Nonsteroidal anti-inflammatory drugs increase cisplatin, paclitaxel and doxorubicin efficacy against human cervix cancer cells

Diana Xochiquetzal Robledo-Cadena[1], Víctor Dávila-Borja[2], Silvia Cecilia Pacheco-Velázquez[1], Stephen J. Ralph[3], Betsy Alejandra Blanco-Carpintero[1], Javier Alejandro Belmont-Díaz[1], Juan Carlos Gallardo- Pérez[1], Rafael Moreno-Sánchez[1] and Sara Rodríguez-Enríquez[1,*]

Table S1

Infra-additive effects of celecoxib, DMC or CasII-gly with vitamin E analogues in HeLa cell two-dimensional cultures.

Drug 1	*assayed doses (μM)*	*Drug 2*	*assayed doses (μM)*	*C values (BTA %) [Range]*	*Experimental values % [Range]*	*Synergism (%) [Range]*
Celecoxib	5-10	α-TOS	0.1-1	26 ± 0.8 [25-27]	19 ± 6 [13-23]	-(7 ± 5) [-(4 -13)]
	10-20	α-TEA	0.1-4	11 ± 9.5 [4-22]	23.5 ± 6 [17-28]	12 ± 10 [3-23.5]
DMC	25-30	α-TOS	0.1-1	42 ± 1.5 [40.5-43.5]	28 ± 2 [26-30]	-(14 ± 2) [-(13-16)]
	30-25	α-TEA	0.1-4	30 ± 6 [26-37]	-(37 ± 5.5) [-(33-43)]	- (66.5 ± 11) [-(60– 80)]
CasII-gly	0.3 – 0.5	α-TOS	0.1 – 1	25 ± 14 [8.5-33]	7 ± 3 [4.5-11]	- (18 ± 13) [-(3 – 28)]
	0.3 – 0.5	α-TEA	0.5 – 4	33± 6 [29-39.5]	16 ± 3 [12-19]	- (17 ± 4) [-(13 – 20)]
	0.3 – 0.5	M-TEA	0.1-1	36± 1.5 [34-37]	19.5 ± 7 [14-28]	-(16 ± 8) [-(7-23)]

Infra-additive effects of celecoxib, DMC or CasII-gly with vitamin E analogues in HeLa MCTS cultures

Preventive protocol						
Drug 1	*assayed doses (nM)*	*Drug 2*	*assayed doses (nM)*	*C values (BTA %) [Range]*	*Experimental values % [Range]*	*Synergism (%) [Range]*
Celecoxib	0.1-0.5	α-TOS	1-10	37 ± 5 [33-43]	19 ± 6 [13-23]	-(18 ± 10) [-(11 – 30)]
	0.5-1	α-TEA	15-25	44 ± 8 [38-53]	23.5 ± 6 [17-28]	-(21 ± 13) [-(12 – 36)]

DMC	1-10	α-TOS	1-10	45 ± 2 [43.5-48]	23 ± 6 [17-29]	-(22 ± 6) [-(15 – 26)]
	5-10	α-TEA	15-25	35 ± 12 [22-47]	19.5 ± 3 [17-22]	-(15 ± 15) [-(0.6 –29)]
CasII-gly	10-30	α-TOS	1-10	26 ± 15 [8.5-35]	16 ± 8 [7-22]	-(10 ± 7) [-(1 - 13)]
	1-5	α-TEA	15-25	35 ± 5 [30-40]	16 ± 4 [12-19]	-(18 ± 8) [-(12 – 28)]
				Curative Protocol		
Drug 1	*assayed doses (μM)*	*Drug 2*	*assayed doses (μM)*	*C values (BTA %) [Range]*	*Experimental values % [Range]*	*Synergism (%) [Range]*
Celecoxib	1-5	α-TOS	10-50	26 ± 0.7 [25-27]	19 ± 6 [13-23]	-(7 ± 5) [-(3 - 13)]
	3-7	α-TEA	25-30	39 ± 7 [34-47]	23.5 ± 6 [17-28]	- (15 ± 13) [-(6 – 30)]
DMC	10-25	α-TOS	10-50	46 ± 7[39-52]	28 ± 2 [26-30]	-(18 ± 8) [-(9 - 25)]
	25-35	α-TEA	25-30	29 ± 5 [26-34.5]	22 ± 4 [17-25]	-(7 ± 9) [-(1 - 17)]
CasII-gly	10-50	α-TOS	10-50	29 ± 7 [20-34]	7 ± 3 [4.5-11]	-(22 ± 6) [-(15 - 28)]
	100-200	α-TEA	24-30	33 ± 3[29-35.5]	16 ± 4 [12-19]	-(16 ± 7) [-(10 - 23)]

For HeLa bidimensional cultures, the IC_{50}values of α-TOS, α-TEA and M-TEA, as single agents, were 1.2 ± 0.46 μM, 4.7 ± 0.7 μM, and 1.2 ± 0.48 μM, respectively. For the MCTS preventive protocol, the IC_{50}values of α-TOS and α-TEA were 10 ± 4 nM and 25 ± 6 nM, respectively. For the MCTS curative protocol, the IC_{50}values of α-TOS and α-TEA were 50 ± 11 μM and 30 ± 2 μM, respectively.

