Supplemental Material to the Manuscript # C-00538-2024R1, accepted for publication in the American Journal of Physiology-Cell Physiology

Title: Dysfunctional mitochondrial bioenergetics sustains drug resistance in cancer cells

Authors: Davide Gnocchi[1], Dragana Nikolic[1], Silvia Russo[2], Maria Laura Matrella[2], Rosa R. Paparella[1], Sujeet Kumar[3], Subhas S. Karki[3], Carlo Sabbà[1], Tiziana Cocco[2], Simona Lobasso[2], and Antonio Mazzocca[1]

Affiliation: [1]Interdisciplinary Department of Medicine, University of Bari Aldo Moro School of Medicine, Piazza G. Cesare, 11 - 70124 Bari, Italy
[2]Department of Translational Biomedicine and Neuroscience (DiBraiN), University of Bari Aldo Moro School of Medicine, Pl. G. Cesare 11, 70124, Bari, Italy
[3]Department of Pharmaceutical Chemistry, Dr Prabhakar B Kore Basic Science Research Center, Off-campus, KLE College of Pharmacy, (A Constituent Unit of KLE Academy of Higher Education and Research, Belagai), 2nd Block, Rajajinagar, Bengaluru, Karnataka State, India

Correspondence: Antonio Mazzocca, M.D. Ph.D., Interdisciplinary Department of Medicine, University of Bari Aldo Moro School of Medicine, Piazza G. Cesare, 11 - 70124 Bari, Italy. Phone: +39 080 5593593
E-mail: antonio.mazzocca@uniba.it

Appendix Figure A1. Dose-response to determine IC50 in parental (P) and resistant (R) tumor cell lines (A-C).

Appendix Figure A2. The metabolic profiling of P and R cell lines was conducted. Bioenergetic function was evaluated using the Seahorse XF24 Analyzer. A representative profile shows the Oxygen Consumption Rate (OCR) (A) and Extracellular Acidification Rate (ECAR) (B) of P- and R-HCT cells, recorded at baseline and following the injection of oligomycin (an ATP synthase inhibitor), FCCP (an uncoupler), rotenone (a complex I inhibitor), antimycin A (a complex III inhibitor), and 2-deoxy glucose (2DG). Each data point represents the mean ± SEM of three technical replicates (n=3). The data, normalized to protein content for Rot+AA-sensitive OCR and 2DG-sensitive ECAR, represent the mean ± SEM of four independent experiments, each with three technical replicates. Representative OCR (C) and ECAR (D) traces are displayed for Huh7 P and R, showing the mean ± SEM of four independent biological replicates, each with three technical replicates (n=4).

Appendix Figure A3. Adding 100 mM L-lactate to the "respiration buffer" used for oxygraphy measurements does not change the pH (A) or osmolarity (B).

Appendix Figure A4. The effect of osmolarity, specifically sucrose, on cellular respiration. The effect of osmolarity on cellular respiration has been tested by repeated additions of 25 mM sucrose, which corresponds to an osmolarity of 25 mOsm. A: Huh7, B: HCT-116, C: MDA-MB-231. Data represents the average of three independent biological replicates (n = 3) with three technical replicates each.

Appendix Figure A5. The effect of L-lactate addition on oxidative and glycolytic function in HCT-116 and Huh7 cells is shown. Bar graphs show basal OCR and basal OCR after the addition of Oligomycin (Oligo) and FCCP in HCT-116 P and R (A) and Huh7 P and R (B). Data represents the average ± SEM of three biological replicates (n=3), each with two technical replicates. ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

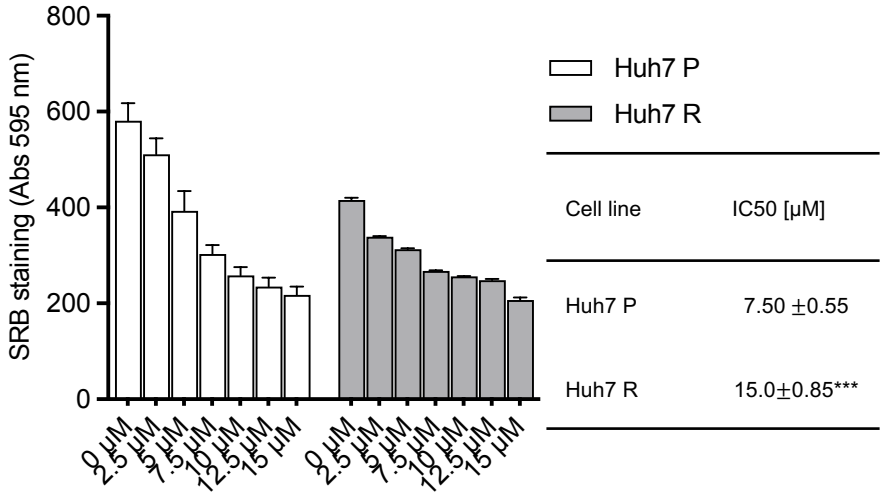
Appendix Figure A6. Tables summarizing the newly synthesized chemical compounds tested in the study.

