (0.16 g, 0.4 mmol), DIPEA (0.21 mL, 1.2 mmol) and DMF (10 mL) was stirred for 24 h at room temperature. The reaction was poured into ice/water (100 mL), and extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with water, brine, dried over anhydrous Na_2SO_4, purified by column chromatography on SiO_2 (DCM: MeOH = 100: 1) to afford **8a** (0.14 g, 49%) as a white solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.93 (s, 1H), 8.77 (t, J = 5.5 Hz, 1H), 8.73 (s, 1H), 8.12 (dd, J = 6.3, 2.5 Hz, 1H), 8.06 (d, J = 8.4 Hz, 2H), 8.00 (d, J = 8.5

Hz, 2H), 7.73-7.76 (m, 1H), 7.61 (t, *J* = 8.8 Hz, 1H), 7.57 (t, *J* = 6.5 Hz, 2H), 7.24 (td, *J* = 7.6, 1.6 Hz, 1H), 7.18 (td, *J* = 7.9, 1.4 Hz, 1H), 6.71 (t, *J* = 5.7 Hz, 1H), 3.56-3.59 (m, 2H), 3.50-3.53 (m, 2H), 1.47 (s, 9H).

***N*^{1}-(2-Aminophenyl)-*N*^{4}-(2-((4-(*N*-(3-bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)amino)ethyl)terephthalamide (10).** A solution of intermediate **8a** (0.17 g, 0.24 mmol) in methanol (10 mL) was treated with 2M NaOH (1.2 mL, 2.4 mmol) and stirred at 40 ^{o}C for 2 h. The reaction mixture was quenched with 6N HCl to pH~7. The solid that precipitated was filtered and washed with water to give intermediate **9a** (0.14 g, 76%), which was used directly for the next step without further purification.

Intermediate **9a** (0.10 g, 0.14 mmol) was dissolved in DCM (15 mL). Trifluoroacetic acid (0.21 mL, 2.87 mmol) was added and the mixture was further stirred for 2 h at 40 ^{o}C. Then, the solution was concentrated in vacuo and the residue was acidified with saturated Na_2CO_3 solution. The solid that precipitated was filtered and purified by column chromatography on SiO_2 (DCM: MeOH = 100: 2) to afford **10** (0.06 g, 77%) as a white solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 11.45 (s, 1H), 9.77 (s, 1H), 8.89 (s, 1H), 8.77 (t, *J* = 5.7 Hz, 1H), 8.09 (s, 1H), 8.08 (s, 1H), 7.98 (s, 1H), 7.97 (s, 1H), 7.19 (t, *J* = 3.7 Hz, 1H), 7.18 (s, 1H), 7.14-7.16 (m, 1H), 7.00 (t, *J* = 7.7 Hz, 1H), 6.78-6.82 (m, 2H), 6.62 (t, *J* = 7.6 Hz, 1H), 6.37 (t, *J* = 6.1 Hz, 1H), 4.94 (s, 2H), 3.53-3.56 (m, 2H), 3.45-3.49 (m, 2H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ : 166.47, 165.18, 156.20, 155.01, 153.43, 143.72, 140.50, 139.73, 138.51, 137.37, 137.21, 128.24, 127.62, 127.27, 127.14, 125.30, 123.50, 121.93, 116.64, 116.47,

116.32, 107.53, 44.06, 38.63; Molecular formula $C_{25}H_{22}BrFN_8O_4$; HRMS (ESI, positive) m/z calcd for $C_{25}H_{23}BrFN_8O_4$ (M + H): 599.0989; found 599.0988; HPLC purity: 95.5%; melting point: 216-218 °C.

Starting from protected and substituted carboxylic acid 7**b**, compounds **11** were prepared according to the synthetic methods for compound **10**.

***N*^{2}-(2-Aminophenyl)-*N*^{5}-(2-((4-(*N*-(3-bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)amino)ethyl)pyridine-2,5-dicarboxamide (11).** White solid (0.16 g, 62%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 11.38 (s, 1H), 10.09 (s, 1H), 9.06 (s, 1H), 8.96 (t, *J* = 5.7 Hz, 1H), 8.85 (s, 1H), 8.39 (dd, *J* = 8.1, 2.1 Hz, 1H), 8.20 (d, *J* = 8.1 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.14 (t, *J* = 8.6 Hz, 1H), 7.10 (dd, *J* = 6.1, 2.5 Hz, 1H); 6.95 (t, *J* = 7.0 Hz, 1H), 6.81 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.74-6.77 (m, 1H), 6.63 (t, *J* = 6.9 Hz, 1H), 6.34 (t, *J* = 6.1 Hz, 1H), 4.89 (s, 2H), 3.51-3.55 (m, 2H), 3.42-3.46 (m, 2H); Molecular formula $C_{24}H_{21}BrFN_9O_4$; HRMS (ESI, positive) m/z calcd for $C_{24}H_{22}BrFN_9O_4$ (M + H): 600.0942; found 600.0958; HPLC purity: 94.1%; melting point: 198-200 °C.

Starting from protected and substituted carboxylic acid 7**c**, compounds **12** were prepared according to the synthetic methods for compound **10**.

***N*^{2}-(2-Aminophenyl)-*N*^{5}-(2-((4-(*N*-(3-bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)amino)ethyl)thiophene-2,5-dicarboxamide (12).** White solid (0.13 g, 64%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 11.43 (s, 1H), 9.83 (s, 1H), 8.87 (s, 1H), 8.78 (t, *J* = 4.9 Hz, 1H), 7.93 (s, 1H), 7.73 (d, *J* = 4.1 Hz, 1H), 7.17 (t, *J* = 8.9 Hz, 1H), 7.12-7.15 (m, 2H), 7.00 (t, *J* = 7.2 Hz, 1H), 6.82 (d, *J* = 7.9 Hz,

1H), 6.75-6.79 (m, 1H), 6.64 (t, *J* = 7.3 Hz, 1H), 6.34 (t, *J* = 6.0 Hz, 1H), 5.22 (s, 2H), 3.46-3.50 (m, 2H), 3.41-3.45 (m, 2H); Molecular formula $C_{23}H_{20}BrFN_8O_4S$; Mass required 605.06 [M + H]; Mass found ESI-MS (m/s): 605.27 [M + H]; HPLC purity: 92.1%; melting point: 208-210 °C.

Starting from protected and substituted carboxylic acid 7**d**, compounds **13** were prepared according to the synthetic methods for compound **10**.

***N*^{1}-(2-Aminophenyl)-*N*^{3}-(2-((4-(*N*-(3-bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)amino)ethyl)isophthalamide (13).** White solid (0.22 g, 71%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 11.41 (s, 1H), 9.88 (s, 1H), 8.84 (s, 1H), 8.76 (t, *J* = 5.3 Hz, 1H), 8.45 (s, 1H), 8.11 (d, *J* = 7.9 Hz, 1H), 8.00 (d, *J* = 7.7 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.21 (d, *J* = 7.6 Hz, 1H), 7.13 (t, *J* = 8.9 Hz, 1H), 7.10 (dd, *J* = 5.9, 2.5 Hz, 1H), 7.02 (t, *J* = 7.5 Hz, 1H), 6.87 (t, *J* = 7.7 Hz, 1H), 6.70-6.75 (m, 2H), 6.33 (t, *J* = 6.1 Hz, 1H), 3.98 (s, 2H), 3.49-3.52 (m, 2H), 3.40-3.44 (m, 2H); Molecular formula $C_{25}H_{22}BrFN_8O_4$; HRMS (ESI, positive) m/z calcd for $C_{25}H_{23}BrFN_8O_4$ (M + H): 599.0989; found 599.0997; HPLC purity: 96.1%; melting point: 181-182 °C.

Methyl 4-((2-((4-(4-(3-bromo-4-fluorophenyl)-5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1,2,5- oxadiazol-3-yl)amino)ethyl)carbamoyl)benzoate (8e). A mixture of 4-(methoxycarbonyl)benzoic acid (**7e**, 0.20 g, 1.1 mmol), intermediate **6** (0.56 g, 1.1 mmol), HATU (0.42 g, 1.1 mmol), DIPEA (0.58 mL, 3.3 mmol) in DMF (10 mL) was stirred for 24 h at room temperature. The reaction was poured into ice/water (100 mL), and extracted with EtOAc (2 × 100 mL). The combined organic layers were

washed with water (100 mL), brine (100 mL), dried over sodium sulfate, purified by column chromatography on SiO_2 (DCM: MeOH = 100: 1) to afford **8e** (0.32 g, 58%) as a white solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.79 (t, J = 5.4 Hz, 1H), 8.12 (dd, J = 6.2, 2.5 Hz, 1H), 8.06 (d, J = 8.3 Hz, 2H), 7.97 (d, J = 8.4 Hz, 2H), 7.73-7.76 (m, 1H), 7.76 (t, J = 8.8 Hz, 1H), 6.69 (t, J = 5.8 Hz, 1H), 3.90 (s, 3H), 3.54-3.57 (m, 2H), 3.48-3.51 (m, 2H); Molecular formula $C_{21}H_{16}BrFN_6O_6$; Mass required 547.04 [M + H]; Mass found ESI-MS (m/s): 547.32 [M + H].

