Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Material

Assessing the differential action on cancer cells of *h*LDH5-inhibitors based on the *N*-hydroxyindole-2-carboxylate (NHI) and malonic (Mal) scaffolds

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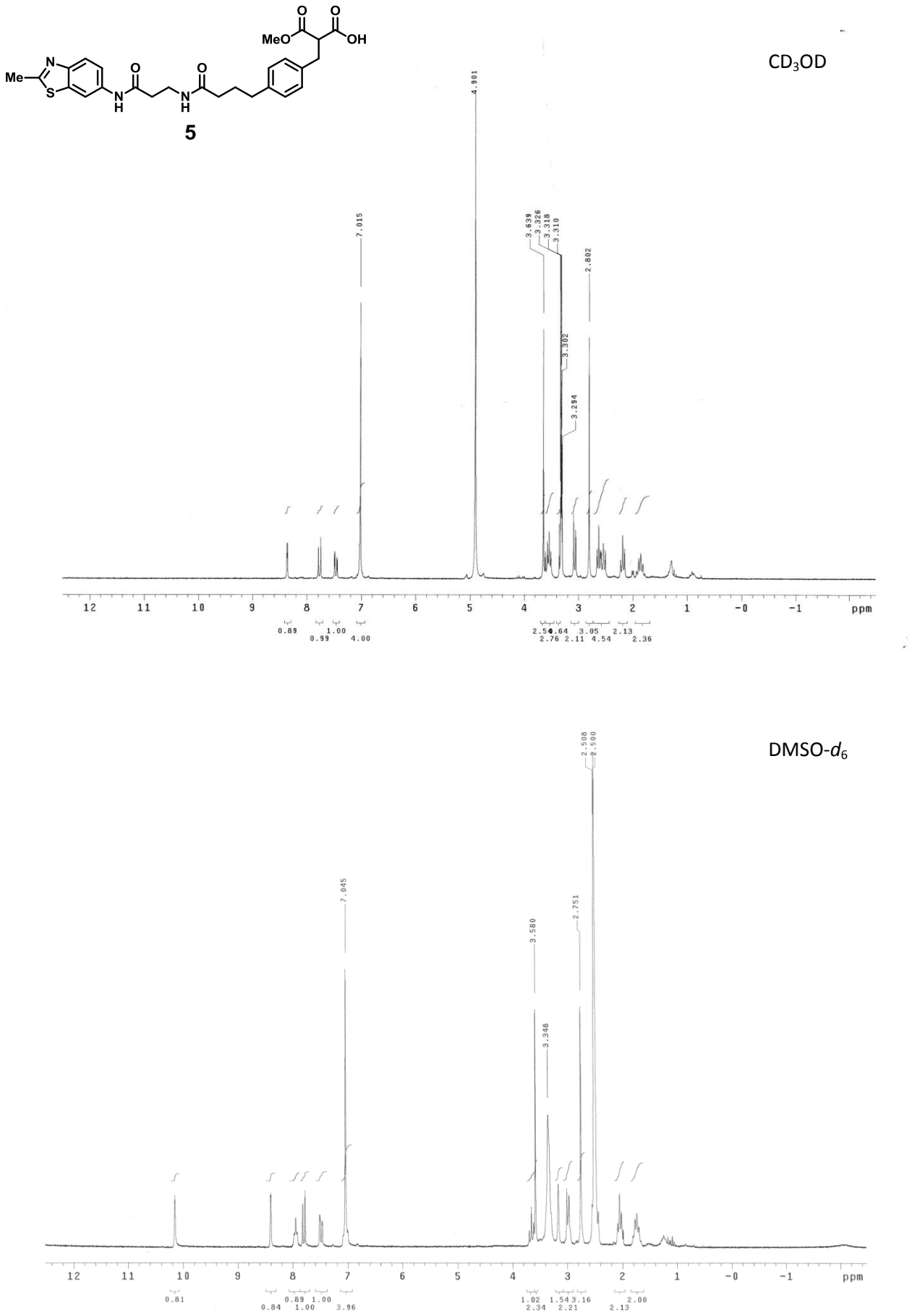


Figure S1. ^{1}H NMR (200 MHz) spectra of compound **5** in CD_3OD (top) and in DMSO-d_6 (bottom).