Appendix Figure A7. A: Lignan-based structure of compounds tested in the study. B: Improvement of OXPHOS efficiency in HK-treated cells for 24 h.

Appendix Figure A8. The effect of chemotherapy drugs, toolkit (TK), and its components on parental (P) cell lines is shown. The figure shows the effects of the drugs, TK, its components (2 μM GNE-140 and 10 μM Honokiol), and the combination of the chemotherapy drug + TK in Huh7 P (A), HCT-116 P (B), MDA-MB-231 P (C), HLE-neo (D), and HepG2 shRNA LPAR6 (E). Data represents the average ± SEM of three biological replicates (n=3), each with two technical replicates. ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

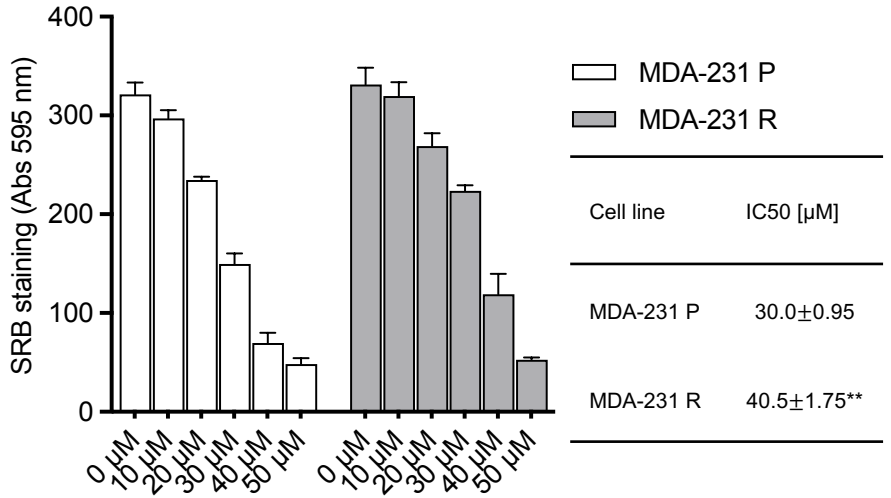
Determine the IC50 in parental (P) and resistant (R) cell lines

A



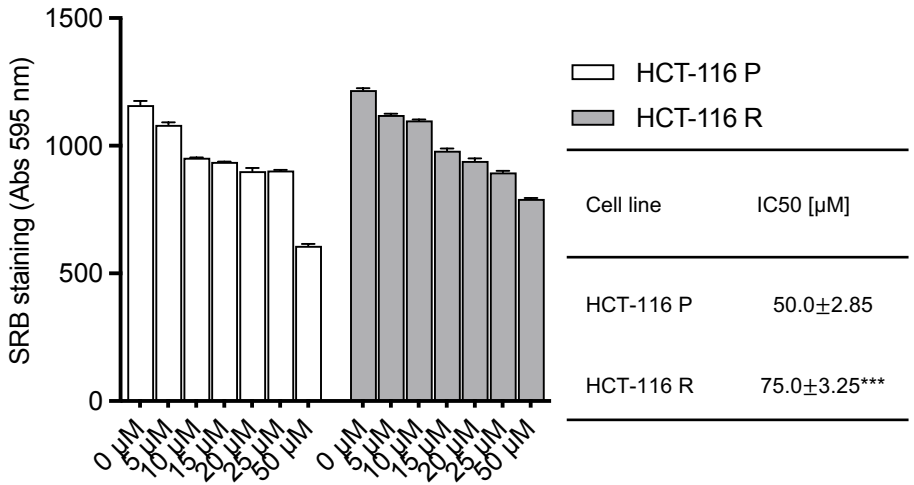
Cell line	IC50 [µM]
Huh7 P	7.50 ±0.55
Huh7 R	15.0±0.85***