***N*^{1}-(2-((4-(*N*-(3-Bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)ami-no)ethyl)-*N*^{4}-hydroxyterephthalamide (14).** To a stirred solution of hydroxylamine hydrochloride (4.67 g, 67 mmol) in MeOH (24 mL) was added a solution of KOH (5.61 g, 100 mmol) in MeOH (12 mL) dropwise at 0 ^{o}C. After addition, the mixture was stirred for 30 min at 0 ^{o}C. The precipitate was filtered and the filtrate formed a solution of free hydroxylamine in MeOH. Then, compound **8e** (0.10 g, 0.18 mmol) was dissolved in above freshly prepared solution of hydroxylamine in MeOH (15 mL). The mixture was stirred at room temperature for 45 min, and then adjusted to pH~7 with acetic acid. The mixture was concentrated and the residue was washed with water to afford compound **14** (0.07 g, 78%) as a white solid. ^{1}H NMR (CD_3OD-d_6, 600 MHz) δ : 7.88 (q, J = 8.4 Hz, 4H), 7.16 (q, J = 2.7 Hz, 1H), 7.05 (t, J = 8.7 Hz, 1H), 6.84-6.87 (m, 1H), 3.70 (t, J = 5.9 Hz, 2H), 3.58 (t, J = 6.0 Hz, 2H); ^{13}C NMR (CD_3OD-d_6, 150 MHz) δ : 168.69, 165.43, 156.04, 155.73, 154.44, 140.70, 139.39, 137.67, 136.52, 135.83, 127.10, 126.73, 126.43, 122.50, 115.30, 107.37, 43.61, 38.55; Molecular formula $C_{19}H_{17}BrFN_7O_5$; HRMS

(ESI, positive) m/z calcd for $C_{19}H_{18}BrFN_7O_5$ (M + H): 522.0537; found 522.0537; HPLC purity: 96.8%; melting point: 172-174 °C.

Starting from carboxylic acid 7**f** and intermediate **6**, compounds **15** were prepared according to the synthetic methods for compound **14**.

(*E*)-Methyl 3-(4-((2-((4-(4-(3-bromo-4-fluorophenyl)-5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)- 1,2,5-oxadiazol-3-yl)amino)ethyl)carbamoyl)phenyl)acrylate (8f). White solid (0.38 g, 53%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 8.68 (t, J = 5.5 Hz, 1H), 8.12 (dd, J = 6.2, 2.5 Hz, 1H), 7.87 (dd, J = 22.2, 8.5 Hz, 4H), 7.73-7.76 (m, 1H), 7.72 (d, J = 16.2 Hz, 1H), 7.62 (t, J = 8.6 Hz, 1H), 6.78 (d, J = 16.1 Hz, 1H), 6.70 (t, J = 5.7 Hz, 1H), 3.76 (s, 3H), 3.53-3.56 (m, 2H), 3.47-3.50 (m, 2H); Molecular formula $C_{23}H_{18}BrFN_6O_6$; Mass required 571.05 [M – H]; Mass found ESI-MS (m/s): 571.10 [M – H].

***N*-(2-((4-(*N*-(3-Bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)ami-no)ethyl)-4-((*E*)-3-(hydroxyamino)-3-oxoprop-1-en-1-yl)benzamide (15).** White solid (0.26 g, 63%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 11.43 (s, 1H), 10.78 (s, 1H), 9.08 (s, 1H), 8.86 (s, 1H), 8.65 (t, J = 5.0 Hz, 1H), 7.86 (s, 1H), 7.85 (s, 1H), 7.64 (s, 1H), 7.63 (s, 1H), 7.48 (d, J = 16.0 Hz, 1H), 7.14 (t, J = 8.9 Hz, 1H), 7.10-7.13 (m, 1H), 6.74-6.80 (m, 1H), 6.54 (d, J = 16.0 Hz, 1H), 6.32 (t, J = 5.8 Hz, 1H), 3.48-3.51 (m, 2H), 3.40-3.44 (m, 2H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ : 166.57, 162.82, 156.18, 154.95, 153.37, 140.51, 139.61, 138.55, 138.15, 135.15, 128.28, 127.68, 125.19, 121.84, 121.65, 116.46, 116.29, 107.57, 107.42, 44.09, 38.59. Molecular formula $C_{21}H_{19}BrFN_7O_5$; HRMS (ESI, positive) m/z calcd for

$C_{21}H_{20}BrFN_7O_5$ (M + H): 550.0673; found 550.0675; HPLC purity: 94.5%; melting point: 168-170 °C.

Starting from carboxylic acid 7**g** and intermediate **6**, compounds **16** were prepared according to the synthetic methods for compound **14**.

***N*-(2-((4-(*N*-(3-Bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)ami-no)ethyl)-3-((*E*)-3-(hydroxyamino)-3-oxoprop-1-en-1-yl)benzamide (16).** White solid (0.28 g, 74%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 11.40 (s, 1H), 10.77 (s, 1H), 9.05 (s, 1H), 8.85 (s, 1H), 8.67 (t, *J* = 5.4 Hz, 1H), 8.01 (s, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.45-7.50 (m, 2H), 7.12 (d, *J* = 8.9 Hz, 1H), 7.10 (dd, *J* = 6.0, 2.7 Hz, 1H), 6.73-6.76 (m, 1H), 6.52 (d, *J* = 15.65 Hz, 1H), 6.31 (t, *J* = 6.0 Hz, 1H), 3.47-3.51 (m, 2H), 3.39-3.43 (m, 2H); Molecular formula $C_{21}H_{19}BrFN_7O_5$; Mass required 550.06 [M + H]; Mass found ESI-MS (m/s): 549.97 [M + H]; HPLC purity: 97.0%; melting point: 174-176 °C.

Starting from carboxylic acid 7**h** and intermediate **6**, compounds **17** were prepared according to the synthetic methods for compound **14**.

Methyl 5-(4-(4-((2-((4-(4-(3-bromo-4-fluorophenyl)-5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1,2,5-oxadiazol-3-yl)amino)ethyl)carbamoyl)phenyl)-1H-1,2,3-triazol-1-yl)pentanoate (8h). White solid (0.23 g, 66%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 8.70 (s, 1H), 8.62 (t, *J* = 5.5 Hz, 1H), 8.11 (dd, *J* = 6.2, 2.5 Hz, 1H), 7.91-7.95 (m, 4H), 7.72-7.75 (m, 1H), 7.60 (t, *J* = 8.7 Hz, 1H), 6.69 (t, *J* = 5.6 Hz, 1H), 4.44 (t, *J* = 7.0 Hz, 2H), 3.59 (s, 3H), 3.53-3.56 (m, 2H), 3.47-3.50 (m, 2H), 2.38 (t, *J* = 7.4 Hz, 2H), 1.90 (q, *J* = 7.8 Hz, 2H), 1.30 (q, *J* = 7.7 Hz, 2H); Molecular

formula $C_{27}H_{25}BrFN_9O_6$; Mass required 670.12 [M + H]; Mass found ESI-MS (m/s): 670.43 [M + H].

(*Z*)-*N*-(2-((4-(*N*-(3-Bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)a-mino)ethyl)-4-(1-(5-(hydroxyamino)-5-oxopentyl)-1*H*-1,2,3-triazol-4-yl)benzamide (17). White solid (0.11 g, 83%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 11.42 (s, 1H), 10.36 (s, 1H), 8.87 (s, 1H), 8.67 (s, 2H), 8.63 (t, *J* = 5.5 Hz, 1H), 7.92 (s, 4H), 7.15 (t, *J* = 8.8 Hz, 1H), 7.12 (dd, *J* = 6.1, 2.8 Hz, 1H), 6.75-6.78 (m, 1H), 6.34 (t, *J* = 6.0 Hz, 1H), 4.41 (t, *J* = 7.0 Hz, 2H), 3.49-3.52 (m, 2H), 3.41-3.45 (m, 2H), 2.00 (t, *J* = 7.2 Hz, 2H), 1.82-1.87 (m, 2H), 1.47-1.52 (m, 2H), ^{13}C NMR (DMSO-d_6, 150 MHz) δ : 169.15, 166.69, 156.19, 155.50, 153.41, 146.01, 140.50, 139.71, 138.52, 133.92, 128.40, 125.27, 122.56, 121.96, 116.47, 116.31, 107.43, 49.78, 44.15, 38.55, 32.04, 29.66, 22.58; Molecular formula $C_{25}H_{26}BrFN_{10}O_5$; HRMS (ESI, positive) m/z calcd for $C_{25}H_{27}BrFN_{10}O_5$ (M + H): 645.1333; found 645.1332; HPLC purity: 93.7%; melting point: 145-146 °C.