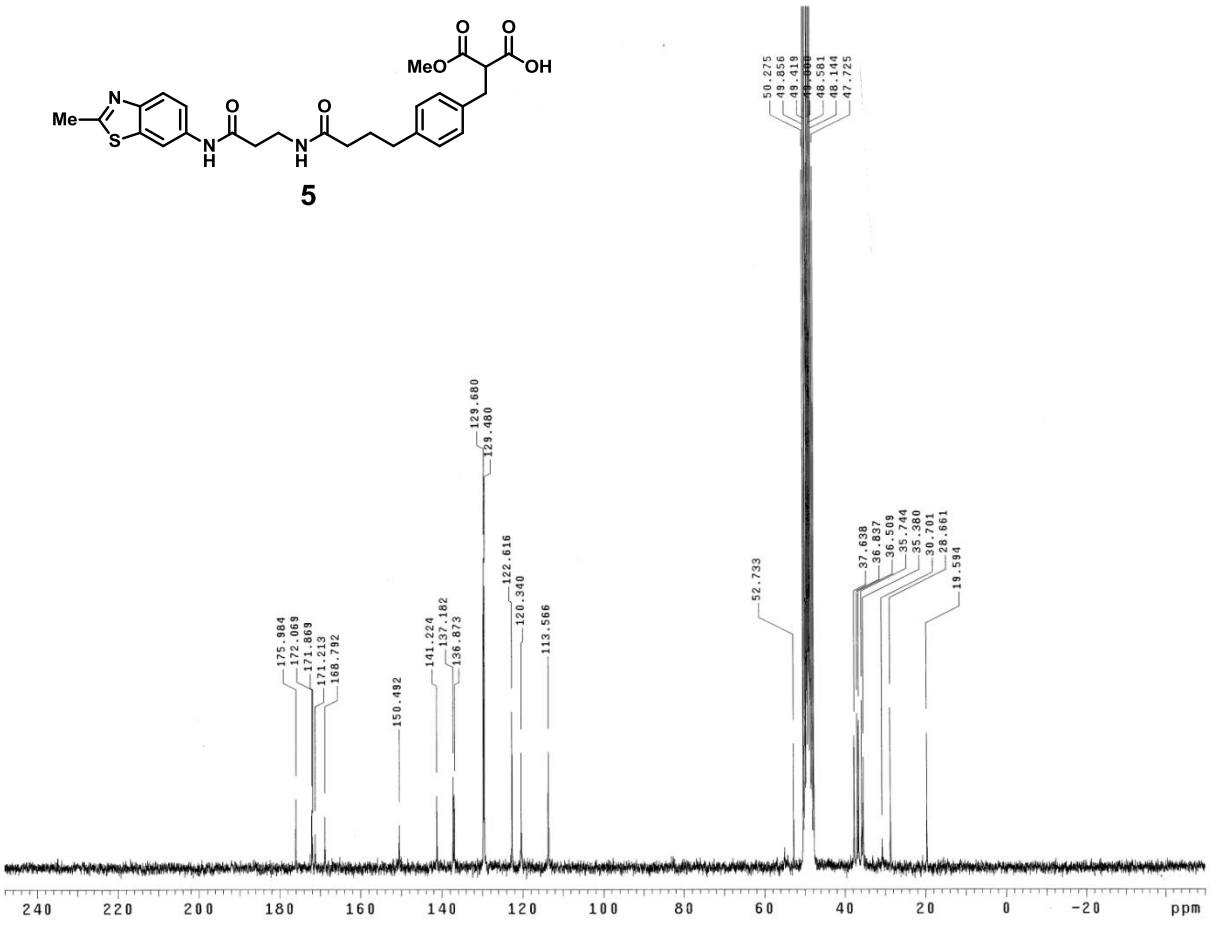


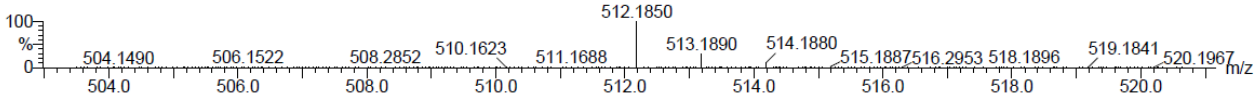
Figure S2. ^{13}C NMR (50 MHz) spectrum of compound **5** in CD_3OD.

Elemental Composition Report **Page 1**

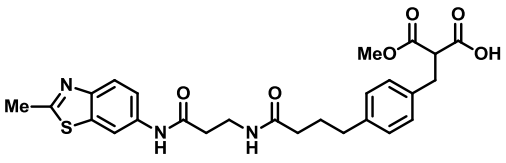
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 600.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
46 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-130 H: 0-250 N: 2-4 O: 5-7 S: 1-1

Calvaresi, Emilia, 071713 267 University of Illnois, SCS, Mass Spectrometry Lab Q-tof UE521
Qtof_48113 412 (11.631) AM (Cen,3, 80.00, Ar,15000.0,716.46,0.70,LS 3); Sm (SG, 2x5.00); Cm (411:430) 1: TOF MS ES+
7.44e+003



Minimum:				-1.5		
Maximum:		5.0	10.0	600.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
512.1850	512.1855	-0.5	-1.0	13.5	2.8	C26 H30 N3 O6 S



