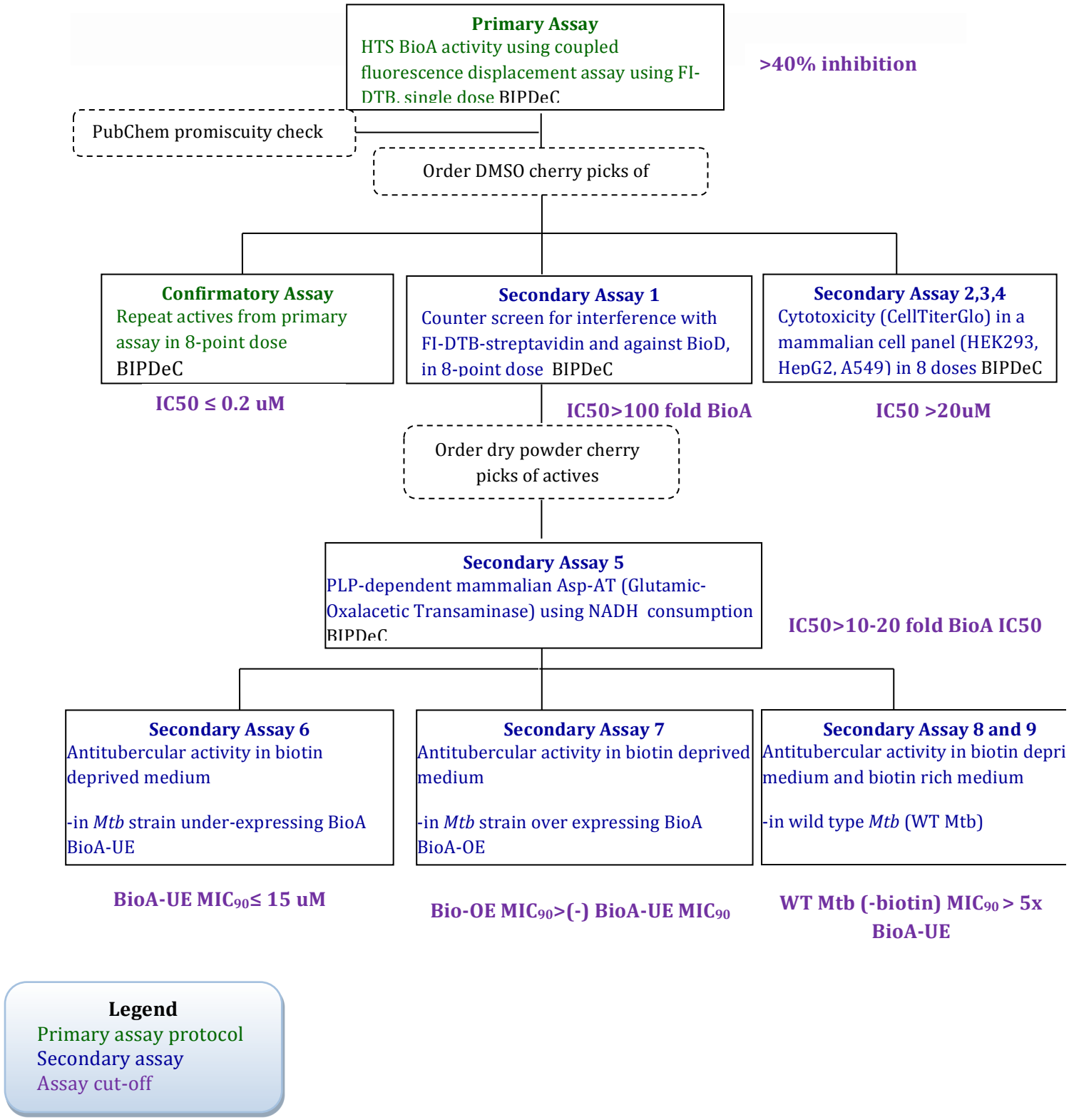
Figure S1. Assay Tree (Related to Figure 3).



Supplemental Table S1 (Related to Table 1). Sheet 1: Complete List of BioA hits from Primary HTS. Sheet 2: Source of Compound and Smiles String. Sheet 3: Scaffold Analysis.

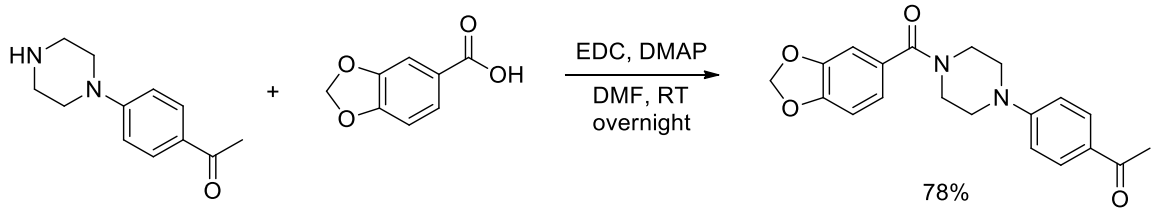
see Excel Spreadsheet

Supplemental Table S2 (Related to Figure 5). Summary of Crystallographic data and Refinement Statistics