Starting from carboxylic acid 7**i** and intermediate **6**, compounds **18** were prepared according to the synthetic methods for compound **14**.

(*Z*)-*N*-(2-((4-(*N*-(3-Bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)a-mino)ethyl)-4-(1-(6-(hydroxyamino)-6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)benzamide (18). White solid (0.14 g, 79%). ^{1}H NMR (CD_3OD-d_6, 600 MHz) δ : 8.44 (s, 1H), 7.90-7.96 (m, 4H), 7.15 (dd, *J* = 5.9, 2.7 Hz, 1H), 7.02 (t, *J* = 8.7 Hz, 1H), 6.83-6.86 (m, 1H), 4.49 (t, *J* = 7.0 Hz, 2H), 3.71 (t, *J* = 5.5 Hz, 2H), 3.58 (t, *J* = 5.7 Hz, 2H), 2.13 (t, *J* = 7.2 Hz, 2H), 1.98-2.02 (m, 2H), 1.68-1.72 (m, 2H), 1.37-1.43

(m, 2H); ^{13}C NMR (CD_3OD-d_6, 150 MHz) δ : 169.77, 167.44, 154.53, 154.25, 152.92, 144.92, 139.17, 137.90, 136.18, 132.10, 126.19, 124.91, 123.70, 121.00, 120.96, 120.14, 113.82, 105.88, 48.42, 42.20, 37.03, 30.56, 27.96, 24.00, 23.11; Molecular formula $C_{26}H_{28}BrFN_{10}O_5$; HRMS (ESI, positive) m/z calcd for $C_{26}H_{29}BrFN_{10}O_5$ (M + H): 661.1469; found 661.1477; HPLC purity: 97.6%; melting point: 160-162 °C.

Starting from carboxylic acid 7**j** and intermediate **6**, compounds **19** were prepared according to the synthetic methods for compound **14**.

Methyl 7-(4-(4-((2-((4-(4-(3-bromo-4-fluorophenyl)-5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1,2,5-oxadiazol-3-yl)amino)ethyl)carbamoyl)phenyl)-1*H*-1,2,3-triazol-1-yl)heptanoate (8j). White solid (0.17 g, 74%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 8.66 (s, 1H), 8.59 (t, *J* = 5.5 Hz, 1H), 8.08 (dd, *J* = 6.2, 2.5 Hz, 1H), 7.88-7.93 (m, 4H), 7.69-7.72 (m, 1H), 7.57 (t, *J* = 8.7 Hz, 1H), 6.66 (t, *J* = 5.7 Hz, 1H), 4.38 (t, *J* = 7.0 Hz, 2H), 4.01 (s, 3H), 3.50-3.54 (m, 2H), 3.44-3.48 (m, 2H), 2.25 (t, *J* = 7.3 Hz, 2H), 1.85 (q, *J* = 7.2 Hz, 2H), 1.49 (q, *J* = 7.4 Hz, 2H), 1.23-1.32 (m, 4H); Molecular formula $C_{29}H_{29}BrFN_9O_6$; Mass required 698.15 [M + H]; Mass found ESI-MS (m/s): 698.32 [M + H].

(*Z*)-*N*-(2-((4-(*N*-(3-Bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)amino)ethyl)-4-(1-(7-(hydroxyamino)-7-oxoheptyl)-1*H*-1,2,3-triazol-4-yl)benzamide (19). White solid (0.07 g, 69%).^{1}H NMR (CD_3OD-d_6, 600 MHz) δ : 8.42 (s, 1H), 7.87-7.93 (m, 4H), 7.12 (dd, *J* = 6.1, 2.8 Hz, 1H), 7.00 (t, *J* = 8.7 Hz, 1H), 6.80-6.83 (m, 1H), 4.46 (t, *J* = 7.0 Hz, 2H), 3.68 (t, *J* = 6.0 Hz, 2H), 3.55 (t, *J* = 5.6 Hz, 2H), 2.08 (t, *J* = 7.6 Hz, 2H), 1.94-2.00 (m, 2H), 1.59-1.64 (m, 2H), 1.35-1.42 (m,

4H); Molecular formula $C_{27}H_{30}BrFN_{10}O_5$; HRMS (ESI, positive) m/z calcd for $C_{27}H_{31}BrFN_{10}O_5$ (M + H): 673.1646; found 673.1640; HPLC purity: 93.9%; melting point: 167-169 °C.

Starting from carboxylic acid 7**k** and intermediate **6**, compounds **20** were prepared according to the synthetic methods for compound **14**.

Methyl 5-((2-((4-(4-(3-bromo-4-fluorophenyl)-5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1,2,5-oxadiazol-3-yl)amino)ethyl)amino)-5-oxopentanoate (8k). White solid (0.16 g, 85%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.11 (dd, J = 6.2, 2.5 Hz, 1H), 7.95 (t, J = 5.2 Hz, 1H), 7.72-7.75 (m, 1H), 7.61 (t, J = 8.7 Hz, 1H), 6.55 (t, J = 5.7 Hz, 1H), 3.59 (s, 3H), 3.2-3.33 (m, 4H), 2.30 (t, J = 7.5 Hz, 2H), 2.10 (t, J = 7.5 Hz, 2H), 1.74 (q, J = 7.3 Hz, 2H); Molecular formula $C_{18}H_{18}BrFN_6O_6$; Mass required 515.05 [M + H]; Mass found ESI-MS (m/s): 515.44 [M + H].

***N*^{1}-(2-((4-(*N*-(3-Bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)amino)ethyl)-*N*^{5}-hydroxyglutaramide (20).** Light yellow solid (0.09 g, 58%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 11.46 (s, 1H), 10.36 (s, 1H), 8.87 (s, 1H), 8.67 (s, 1H), 7.94 (s, 1H), 7.20 (t, J = 8.8 Hz, 1H), 7.13 (dd, J = 6.1, 2.7 Hz, 1H), 6.76-6.79 (m, 1H), 6.21 (t, J = 5.3 Hz, 1H), 3.28 (t, J = 7.0 Hz, 4H), 2.08 (t, J = 7.7 Hz, 2H), 1.97 (t, J = 7.7 Hz, 2H), 1.69-1.75 (m, 2H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ : 172.02, 168.78, 155.62, 154.54, 152.95, 139.94, 139.26, 138.02, 124.84, 121.48, 115.90, 107.00, 43.72, 37.33, 34.69, 31.72, 21.32; Molecular formula $C_{16}H_{19}BrFN_7O_5$; HRMS (ESI, positive) m/z calcd for $C_{16}H_{20}BrFN_7O_5$ (M + H): 490.0673; found 490.0675; HPLC purity: 92.1%; melting point: 118-120 °C.

Starting from carboxylic acid 7**l** and intermediate **6**, compounds **21** were prepared according to the synthetic methods for compound **14**.

Methyl 6-((2-((4-(4-(3-bromo-4-fluorophenyl)-5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1,2,5-oxadiazol-3-yl)amino)ethyl)amino)-6-oxohexanoate (8l). White solid (0.12 g, 61%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.10 (d, J = 4.1 Hz, 1H), 7.92 (s, 1H), 7.71-7.73 (m, 1H), 7.60 (t, J = 8.7 Hz, 1H), 6.53 (s, 1H), 3.57 (s, 3H), 3.27-3.32 (m, 4H), 2.28 (s, 2H), 2.05 (s, 2H), 1.48 (s, 4H); Molecular formula $C_{19}H_{20}BrFN_6O_6$; Mass required 529.07 [M + H]; Mass found ESI-MS (m/s): 529.13 [M + H].

***N*^{1}-(2-((4-(*N*-(3-Bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)amino)ethyl)-*N*^{6}-hydroxyadipamide (21).** Light yellow solid (0.06 g, 57%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 11.49 (s, 1H), 10.36 1H), 8.87 (s, 1H), 8.67 (s, 1H), 7.93 (s, 1H), 7.19 (t, J = 8.8 Hz, 1H), 7.13 (dd, J = 6.0, 2.6 Hz, 1H), 6.77-6.80 (m, 1H), 6.22 (s, 1H), 3.27 (s, 4H), 2.07 (s, 2H), 1.94 (s, 2H), 1.47 (s, 4H); Molecular formula $C_{17}H_{21}BrFN_7O_5$; Mass required 504.08 [M + H]; Mass found ESI-MS (m/s): 504.32 [M + H]; HPLC purity: 96.4%; melting point: 123-125 °C.