5

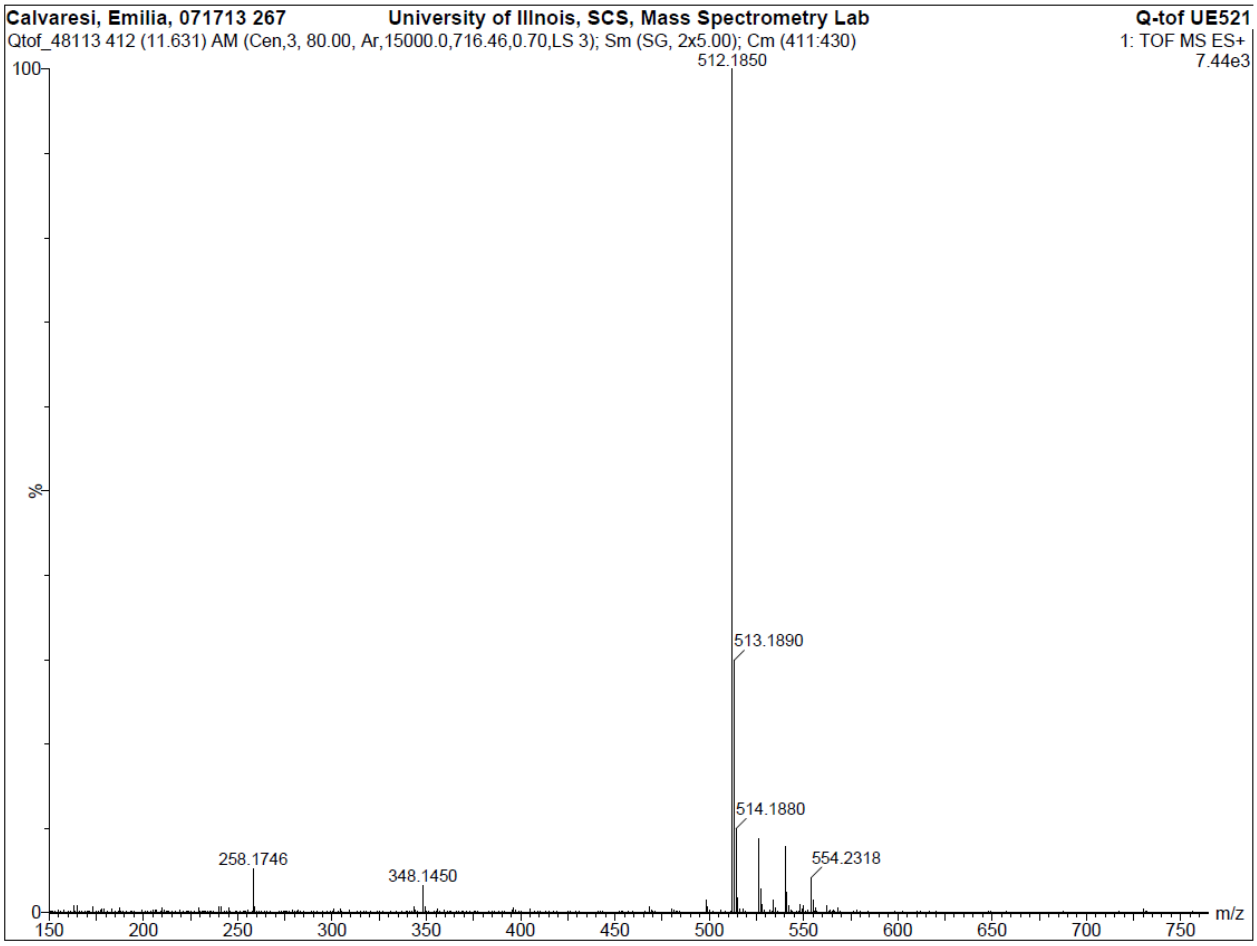


Figure S3. HRMS data and spectrum of compound **5**.

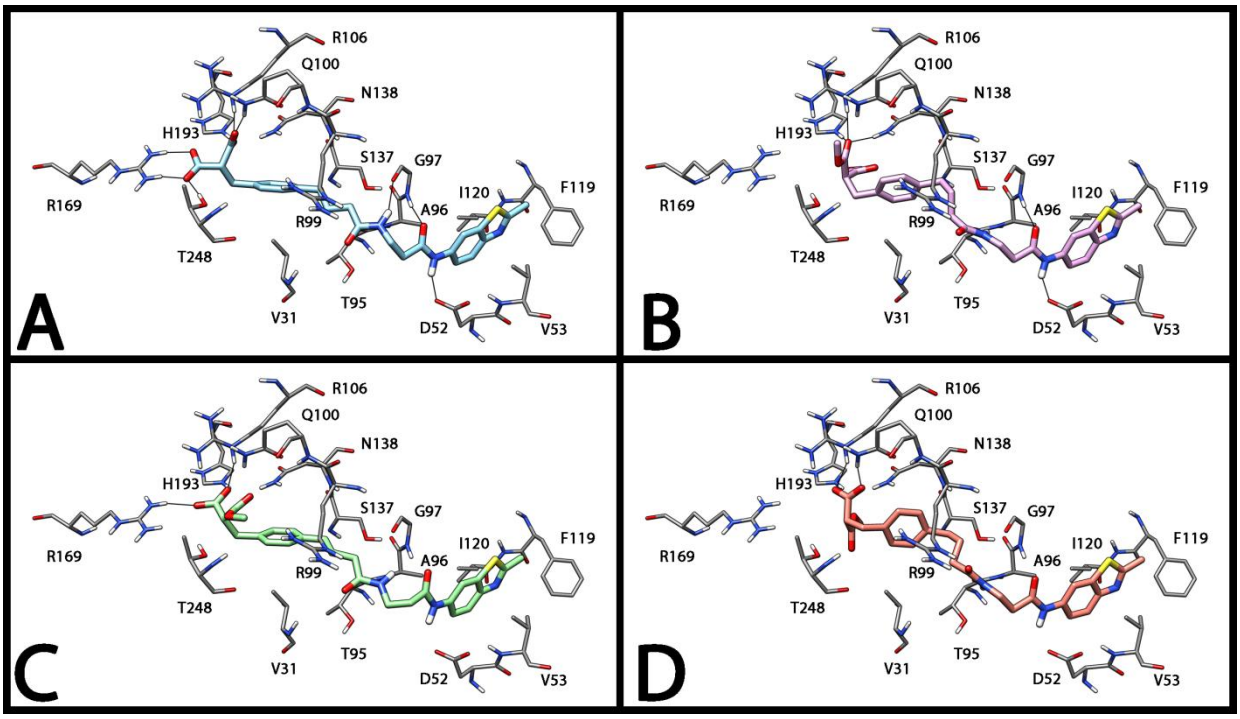


Figure S4. Binding poses of compounds **3-5** in the active site of LDH-A. X-Ray structure of the complex between compound **3** and human LDH-A (**A**, PDB code: 4AJP) and docking analysis of compound **4** (**B**) and the rapidly interconvertible *R* and *S* enantiomers of compound **5** (**C** and **D**, respectively) in LDH-A's active site.