B



Cell line	IC50 [µM]
MDA-231 P	30.0±0.95
MDA-231 R	40.5±1.75**

C



Cell line	IC50 [µM]
HCT-116 P	50.0±2.85
HCT-116 R	75.0±3.25***

Figure A1

Metabolic profiling of Parental (P) vs Resistant (R) cell lines

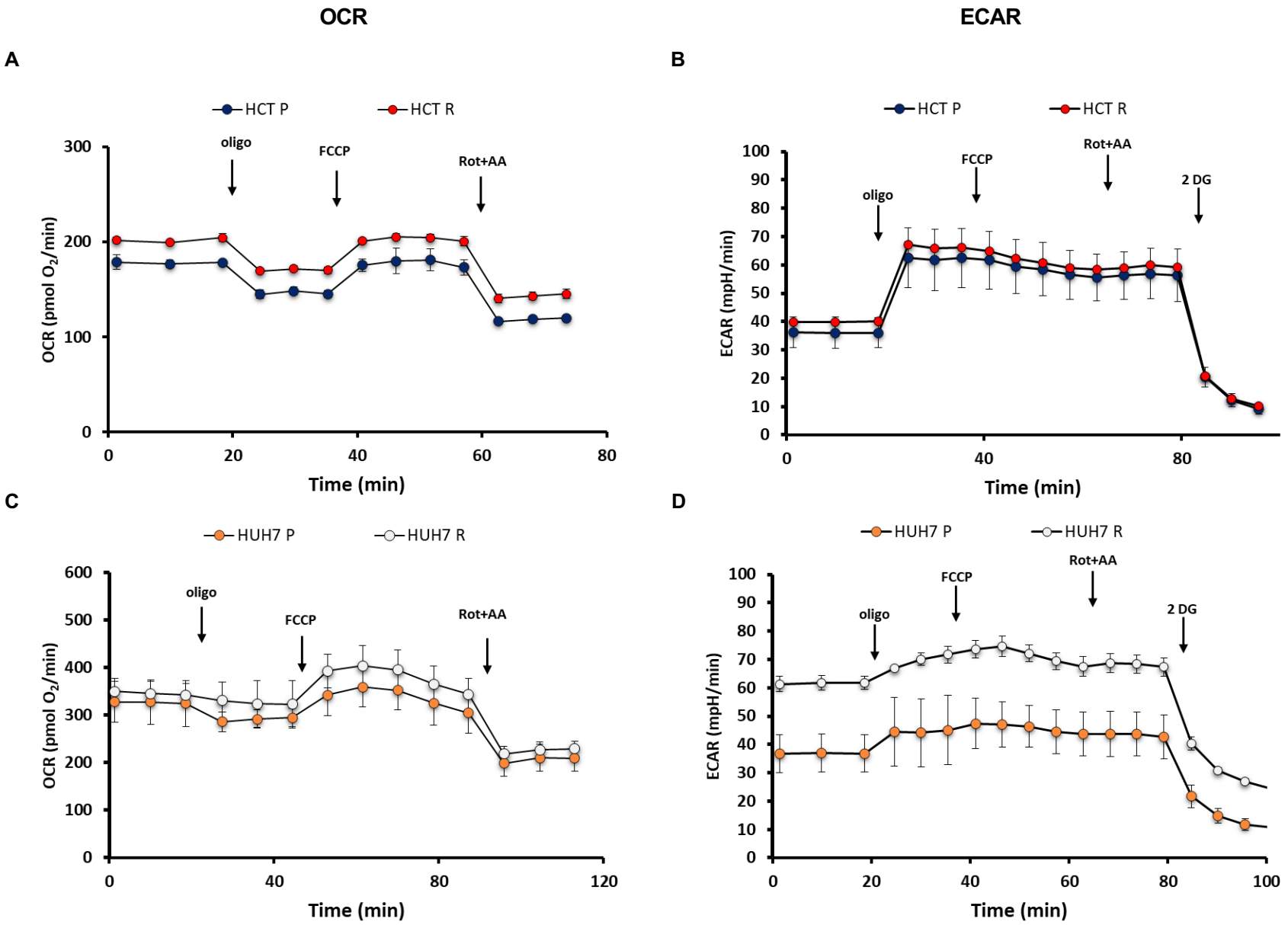


Figure A2

Effect of 100 mM L-lactate on the osmolarity and pH of the “respiration buffer”