Starting from carboxylic acid 7**m** and intermediate **6**, compounds **22** were prepared according to the synthetic methods for compound **14**.

***N*^{1}-(2-((4-(*N*-(3-Bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)amino)ethyl)-*N*^{7}-hydroxyheptanediamide (22).** Light yellow solid (0.04 g, 63%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ : 11.46 (s, 1H), 10.31 (s, 1H), 8.86 (s, 1H), 8.64 (s, 1H), 7.91 (s, 1H), 7.18 (t, J = 8.8 Hz, 1H), 7.12 (dd, J = 6.0, 2.8 Hz, 1H),

6.76-6.78 (m, 1H), 6.21 (t, *J* = 5.4 Hz, 1H), 3.26 (t, *J* = 6.5 Hz, 4H), 2.05 (t, *J* = 7.5 Hz, 2H), 1.91 (t, *J* = 7.4 Hz, 2H), 1.43-1.48 (m, 4H), 1.19-1.23 (m, 2H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ : 172.98, 169.60, 156.11, 155.00, 153.42, 140.42, 139.72, 138.52, 125.28, 121.92, 116.40, 107.48, 44.25, 37.74, 35.73, 32.63, 28.74, 25.41; Molecular formula $C_{18}H_{23}BrFN_7O_5$; HRMS (ESI, positive) m/z calcd for $C_{18}H_{24}BrFN_7O_5$ (M + H): 518.0986; found 518.0994; HPLC purity: 98.2%; melting point: 129-130 °C.

Starting from carboxylic acid 7**n** and intermediate **6**, compounds **23** were prepared according to the synthetic methods for compound **14**.

***N*^{1}-(2-((4-(*N*-(3-Bromo-4-fluorophenyl)-*N*'-hydroxycarbamimidoyl)-1,2,5-oxadiazol-3-yl)ami-no)ethyl)-*N*^{8}-hydroxyoctanediamide (23).** Light yellow solid (0.13 g, 57%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 11.44 (s, 1H), 10.42 (s, 1H), 8.87 (s, 1H), 8.63 (s, 1H), 7.92 (s, 1H), 7.17 (t, *J* = 8.7 Hz, 1H), 7.10 (dd, *J* = 6.0, 2.6 Hz, 1H), 6.74-6.77 (m, 1H), 6.22 (s, 1H), 3.25 (s, 4H), 2.03 (t, *J* = 7.6 Hz, 2H), 1.91 (t, *J* = 7.2 Hz, 2H), 1.42-1.46 (m, 4H), 1.19 (s, 4H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ : 172.49, 168.98, 155.61, 154.45, 152.87, 139.93, 139.15, 138.04, 124.71, 121.35, 115.90, 107.00, 43.75, 37.24, 35.30, 32.15, 28.35, 28.30, 25.04, 24.96. Molecular formula $C_{19}H_{25}BrFN_7O_5$; HRMS (ESI, positive) m/z calcd for $C_{19}H_{26}BrFN_7O_5$ (M + H): 532.1142; found 532.1135; HPLC purity: 95.6%; melting point: 135-130 °C.

Molecular Modeling

Molecular modeling experiments were carried out with the Schrödinger Maestro 9.0 package. The crystal structure of IDO1 in complex with Amg-1 was obtained from

protein database bank (PDB ID 4PK5 or 4BKX)[2] and prepared for docking using Protein Preparation Wizard. During this process, waters were removed and hydrogens were added to the structure. The resulting structure was refined by OPLS3 force-field with the hydrogens only. Then, Receptor Grid Preparation was used to generate the protein grid for docking experiments. Amg-1 was picked for ligand identification. Fe in heme was selected which could participate in metal-ligand interactions. Other parameters were set default. Ligand Docking option was used for docking performance. Under Setting, SP (standard precision), "Dock flexibly", "Sample nitrogen inversions", "Sample ring conformation" and "Epik state penalties" to docking score were selected.

Conformations were generated and scored using glide score as fitness function. The best conformation was chosen to analyse the ligand–protein interaction.

HDAC1 Enzyme Activity Assay.

The HDAC1 enzyme was purchase from Abcam (#AB101661). The enzymatic reactions were conducted at 37 ^{o}C for 30 minutes. The reaction mixture contained 25 mM Tris (pH 8.0), 1 mM $MgCl_2$, 0.1 mg/ml BSA, 137 mM NaCl, 2.7 mM KCl, HDAC1 and the enzyme substrate in a final volume of 50 µL. The HDAC1 protein was incubated with a commercially available fluorogenic HDAC1 substrate (#BPS50037) at a concentration equivalent to the substrate Km (8 µM for HDAC1). The compounds were diluted in 10% DMSO and 5 µL of the dilution was added to a 50 µL reaction so that the final concentration of DMSO was 1% in all of reactions. The assay was performed by quantitating the fluorescent product amount of in

solution following an enzyme reaction. Fluorescence was then analyzed with an excitation of 350-360 nm and an emission wavelength of 450-460 nm at Spectra Max M5 microtiter plate reader. The IC_{50} values were calculated using nonlinear regression with normalized dose-response fit using Prism GraphPad sofeware. *K*i values were calculated based on the Cheng-Prusoff equation, *K*i = IC_{50}/(1 + ([S]/*K*m). [S], substrate concentration; *K*m, Michaelis constant.

HDAC2, HDAC3, HDAC4, HDAC6 and HDAC8 Enzyme Activity Assays.

All of the enzymatic reactions were conducted at 37 ^{o}C for 30 minutes. The 50 uL reaction mixture contains 25 mM Tris, pH 8.0, 1 mM $MgCl_2$, 0.1 mg/ml BSA, 137mM NaCl, 2.7 mM KCl, HDAC and the enzyme substrate. The compounds were diluted in 10% DMSO and 5 uL of the dilution was added to a 50 uL reaction so that the final concentration of DMSO is 1% in all of reactions. The assay was performed by quantitating the fluorescent product amount of in solution following a enzyme reaction. Fluorescence is then analyzed with an excitation of 350-360 nm and an emission wavelength of 450-460 nm at SpectraMax M5 microtiter plate reader. The IC50 values were calculated using nonlinear regression with normalized dose-response fit using Prism GraphPad sofeware.

Assay	Enzyme Used (ng) / Reaction	Substrate
HDAC2	7.5	10 µM HDAC Substrate 3
HDAC3/NCOR2	1.3	10 µM HDAC Substrate 3
HDAC4	0.05	2 mM HDAC Substrate Class 2a
HDAC6	15	10 µM HDAC Substrate 3

HDAC8	22	2 μM HDAC Substrate 3

Determination of *in vitro* metabolic stability in male mouse liver microsome.

Incubation mixtures consisted of microsome protein (final: 0.5 mg microsome protein/mL), compound **10** in acetonitrile (final: 1 μM), and phosphate buffer (pH 7.4). The mixture was first shaken for 5 min for preincubation in a shaking water bath at 37 ^{o}C. Reaction was initiated by adding NADPH to obtain a final concentration of 2 mM NADPH in the mixture. The total volume of the reaction mixture was 500 μL. For metabolic stability studies, aliquots of 50 μL of the incubation sample mixture were collected at 0, 5, 10, 15, 30, 45 and 60 min. After collection of samples, the reaction was terminated with 100 μL of chilled acetonitrile containing the internal standard. The mixture was then centrifuged at 10,000 X g to remove the protein and the supernatant was subsequently applied to LC-MS/MS analysis.

IDO1 Enzyme Activity Assay.[3]

IDO1 catalyzes the oxidative cleavage of the pyrrole ring of the indole nucleus of tryptophan to yield *N*'-formylkynurenine. The assays were performed at room temperature as described in the literature using 20 nM IDO1 (NOVUS BioscienceInc. # H00003620-P01) and 2 mM D-Trp in the presence of 20 mM ascorbate, 3.5 uM methylene blue and 0.2 mg/mL catalase in 50 mM potassium phosphate buffer (pH 6.5). The initial reaction rates were recorded by continuously following the absorbance increase at 321 nm due to the formation of *N*'-formlylkynurenine. The IC_{50} values were calculated using nonlinear regression with normalized dose-response

fit using Prism GraphPad software.