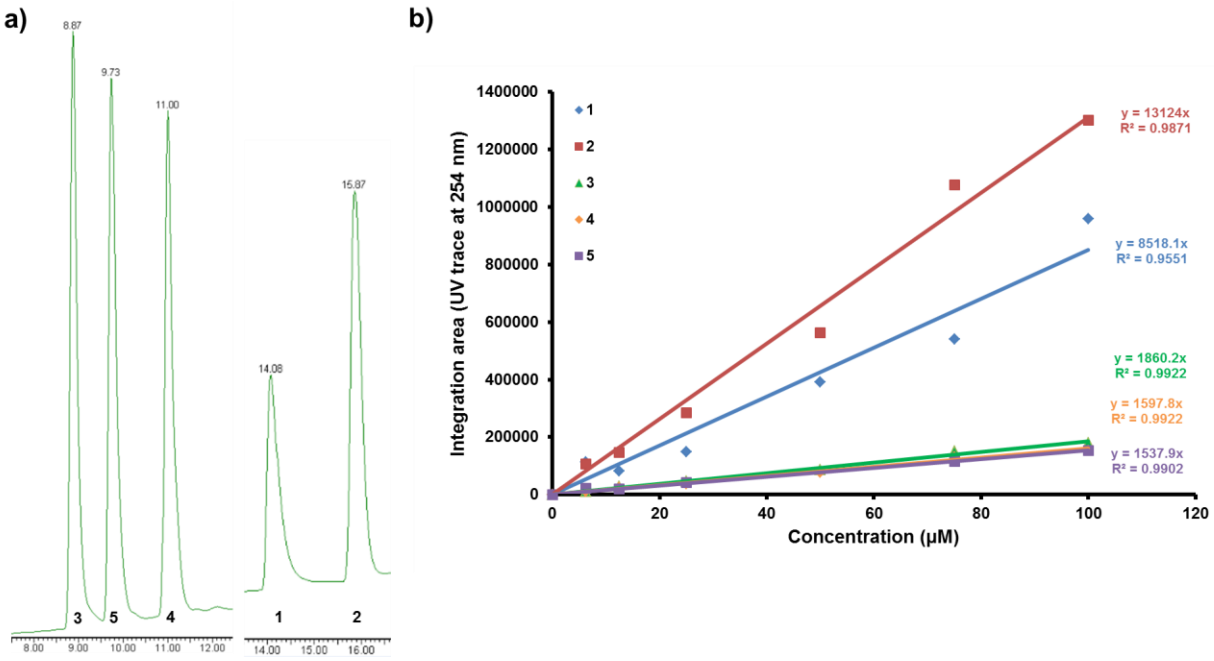


Figure S5. LC-MS separation of compounds **1**-**5**, and calibration of compound concentration to integration area. (a) A representative UV trace at 254 nm, collected simultaneously with the total ion chromatograph (TIC), of a standard sample containing 100 μM concentrations of all five compounds prepared in methanol, demonstrating that the running parameters allow for clear and distinct resolution of each compound of interest. The UV trace shows compound **3** eluting at 8.9 minutes, compound **5** eluting at 9.7 minutes, compound **4** eluting at 11.0 minutes, compound **1** eluting at 14.1 minutes, and compound **2** eluting at 15.9 minutes. (b) UV trace integration areas attained by LC-MS for known concentrations of compounds **1**-**5** were plotted against concentrations. From these data points, linear equations were calculated to represent the relationship between concentration and integration area.