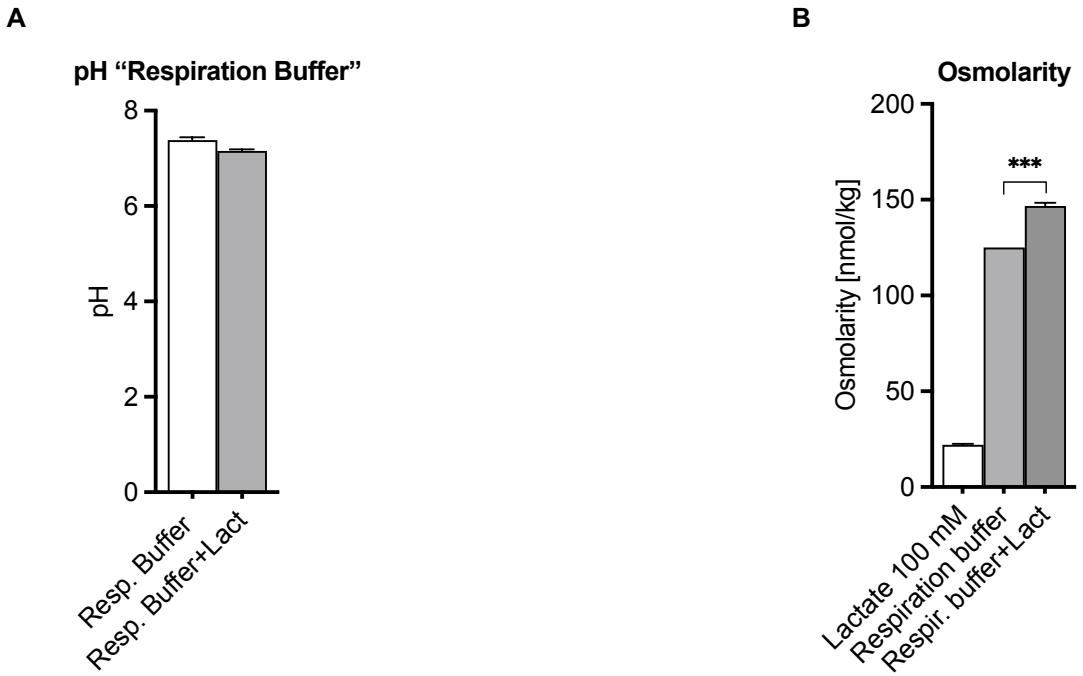


Figure A3

Effect of sucrose-mediated osmolarity on cellular respiration

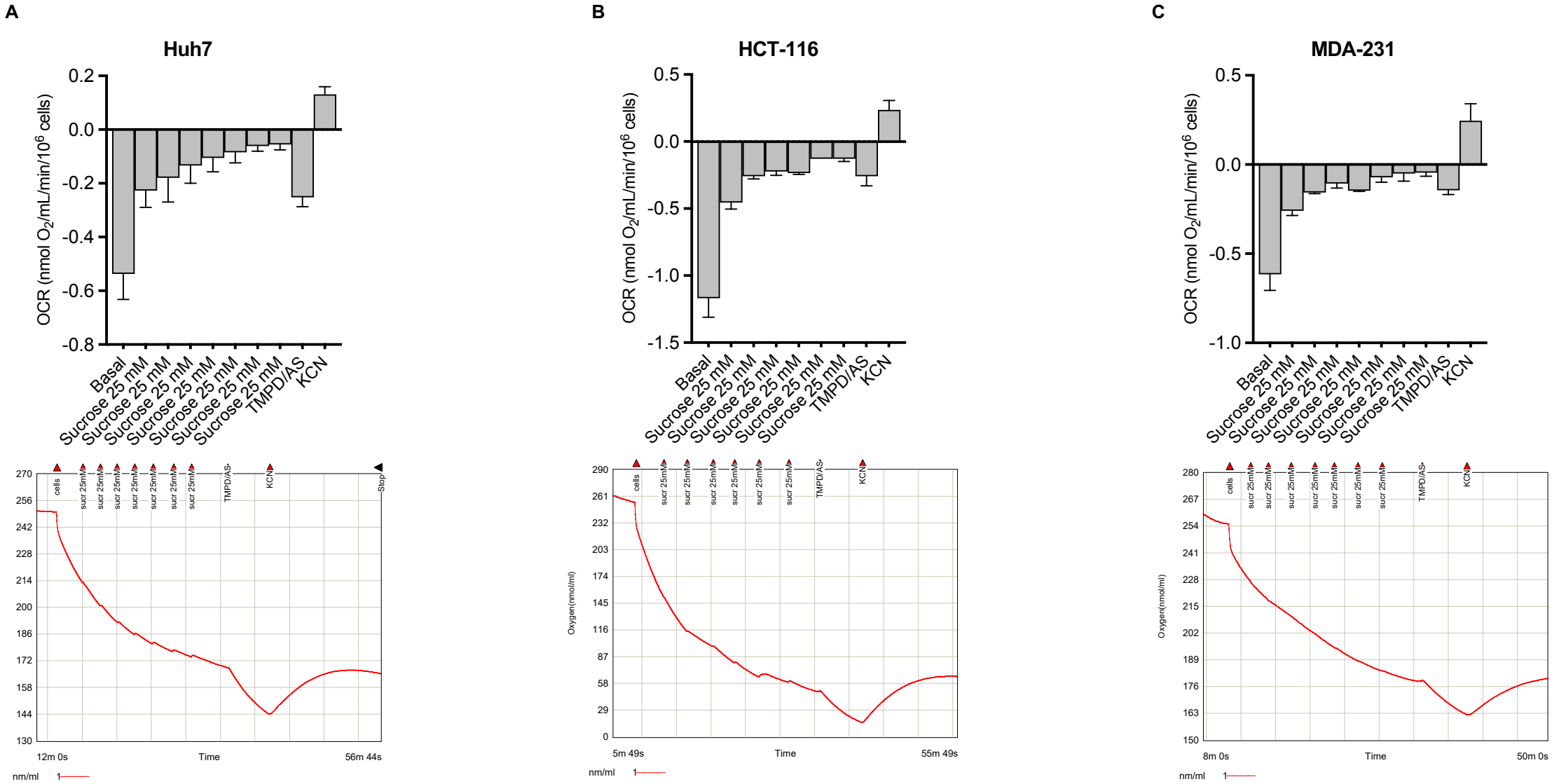
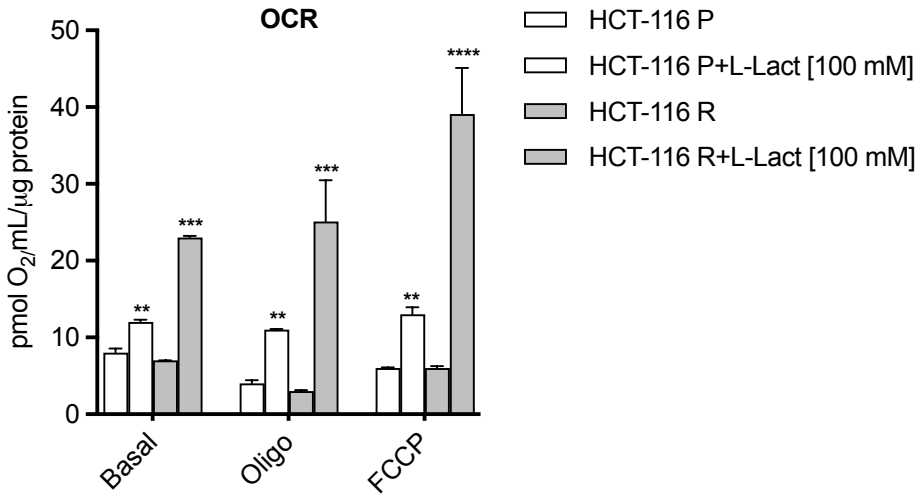


Figure A4

Effects of L-lactate addiction on oxidative and glycolytic functions of HCT-116 and Huh7 cells