IDO1 Cellular Assay.[3]

HeLa cells were seeded in 96-well culture plates at a density of 6×10^3 per well. On the next day, human IFN-γ (Sangon Biotech # C600039, 50 ng/mL) and compounds in a total volume of 200 μL culture medium containing 15 μg/mL of L-tryptophan were added to the cells. After incubation for 48 hours, 140 μL of the supernatant was mixed with 10 μL of 6.1N trichloroacetic acid and the mixture was incubated for 30 min at 50 ^{o}C to hydrolyze *N*'-formlylkynurenine produced by IDO1 to kynurenine. The reaction mixture was then centrifuged for 10 minutes at 2500 rpm to remove sediments. 100 μL of the supernatant was mixed with 100 μL of 2% (w/v) p-dimethylaminobenzaldehyde in acetic acid and measured at 480 nm. The data was processed using nonlinear regression to generate EC_{50} values (Prism Graphpad).

***In vitro* Cytotoxicity Assay.**

This assay was determined by the Cell Counting Kit-8 (CCK-8) method. Cells were plated in 96-well microtiter plates at a density of $6\sim10 \times 10^3$/well and incubated in a humidified atmosphere with 5% CO_2 at 37 ^{o}C for 24 h. Test compounds were added onto triplicate wells with different concentrations and 0.1% DMSO for control. After they had been incubated for 48 h, 10 μL of the CCK-8 solution was added to each well and the plate was incubated for additional 1-4 h in the incubator. The absorbance (OD) was read on a WellscanMK-2 microplate reader (Labsystems) at 450 nm. The concentration causing 50% inhibition of cell growth (IC_{50}) was determined by the Logit method. All experiments were performed three times.

***In vitro* Cell Apoptosis Assay.**

HCT-116 (obtained from Shcellbank, 3×10^5 /well) cells were incubated in six-well plates for 24 h, and then treated with 0.1% DMSO (as control), various concentrations of compounds **10**, **15** or **23** in fresh growth medium. After 48 h, the cells were then harvested by trypsinization and washed twice with cold PBS. After centrifugation and removal of the supernatants, cells were resuspended in 400 μL of 1 × binding buffer, which was then added to 5 μL of annexin V-FITC and incubated at room temperature for 15 min. After adding 10 μL of PI, the cells were incubated at room temperature for another 15 min in the dark. The stained cells were analyzed by a flow cytometer (BD Accuri C6).

***In vitro* Cell Cycle Assay.**

HCT-116 (3×10^5 /well) cells were incubated in six-well plates (Corning) for 24 h, and then treated with 0.1% DMSO (as control), various concentrations of compounds **10**, **15** or **23** for 0-48 h. The treated cells were collected, resuspended, and incubated for 30 min at 37 ^{o}C with 25 μg/mL PI and 10 μg/mL RNase buffer. For each sample, at least 1×10^4 cells were analyzed using flow cytometry (BD Accuri C6).

Western Blotting Analysis.

HCT-116 (3×10^6/well) cells were incubated in six-well plates for overnight, and then treated with DMSO (as solvent control), the different concentration 1, 5 and 10 μM of **10**. After 12 h, the cells were harvested and washed with PBS (0.1 M, pH 7.4), centrifuged, and resuspended in cell lysis solution containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1% Triton X-100 and several protein inhibitors such as sodium

pyrophosphate, b-glycerophosphate, EDTA, Na_3PO_4 and leupeptin (Beyotime Biotech, China) for 30 min, then centrifuged for 15 min at 12 000 rpm at 4 ^oC, and the supernatant was the whole-cell extracts.Total proteinextracts (20 μg per lane) were separated by 10% SDS polyacrylamide gel electrophoresis and transferred onto PVDF membranes (Cat. IPVH00010, Millipore). Membrane was blocked with 5% BSA in TBS-T (10 mM Tris [pH 7.4], 150 mM NaCl, and 0.1% Tween20) for 1h at room temperature, then incubated with a 1:10000 dilution of primary antibody overnight at 4 ^oC. Then the membrane was washed 10 min (×3) and incubated at 1:5000 dilution of antirabbit secondary antibodies for 1.5 h at room temperature. After washing with TBST for 3 times, blots were scanned on a LI-COR Odyssey imaging system. The protein levels were quantified by the gray values of the bands in the resulting images using the control group as the standard.

***In vivo* Pharmacokinetic Assay.**

Male Sprague-Dawley rats, weighing 250–350 g each (8–10 weeks old), were purchased from Shanghai Laboratory Animal Center, SLAC. A single 100 mg/kg dose of compound **2** or **10** was administered to group of 3 rats each oral gavage (po). A single 2 mg/kg dose of compound **10** was administered to group of 3 rats each oral gavage (iv). At 0 (prior to dosing), 30 min,1 h, 2 h, 4 h, 8 h and 24 h after dosing, blood was collected via the jugular-vein cannula into pre-cold EDTA-3K tubes. Blood sample was centrifuged at 4 ^oC (2000 g, 5 min) to obtain plasma within 15 min after sample collection. Then 60 μL homogenized solution added with 240 μL IS (500 ng/mL) in MeOH-1% trifluoroacetic acid. The mixture was vortexed for 5 min and

centrifuged at 5800 rpm for 10 min. Aliquots (50 μL) of supernatant were diluted with aliquots (50 μL) H_2O and then an aliquot of 2 μL supernatant was injected onto the LC-MS/MS (API 6500) system. Data were acquired via multiple reactions monitoring. Plasma concentration–time data were analyzed by non-compartmental analysis to obtain pharmacokinetic parameters.

***In vivo* Pharmacodynamic Assay.**

Compound **10** at a dose of 100 mg/kg were administered by oral gavage to C57BL/6 male mice (certificate SCXK-2013-0018, n = 4 animals/group). Food will be removed for 2 h prior to study start and animals will be kept off food for the duration of the study. At various time points, mice were euthanized and blood was collected via retro-orbital puncture (under anesthesia with Isoflurane) into pre-cold EDTA-3K tubes. Blood sample was centrifuged at 4 ^{o}C (2000 g, 5 min) to obtain plasma within 15 min after sample collection. Then 60 μL homogenized solution added with 240 uL IS (500 ng/mL) in MeOH-1% trifluoroacetic acid. The mixture was vortexed for 5 min and centrifuged at 5800 rpm for 10 min. Aliquots (50 μL) of supernatant were diluted with aliquots (50 μL) H_2O and then an aliquot of 2 μL supernatant was injected onto the LC-MS/MS (API 6500) system. The levels of kynurenine (kyn) were measured. All experimental protocols were approved by the institutional Animal Studies Committee, and all murine experiments were conducted in compliance with institutional guidelines for the use of research animals.

***In vivo* Antitumor Activity Assay.**

C57BL/6 female mice (certificate SCXK-2013-0016, weighing 18~20 g) were

purchased from Shanghai Laboratory Animal Center, SLAC. The LLC cancer cells used for implantation were harvested during log phase growth and resuspended in phosphate-buffer saline. Each mice were inoculated s.c. into the right forelimb with 1 × 10^6 tumor cells. When tumor volumes approached ~50 mm^3, the mice were assigned into groups randomly and treated orally with compound **10**, SAHA, or INCB24360, and the blank control group received an equal volume of water containing 0.5% carboxyl methyl cellulose and 0.5% Tween 80 twice every day. Compounds were reconstituted in 0.5% carboxyl methyl cellulose and 0.5% Tween 80 in water. Tumor volumes were monitored by caliper measurement of the length and width and calculated using the formula of TV = $^1/_2 \times a \times b^2$, where a is the tumor length and b is the width. Tumor volumes and body weights were monitored every 4 days over the course of treatment. After 14 days of treatment, the mice were sacrificed. All experimental protocols were approved by the institutional Animal Studies Committee, and all murine experiments were conducted in compliance with institutional guidelines for the use of research animals.