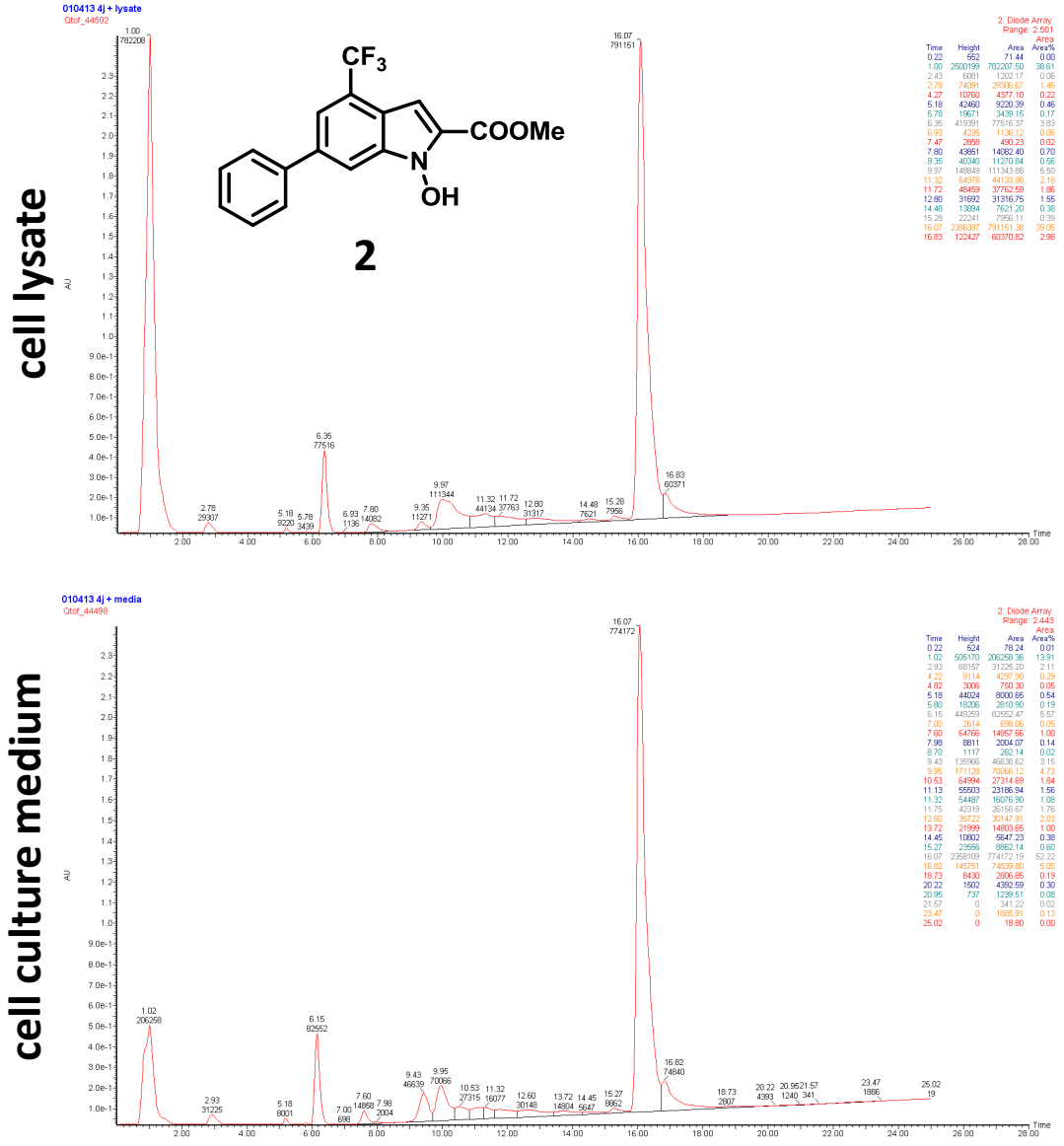


Figure S6. Stability assays of compound **2**. Compound **2** was incubated in freshly-generated HeLa cell lysate in RPMI 1640 growth media, or growth media alone, at 37 °C for 30 minutes, to mimic conditions used in the whole cell treatment experiment (Figure 3). This experiment was performed concurrently with the incubations of compound **4** in HeLa lysate (Figure S8) and RPMI media (Figure S9), using the same batches of lysate and media to assess both compounds. Each sample was then visualized by LC-MS, with UV traces at 254 nm during the run shown above. As shown in the UV traces, no cleavage of compound **2** to its free acid analog (compound **1**), or any other species, was observed in either incubation.