A



B

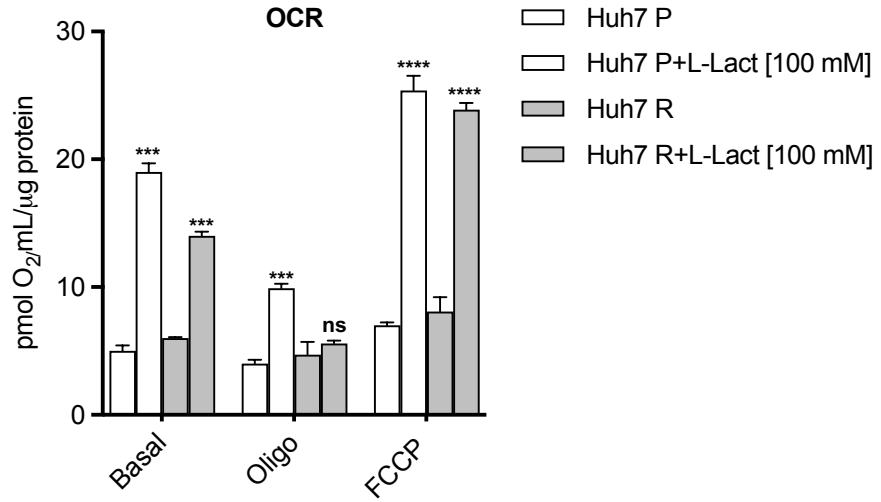
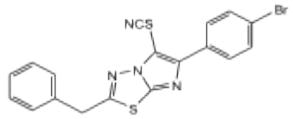
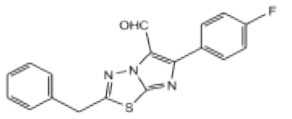
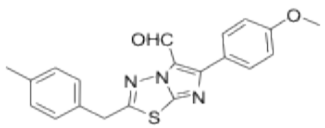
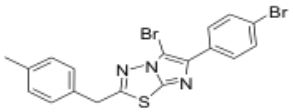
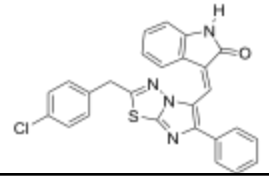
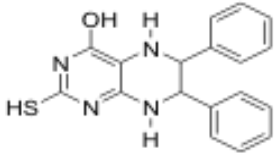