References

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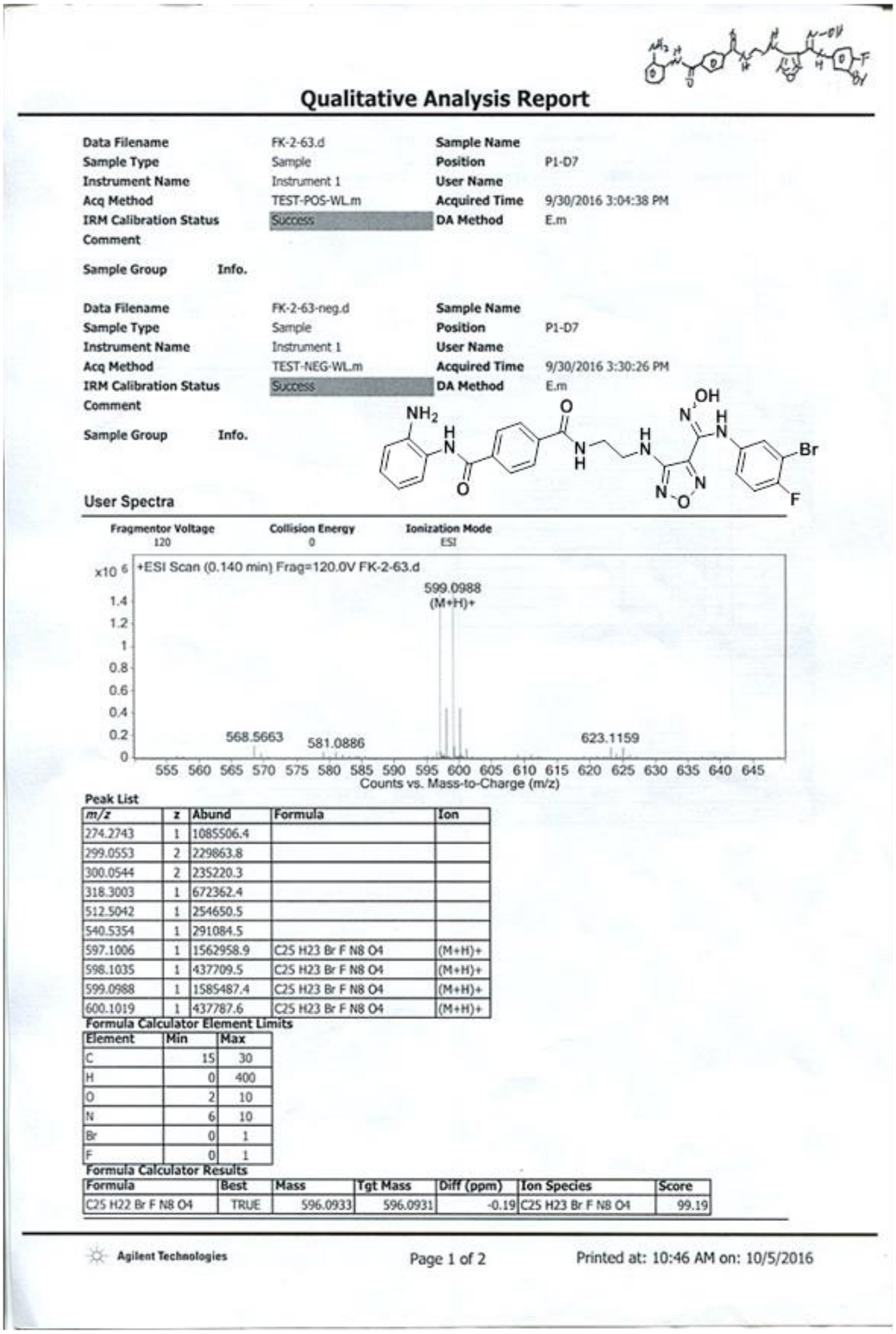
Copies of the HRMS for representative compounds



Qualitative Analysis Report

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Comment			
Sample Group	**Info.**		

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Comment			
Sample Group	**Info.**		

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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Peak List

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299.0553	2	229863.8		
300.0544	2	235220.3		
318.3003	1	672362.4		
512.5042	1	254650.5		
540.5354	1	291084.5		
597.1006	1	1562958.9	C25 H23 Br F N8 O4	(M+H)+
598.1035	1	437709.5	C25 H23 Br F N8 O4	(M+H)+
599.0988	1	1585487.4	C25 H23 Br F N8 O4	(M+H)+
600.1019	1	437787.6	C25 H23 Br F N8 O4	(M+H)+

Formula Calculator Element Limits

Element	Min	Max
C	15	30
H	0	400
O	2	10
N	6	10
Br	0	1
F	0	1

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C25 H22 Br F N8 O4	TRUE	596.0933	596.0931	-0.19	C25 H23 Br F N8 O4	99.19

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Qualitative Analysis Report

Data Filename	F11.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A4
Instrument Name	Instrument 1	**User Name**	
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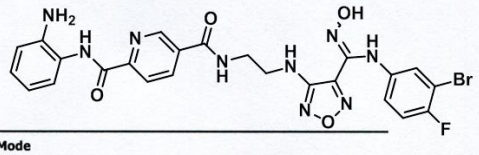
IRM Calibration Status	Success	**DA Method**	E.m
Comment			
Sample Group	**Info.**		



User Spectra

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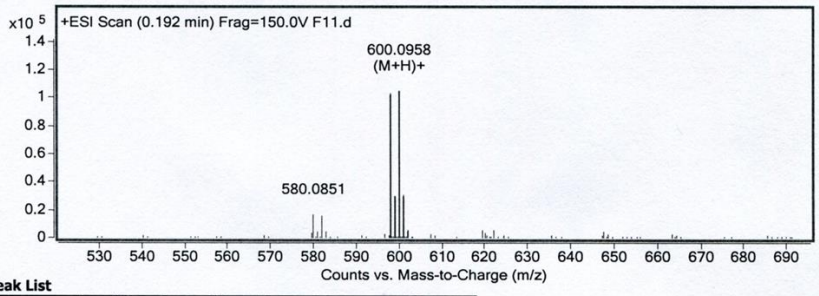

Peak List

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300.5525	2	47418		
598.0975	1	103294.4	C24 H22 Br F N9 O4	(M+H)+
599.1003	1	28592.6	C24 H22 Br F N9 O4	(M+H)+
600.0958	1	104848.5	C24 H22 Br F N9 O4	(M+H)+
601.0967	1	30879.3	C24 H22 Br F N9 O4	(M+H)+
922.0098		24530.3		

Formula Calculator Element Limits

Element	Min	Max
C	0	100
H	0	100
O	0	20
N	7	9
Br	0	1
F	0	1

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
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--- End Of Report ---

Qualitative Analysis Report

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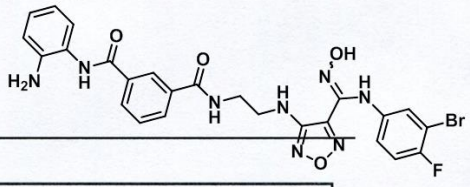
Comment			

Sample Group **Info.**



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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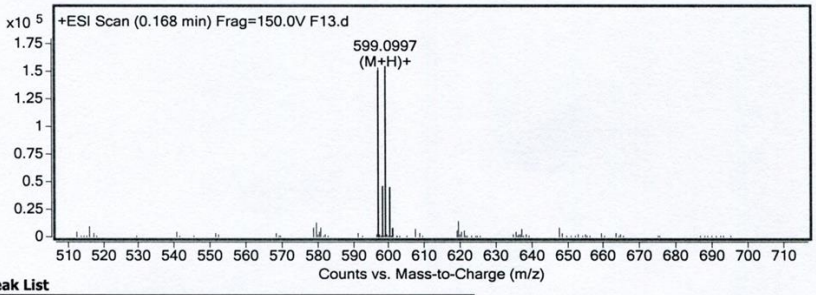

Peak List

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437.1939	1	66623.6		
453.1674	1	91591		
597.1016	1	153864.4	C25 H23 Br F N8 O4	(M+H)+
598.1042	1	41925.4	C25 H23 Br F N8 O4	(M+H)+
599.0997	1	154741	C25 H23 Br F N8 O4	(M+H)+
600.1018	1	45480.3	C25 H23 Br F N8 O4	(M+H)+

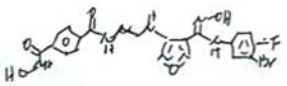
Formula Calculator Element Limits

Element	Min	Max
C	0	100
H	0	100
O	0	20
N	8	8
Br	0	1
F	0	1
S	0	1

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C25 H22 Br F N8 O4	TRUE	596.0942	596.0931	-1.72	C25 H23 Br F N8 O4	97.46

--- End Of Report ---



Qualitative Analysis Report

Data Filename	FK-2-75.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-D6
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	9/30/2016 3:02:50 PM
IRM Calibration Status	Success	**DA Method**	E.m
Comment			