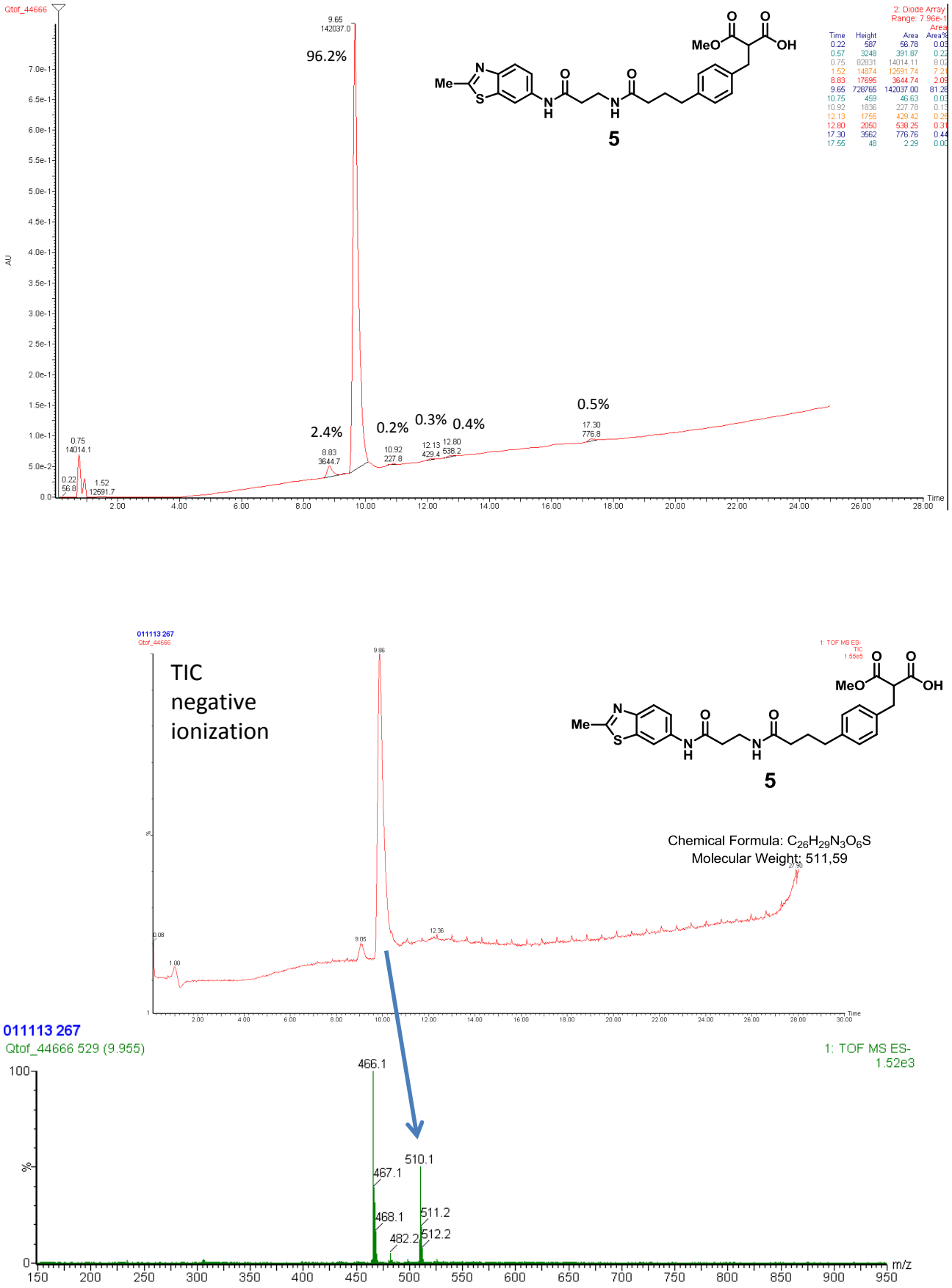


Figure S7. LC-UV (with integrated peak areas) and LC-MS chromatograms of compound **5**.

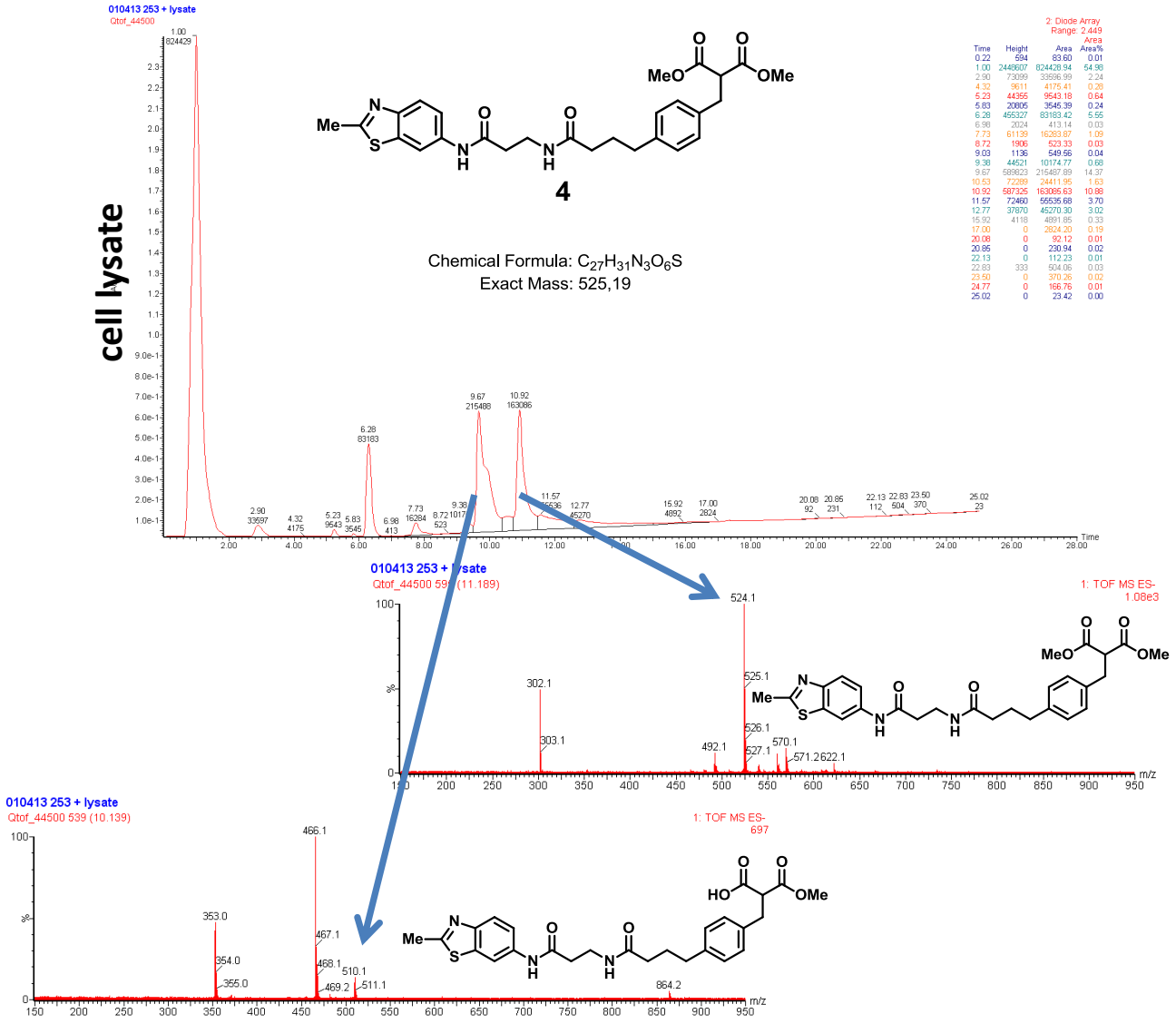


Figure S8. Stability assays of compound **4** in HeLa cell lysate. Compound **4** was incubated in freshly-generated HeLa cell lysate (in RPMI 1640 growth media) at 37 °C for 30 minutes, to mimic the conditions used in the whole cell treatment experiment (Figure 3). The sample was then visualized by LC-MS, with UV traces at 254 nm during the run shown above, and the mass spectra of compound **4**'s main peak and demethylated cleavage peak (compound **5**) shown below.