Figure A5

Table A1. Newly synthesized chemical compounds tested in the study

Identification label (trivial name)	FW formula	Structure
XET 2-benzyl-6-(4-bromophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl thiocyanate	427,34 $C_{18}H_{11}BrN_4S_2$	
XBF 2-benzyl-6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde	337,37 $C_{18}H_{12}FN_3OS$	
SKS-106 6-(4-methoxyphenyl)-2-[(4-methylphenyl)methyl]imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde	463,19 $C_{20}H_{17}N_3O_2S$	
SKS-194 5-bromo-6-(4-bromophenyl)-2-[(4-methylphenyl)methyl]imidazo[2,1-b][1,3,4]thiadiazole	404.08 $C_{18}H_{13}Br_2N_3S$	
SKS-63 (3E)-3-({2-[(4-chlorophenyl)methyl]-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-yl}methylidene)-1,3-dihydro-2H-indol-2-one	468,96 $C_{26}H_{17}ClN_4OS$	
SCR-7(P) [5,6-bis(benzylideneamino)-2-mercapto-pyrimidin-4-ol]	336,41 $C_{18}H_{16}N_4OS$	

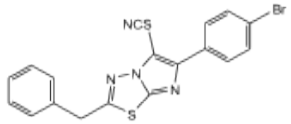
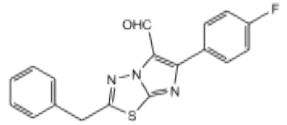
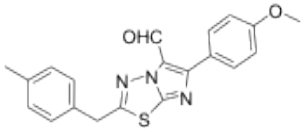
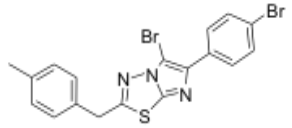
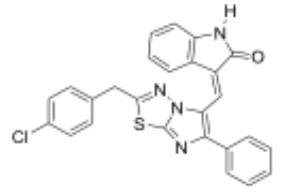
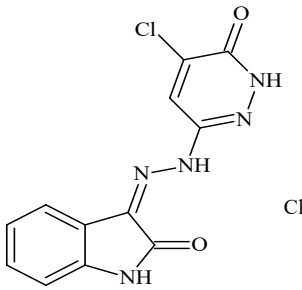
Identification label (trivial name)	FW formula	Structure
55-28 (E)-1,2,3-trimethoxy-5-(4-methylstyryl)benzene	284,35 $C_{18}H_20O_3$	
SKS-155 (3Z)-3-[2-(5-chloro-6-oxo-1,6-dihydropyridazin-4-yl)hydrazinylidene]-1,3-dihydro-2H-indol-2-one	289,68 $C_{12}H_8ClN_5O_2$	
SKS-159 (3Z)-5-chloro-3-[2-(5-chloro-6-oxo-1,6-dihydropyridazin-4-yl)hydrazinylidene]-1,3-dihydro-2H-indol-2-one	324,12 $C_{12}H_7Cl_2N_5O2$	
AD-05	422,48 $C_{26}H_{22}N_4O_2$	
AD-22	436,51 $C_{27}H_{24}N_4O_2$	

Figure A6

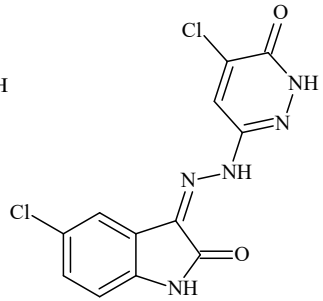
Lignan-based structures of some of the compounds used in the study