Inhibitor	**18**	**15**	**14-Cl**
PDB Accession Code	4W1V	4W1W	4W1X
Cell dimensions			
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a, *b*, *c* (Å)	63.04, 66.24, 204.24	63.08, 66.21, 204.44	63.13, 66.04, 203.56
Molecules per ASU	2	2	2
Data Summary			
X-Ray source	Rigaku 007-MaxFlux	ALS	ALS
Detector	Saturn 944$^+$ CCD	NOIR-1 CCD	NOIR-1 CCD
Wavelength (Å)	1.541	1.000	1.000
Resolution (Å) (highest shell)	102.12-2.24 (2.14-2.24)	63.08-1.80 (1.90-1.80)	47.33-1.80 (1.86-1.80)
R_{merge}	0.089 (0.471)	0.076 (0.560)	0.098 (0.529)
Mean I/σ_I	10.2 (1.7)	19.8 (2.6)	9.2 (1.6)
Completeness	96.1% (81.2%)	90.5% (58.7%)	96.8% (79.0%)
Multiplicity	3.20 (1.90)	6.6 (4.2)	6.06 (2.89)
Observations	133962	477296	468895
Unique reflections	41863	72681	77334
Mosaicity	0.14	0.12	0.4
Refinement			
Resolution (Å)	28.189-2.244	102.00-1.90	47.33-1.80
R_{work}/R_{free}	0.1768/0.2086	0.182/0.221	0.193/0.223
Modeled atoms	6938	7422	7138
Modeled water	383	738	481
Average B value (Å^2)			
Protein atoms	20.48	22.85	21.11
Ligand atoms	21.29	22.50	20.14
PLP atoms	13.33	18.84	16.68
Water O atoms	23.95	33.71	27.71
Ramachandran plot			
Favored	96.63%	96.63%	96.42%
Allowed	2.67%	2.37%	2.97%
Disallowed	0.70%	1.00%	0.62%
R.M.S deviations from ideal geometry			
Bond lengths (Å)	0.003	0.007	0.007
Bond angles (°)	0.948	1.264	1.190

Mammalian Cell Cytotoxicity Assays. HepG2, HEK293, and NIH3T3 cells (ATCC) were cultured in Dulbecco's modified Eagle's medium with 10% FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. Confluent cells were trypsinized, and seeded in 40 μL in 384-well plates at 5000 cells/well and incubated at 37 °C (5% CO_2, 95% humidity) for 24 h. Next, 100 nL of each compound in DMSO was pin transfered to afford final concentrations ranging from 20.0-0.156 μM. The proteasome inhibitor MG132 (Tocris) was used as a positive control for HEK293 and NIH3T3 cells while mitoxantrone dihydrochloride was used for HepG2 cells. After 3 days at 37 °C, the plate was removed from the incubator to cool for 15 min, then 20 μL of 50% Promega CellTiterGlo reagent (diluted 1:1 with PBS, pH 7.4) was added and the plate was incubated at rt for 5 min. Luminescence was read on a Perkin-Elmer EnVision with US LUM settings for 0.1 sec per well.

Aspartate aminotransferase counter-screen. Inhibition of aspartate transaminase (AST) was determined using the EnzyChrom™ Aspartate Transaminase Assay Kit (EnzyChrom) according to the manufacturers instructions. Briefly, 5 μL of each compound (10 mM DMSO stock solution) was transferred to a 384 well assay plate. Next, 50 μL of assay solution was dispensed to each well (250 nL EnzyChrom™ AST assay kit cofactor, 250 nL EnzyChrom™ assay kit enzyme mix, and 1 uL EnzyChrom™ assay kit NADH Solution in 50 uL EnzyChrom™ assay kit assay buffer) followed by 5 μL of plasma (5 μL of water to the standard and blank wells) and the plate was tapped to mix the compounds and reagents. Blank (negative control) and standard control wells contain 5 μL of DMSO only. The plate was read at 340 nm on a spectrophotometer at 0 and 10 min. For each sample the rate of NADH consumption was calculated by subtracting the OD_{340} at 10 min from the OD_{340} at time 0 (ΔOD).



1-{4-[4-(Benzo[*d*][1,3]dioxole-5-carbonyl)piperazin-1-yl]phenyl}ethanone (14). To a solution of piperonylic acid (66 mg, 0.4 mmol, 1.0 equiv) and 4′-piperazinoacetophenone (98 mg, 0.48 mmol, 1.2 equiv) in DMF (3 mL) was added EDC (77 mg, 0.4 mmol, 1.0 equiv) followed by DMAP (49 mg, 0.4 mmol, 1.0 equiv) at room temperature. The mixture was stirred at room temperature overnight then partitioned between H_2O (10 mL) and EtOAc (10 mL). The organic layer was washed with H_2O (2 × 10 mL), saturated aqueous NaCl (10 mL), and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (1:1 hexane–EtOAc) to afford the title compound (110 mg, 78% yield) as white solid: R_f = 0.23 (1:1 hexane–EtOAc), ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.48 (s, 3H), 3.34 (br s, 4H), 3.73 (br s, 4H), 5.97 (s, 2H), 6.79–6.85 (m, 3H), 6.91–6.94 (m, 2H), 7.84 (d, J = 8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.0, 47.6*, 47.7*, 101.4, 107.9, 108.1, 113.8, 121.6, 128.2, 128.7, 130.2, 147.6, 148.9, 153.6, 169.8, 196.3 (*C signals not observed in 1-D ^{13}C NMR, but through an HMQC experiment due to nitrogen quadrupole broadening); LRMS (ESI+) 353.17 $[M + H]^+$.