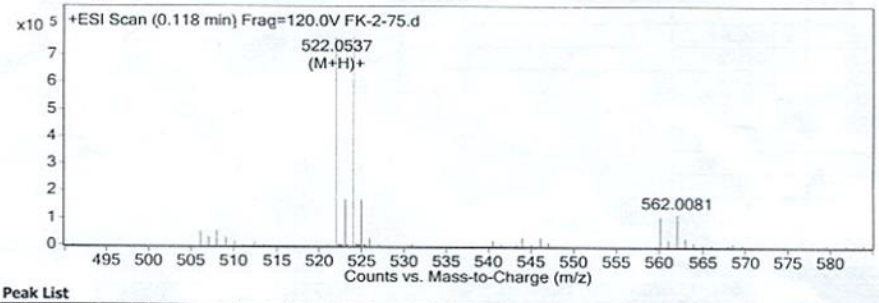
Sample Group **Info.**

Data Filename	FK-2-75-neg.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-D6
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-NEG-WL.m	**Acquired Time**	9/30/2016 3:28:48 PM
IRM Calibration Status	Success	**DA Method**	E.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI

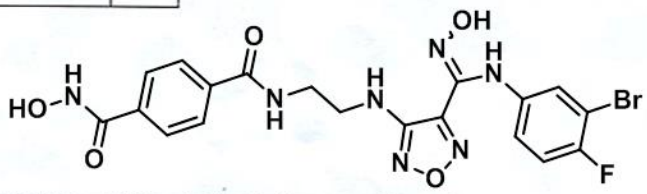


Peak List

m/z	z	Abund	Formula	Ion
274.2748		207078.3		
453.1684	1	351079		
454.1713	1	90252.8		
522.0537	1	770069.6	C19 H18 Br F N7 O5	(M+H)+
523.0574	1	170299.8	C19 H18 Br F N7 O5	(M+H)+
524.0522	1	767054.8	C19 H18 Br F N7 O5	(M+H)+
525.0552	1	168758.4	C19 H18 Br F N7 O5	(M+H)+
560.0096		105117.7		
562.0081		119610.5		
599.2947		124791.6		

Formula Calculator Element Limits

Element	Min	Max
C	15	20
H	0	400
O	2	10
N	5	10
Br	0	1
F	0	1

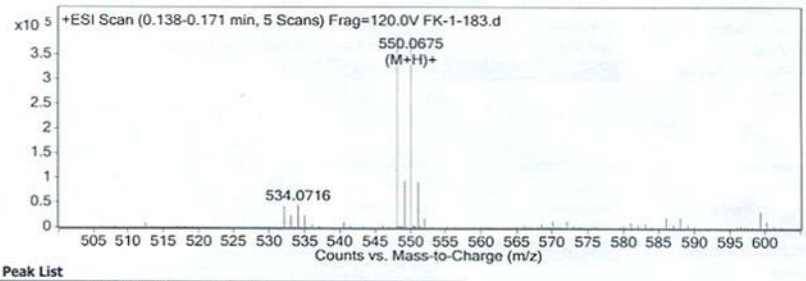


Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C19 H17 Br F N7 O5	TRUE	521.0466	521.0459	-1.39	C19 H18 Br F N7 O5	97.73

Qualitative Analysis Report

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
121.0509		68302.6		
274.2743		106374.8		
534.0716		44140.1		
548.0689	1	387928.9	C21 H20 Br F N7 O5	(M+H)+
549.0721	1	94985.8	C21 H20 Br F N7 O5	(M+H)+
550.0675	1	388614.4	C21 H20 Br F N7 O5	(M+H)+
551.0701	1	92122.2	C21 H20 Br F N7 O5	(M+H)+
922.0098		52418.8		

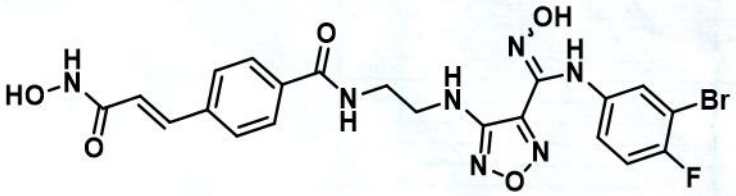
Formula Calculator Element Limits

Element	Min	Max
C	20	27
H	0	400
O	5	10
N	6	10
Br	0	1
F	0	1

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C21 H19 Br F N7 O5	TRUE	547.0618	547.0615	-0.56	C21 H20 Br F N7 O5	99.28

--- End Of Report ---



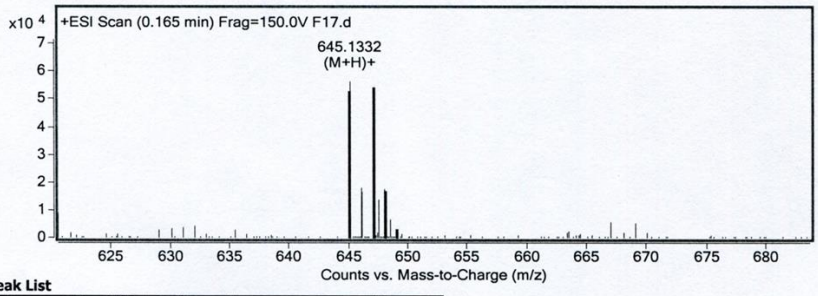
Qualitative Analysis Report

Data Filename	F17.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A8
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	2/3/2018 4:43:25 PM
IRM Calibration Status	Success	**DA Method**	E.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
150	0	ESI

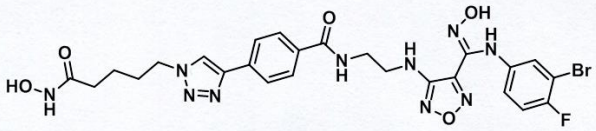


Peak List

m/z	z	Abund	Formula	Ion
121.0509		54580.9		
122.9637		62157		
274.2744		48170.2		
302.3051		20235.7		
437.194	1	55010.1		
453.1676		22775.2		
619.5267		20347.6		
645.1332	1	56430.6	C25 H27 Br F N10 O5	(M+H)+
647.1325	1	54175.3	C25 H27 Br F N10 O5	(M+H)+
922.0098		24342.6		

Formula Calculator Element Limits

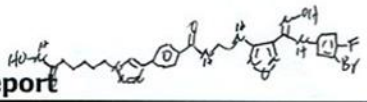
Element	Min	Max
C	0	100
H	0	100
O	0	20
N	10	10
Br	0	1
F	0	1



Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C25 H26 Br F N10 O5	TRUE	644.1263	644.1255	-1.28	C25 H27 Br F N10 O5	98.04

--- End Of Report ---



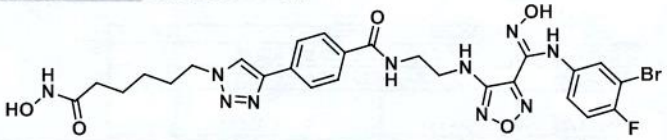
Qualitative Analysis Report

Data Filename	FK-2-97.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-D9
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	9/30/2016 3:07:53 PM
IRM Calibration Status	Success	**DA Method**	E.m
Comment			

Sample Group **Info.**

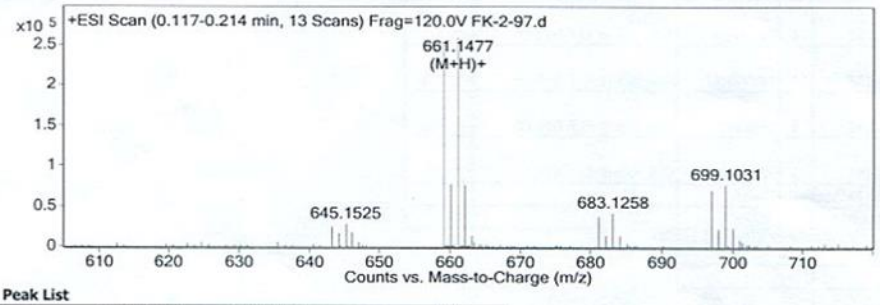
Data Filename	FK-2-97-neg.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-D9
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-NEG-WL.m	**Acquired Time**	9/30/2016 3:33:42 PM
IRM Calibration Status	Some Ions Missed	**DA Method**	E.m
Comment			

Sample Group **Info.**



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
274.2743	1	391127.3		
302.3056	1	83769.5		
318.3006	1	203607.8		
512.5041	1	108856.3		
540.5356	1	125043.3		
659.1493	1	250954.4	C26 H29 Br F N10 O5	(M+H)+
660.1517	1	77371.2	C26 H29 Br F N10 O5	(M+H)+
661.1477	1	258888	C26 H29 Br F N10 O5	(M+H)+
662.1498	1	75956.1	C26 H29 Br F N10 O5	(M+H)+
699.1031	1	76646.3		

Formula Calculator Element Limits

Element	Min	Max
C	15	27
H	0	400
O	2	10
N	6	10
Br	0	1
F	0	1

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C26 H28 Br F N10 O5	TRUE	658.142	658.1412	-1.33	C26 H29 Br F N10 O5	98.43