A

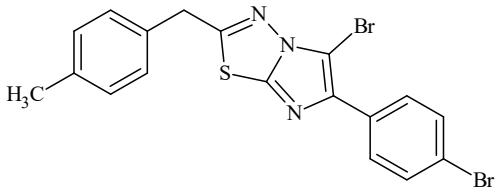
SKS 155



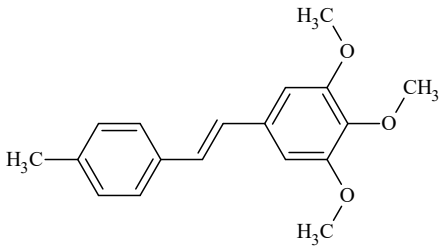
SKS 159



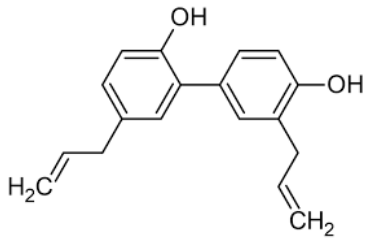
SKS 194



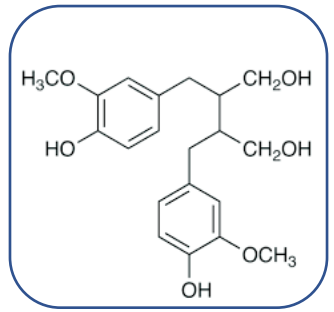
55-28



Honokiol

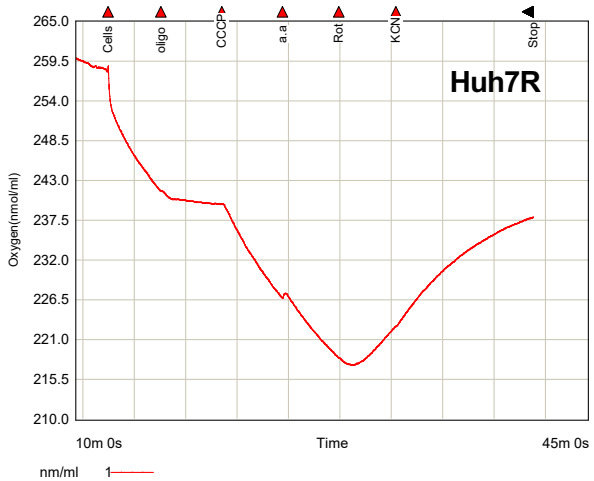


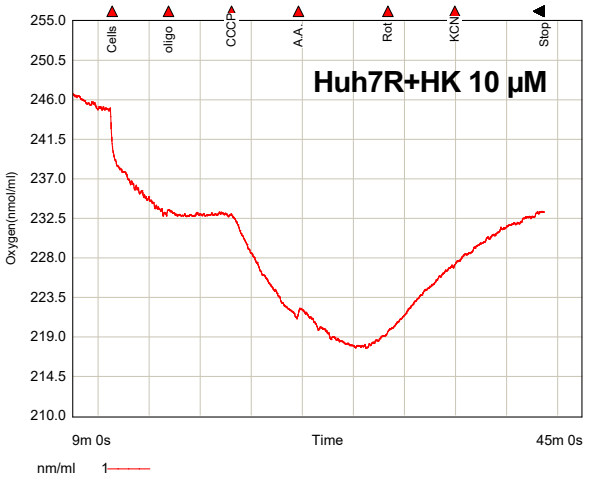