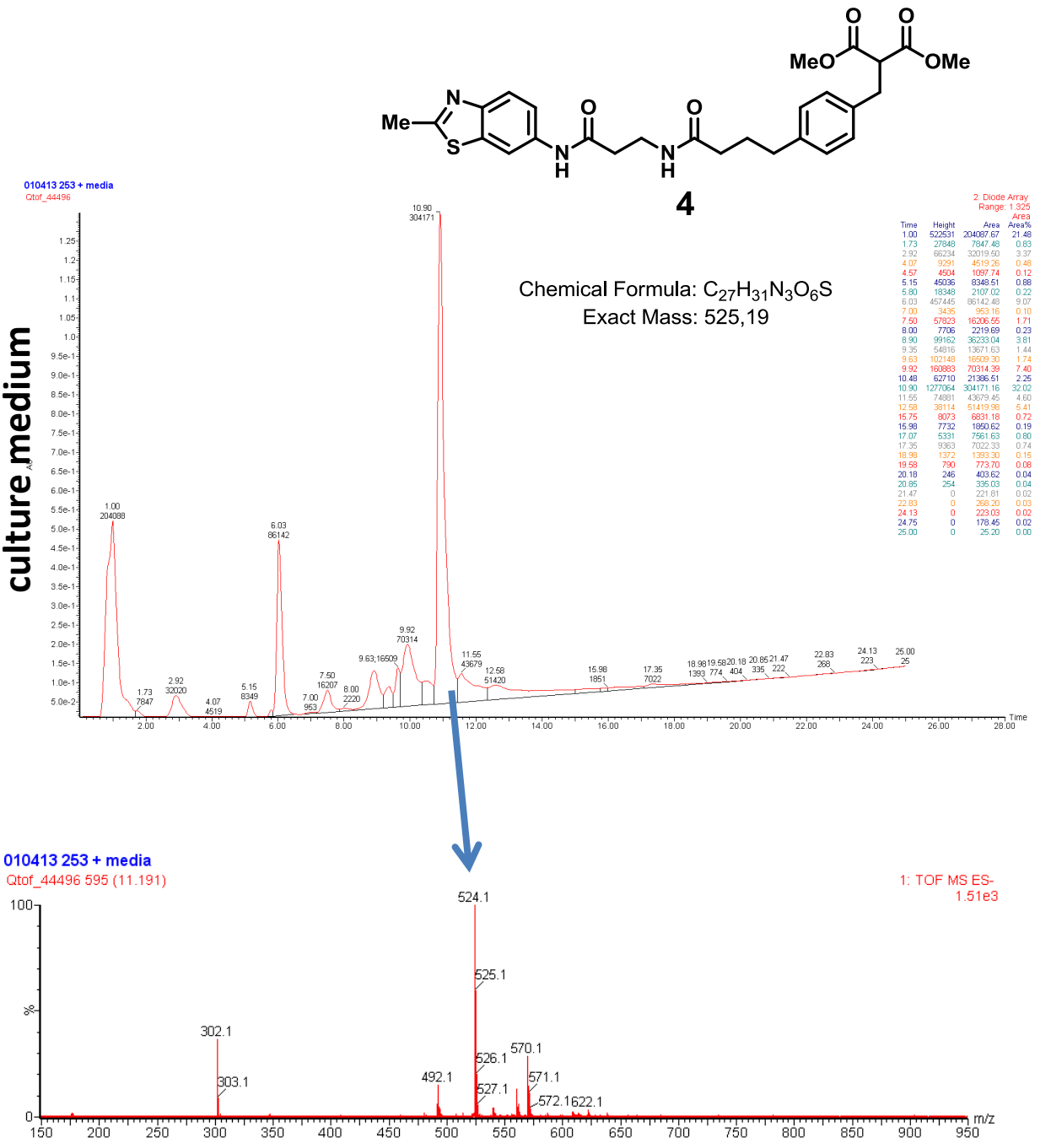


Figure S9. Stability assays of compound **4** in culture media. Compound **4** was incubated in RPMI 1640 growth media at 37 °C for 30 minutes, to mimic conditions used in the whole cell treatment experiment (Figure 3). This incubation in media was done concurrently with the incubation in HeLa cell lysate shown previously (Figure S8). The sample was then visualized by LC-MS, with UV traces at 254 nm during the run shown above, and the mass spectrum of compound **4**'s peak shown below. No cleavage of compound **4** to compound **5** was observed in RPMI media.