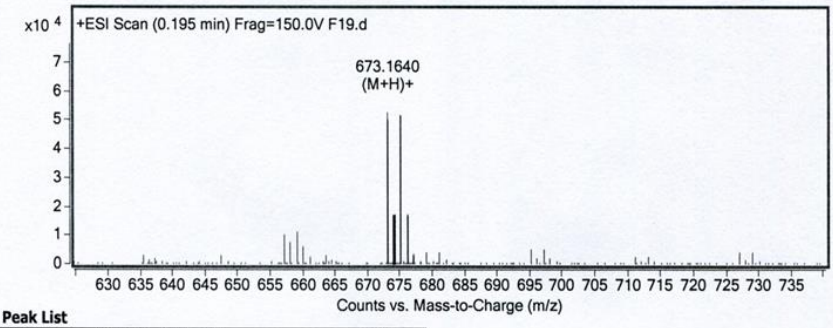
Qualitative Analysis Report

Data Filename	F19.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A9
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	2/3/2018 4:44:59 PM
IRM Calibration Status	Success	**DA Method**	E.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
150	0	ESI

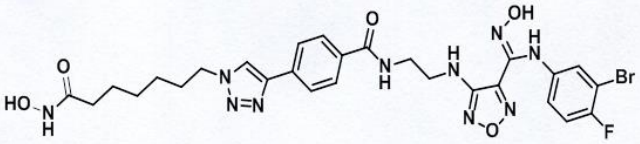


Peak List

m/z	Abund	Formula	Ion
121.0509	71154.2		
122.9641	58140.2		
673.164	52801.2	C27 H31 Br F N10 O5	(M+H)+
675.1632	51880.2		

Formula Calculator Element Limits

Element	Min	Max
C	0	100
H	0	100
O	0	20
N	10	10
Br	0	1
F	0	1



Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C27 H30 Br F N10 O5	TRUE	672.1571	672.1568	-0.4	C27 H31 Br F N10 O5	98.45

--- End Of Report ---

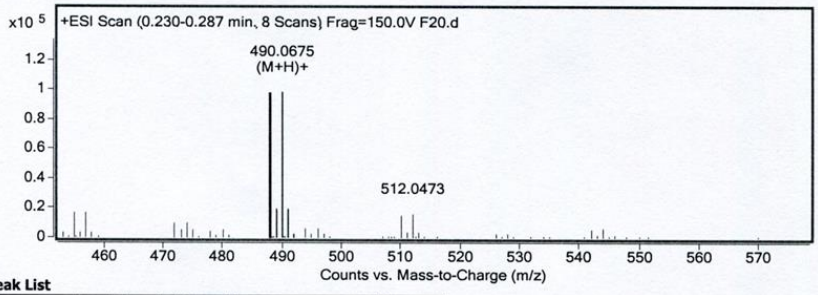
Qualitative Analysis Report

Data Filename	F20.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A7
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	2/3/2018 4:41:50 PM
IRM Calibration Status	Success	**DA Method**	E.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
150	0	ESI

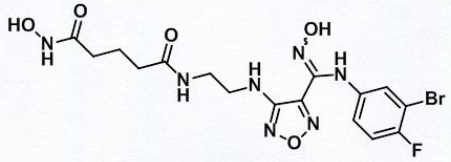


Peak List

m/z	z	Abund	Formula	Ion
121.051		70073.6		
122.9641		49105.1		
455.0473		16664.5		
457.0458		16434.7		
488.0694	1	96150.3	C16 H20 Br F N7 O5	(M+H)+
489.0718	1	19433.9	C16 H20 Br F N7 O5	(M+H)+
490.0675	1	98023.4	C16 H20 Br F N7 O5	(M+H)+
491.0698	1	17904.7	C16 H20 Br F N7 O5	(M+H)+
512.0473		16097.3		
922.0101		25762.4		

Formula Calculator Element Limits

Element	Min	Max
C	0	100
H	0	100
O	0	20
N	7	7
Br	0	1
F	1	1



Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C16 H19 Br F N7 O5	TRUE	487.0621	487.0615	-1.21	C16 H20 Br F N7 O5	99.07

--- End Of Report ---

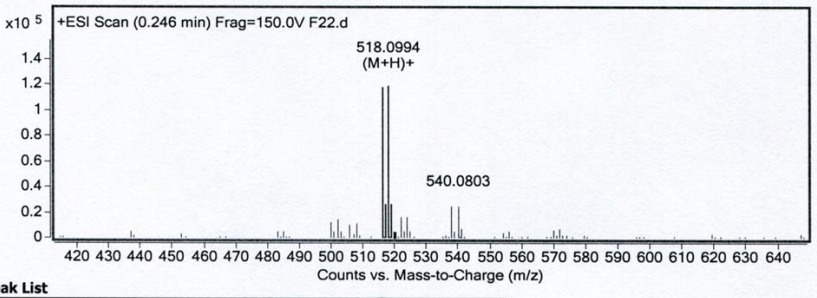
Qualitative Analysis Report

Data Filename	F22.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B1
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	2/3/2018 4:46:34 PM
IRM Calibration Status	Success	**DA Method**	E.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
150	0	ESI

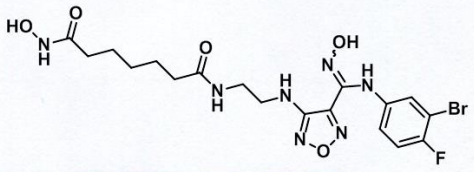


Peak List

m/z	z	Abund	Formula	Ion
121.0509		69798.1		
122.9642		47706.9		
516.1009	1	114266.2	C18 H24 Br F N7 O5	(M+H)+
517.104	1	23416	C18 H24 Br F N7 O5	(M+H)+
518.0994	1	119018.6	C18 H24 Br F N7 O5	(M+H)+
519.1023	1	22307.9	C18 H24 Br F N7 O5	(M+H)+
538.0827		24820.9	C18 H23 Br F N7 Na O5	(M+Na)+
540.0803		24963.6		
922.0098		26217.5		

Formula Calculator Element Limits

Element	Min	Max
C	0	100
H	0	100
O	0	20
N	7	7
Br	0	1
F	0	1



Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C18 H23 Br F N7 O5	TRUE	515.0938	515.0928	-1.94	C18 H24 Br F N7 O5	96.97
C18 H23 Br F N7 O5	TRUE	515.0931	515.0928	-0.62	C18 H23 Br F N7 Na O5	95.44

--- End Of Report ---

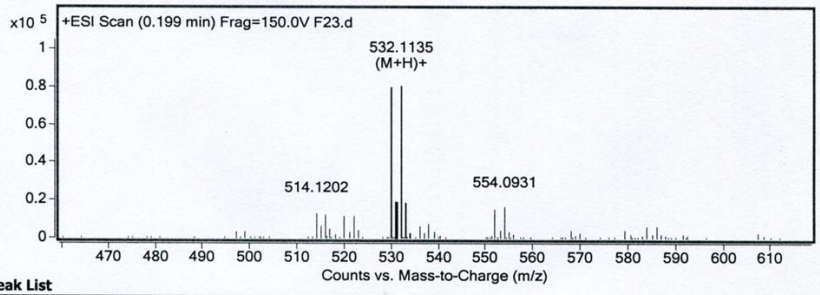
Qualitative Analysis Report

Data Filename	F23.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B2
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	2/3/2018 4:48:08 PM
IRM Calibration Status	Success	**DA Method**	E.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
150	0	ESI

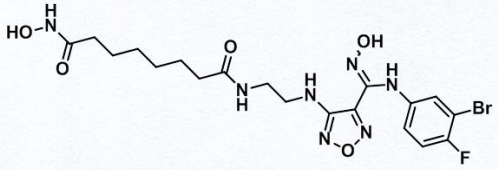


Peak List

m/z	z	Abund	Formula	Ion
121.0509		57670.1		
122.9636		57260.5		
274.2736		16886		
338.3412	1	38883.8		
437.1933		21683.6		
530.1158	1	80103.3	C19 H26 Br F N7 O5	(M+H)+
531.1171	1	19177.1	C19 H26 Br F N7 O5	(M+H)+
532.1135	1	80555.2	C19 H26 Br F N7 O5	(M+H)+
533.1159	1	17132.2	C19 H26 Br F N7 O5	(M+H)+
922.0098		22204		

Formula Calculator Element Limits

Element	Min	Max
C	0	100
H	0	100
O	0	20
N	7	7
Br	0	1
F	0	1



Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C19 H25 Br F N7 O5	TRUE	529.1082	529.1085	0.42	C19 H26 Br F N7 O5	97.97

--- End Of Report ---