Lignans

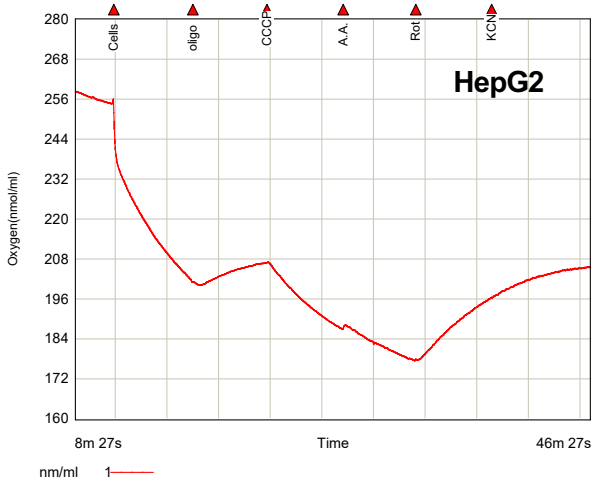


B

HK improves OXPHOS efficiency







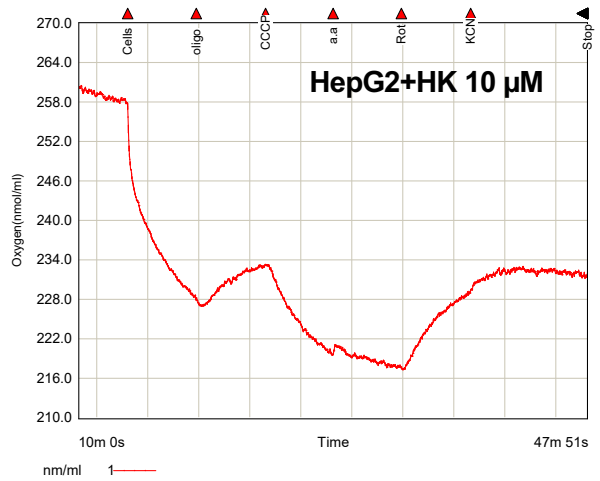


Figure A7

Effects of drugs, toolkits (TK), and TK components on parental (P) cell lines

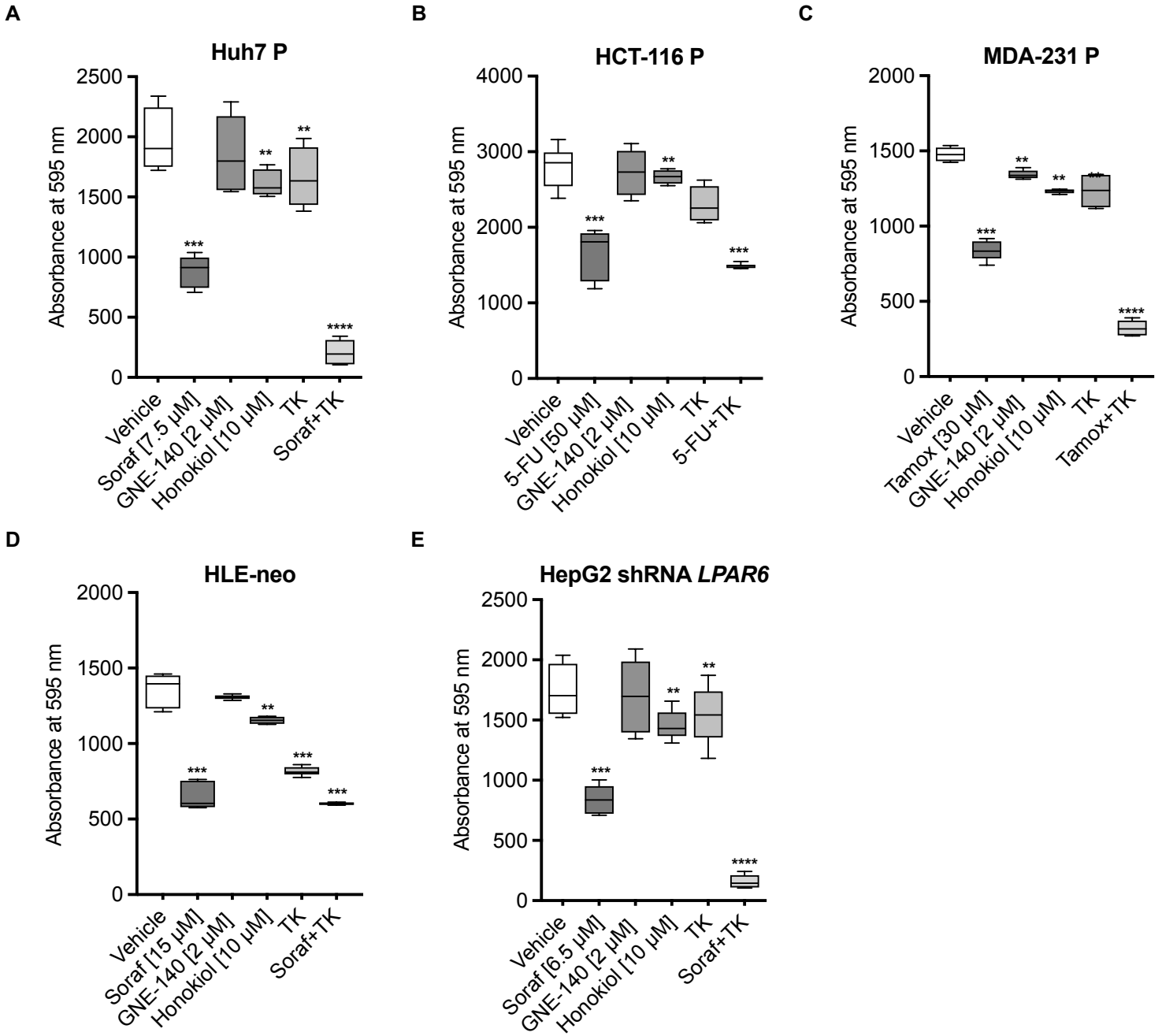


Figure A8