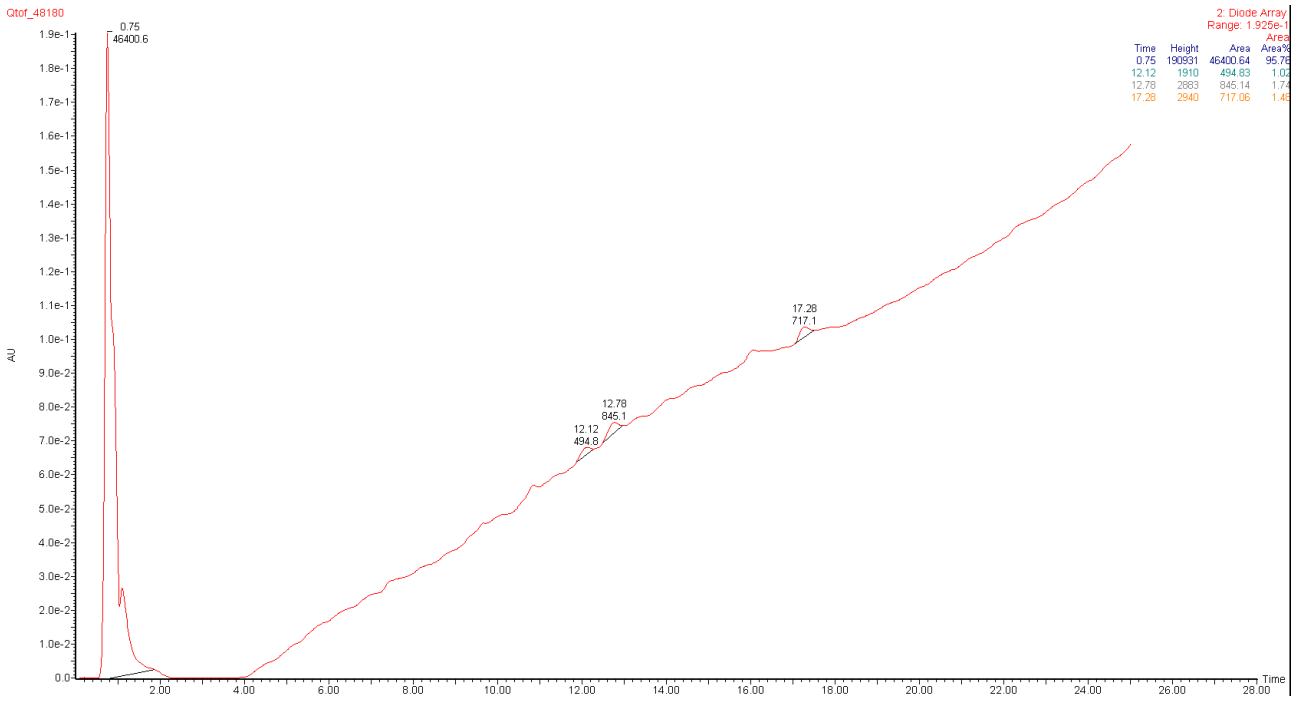


Figure S10. Cell uptake assay of compound **5** by HeLa cells. The LC-UV trace at 254 nm of t HeLa cell lysate after treatment with a 500 µM concentration of **5** for 30 minutes is shown No significant amounts of compound **5** were detected in the intracellular fraction, suggesting that compound **5** is poorly cell-permeable.

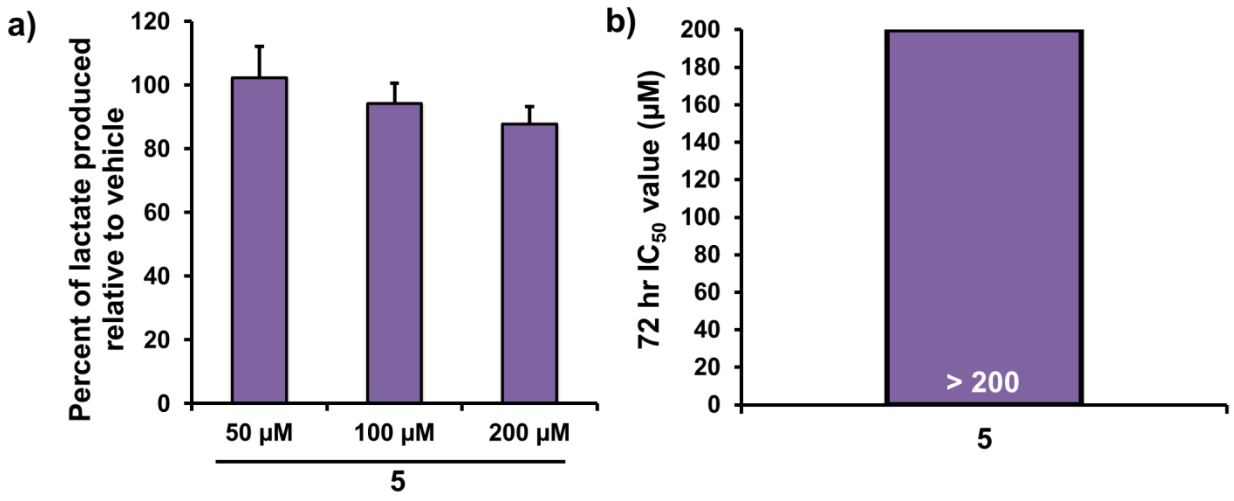


Figure S11. Compound **5**, though an active in vitro inhibitor of LDH-A and a rapidly formed species in cells treated with prodrug compound **4**, is unable to lead to a reduction in lactate production in cells or induce cancer cell death in its own right, likely due to its poor cell permeability (Figure S10). (a) HeLa human cervical carcinoma cells were treated with 50-200 µM concentrations of compound **5** for 8 hours. Cell culture media was extracted from each treatment, concentrated, and derivatized for GC-MS detection and quantification of lactate. No substantial reduction in lactate production was observed upon treatment with any concentration of compound **5** assessed. Error bars denote standard error from three independent experiments. (b) Anticancer potency of compound **5**. HeLa cells were treated with 0.0316-200 µM concentrations of **5** for 72 hours at 37 °C, after which cell death was quantified using the SRB assay. The 72 hour IC_{50} value of compound **5** (the concentration required to kill 50% of cells) was found to be > 200 µM; in fact, no substantial cell death was observed at any concentration used.