Abbreviations: α-TOS, α-tocopheryl succinate; α-TEA, α-tocopherol ether linked acetic acid analog; M-TEA,methoxy-tocopheryloxyacetic acid. The Bliss-Type Additivism (BTA) was calculated as outlined in the Material and Methods section.

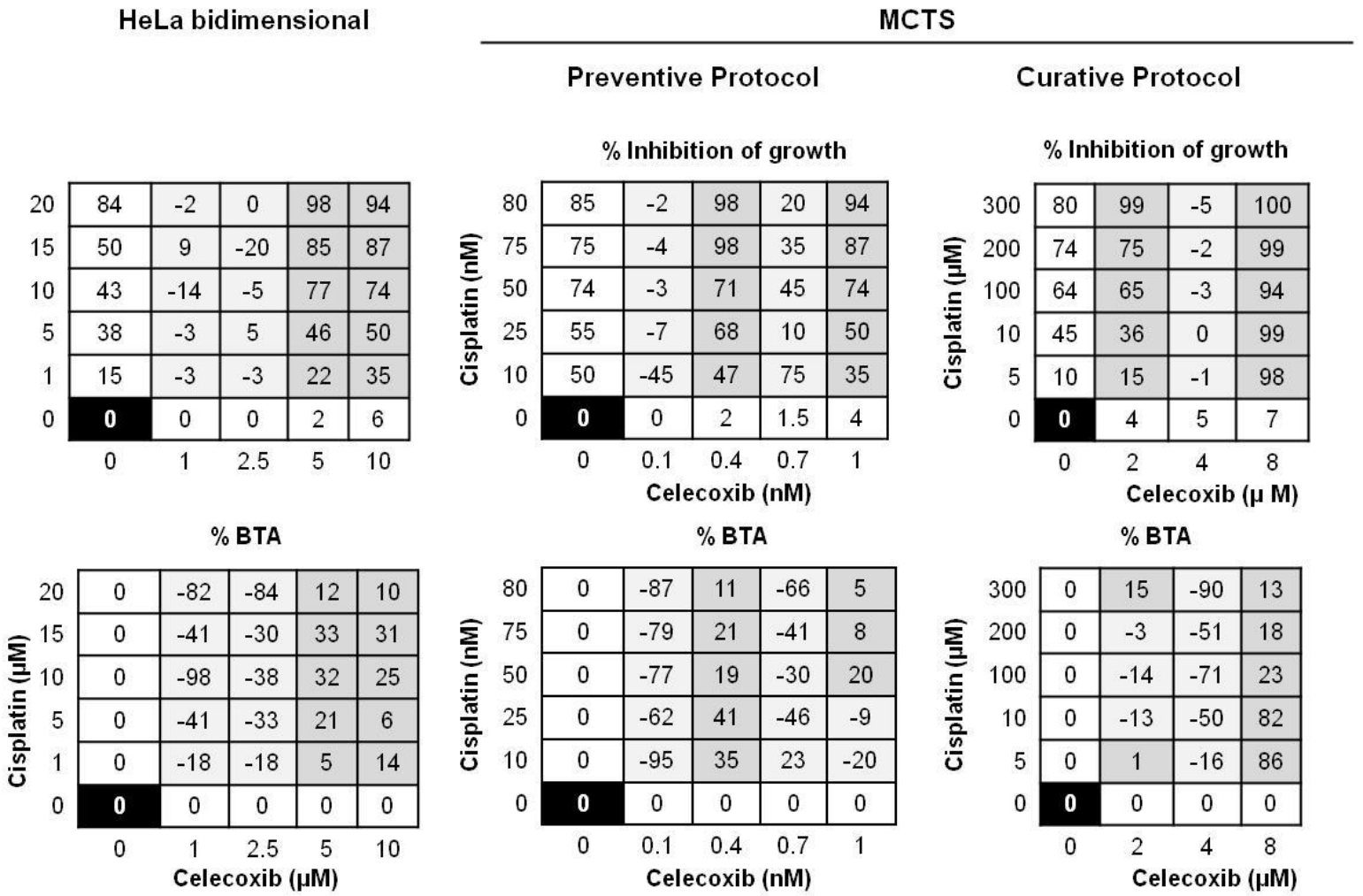
Table S2

Synergistic effects of NSAIDs with canonical anti-cancer drugs at sub-IC50 concentrations in bidimensional HeLa cell cultures as shown by using the Combination Index (CI) value

Drug 1	*assayed doses (μM)*	*Drug 2*	*assayed doses (μM)*	*CI [Range]*
Celecoxib	5-10	Cisplatin	2-5	0.2 ± 0.10 [0.1-0.3]
	5-10	Paclitaxel	11-15	0.65 ± 0.2 [0.4-0.9]
	5-10	Doxorubicin	10-20	0.7 ± 0.1 [0.5-0.8]
DMC	10-15	Cisplatin	2-5	0.2 ± 0.01 [0.19-0.22]
	20-25	Paclitaxel	20-21	0.8 ± 0.09 [0.7-0.9]
	20-25	Doxorubicin	10-20	0.9 ± 0.1 [0.8-1]
CasII-gly	0.5-1	Cisplatin	50-100	0.7 ± 0.2 [0.5-0.9]
	0.5-1	Paclitaxel	10-20	0.55 ± 0.2 [0.35-0.75]
	0.5-1	Doxorubicin	10-20	0.9 ± 0.02 [0.85-0.9]

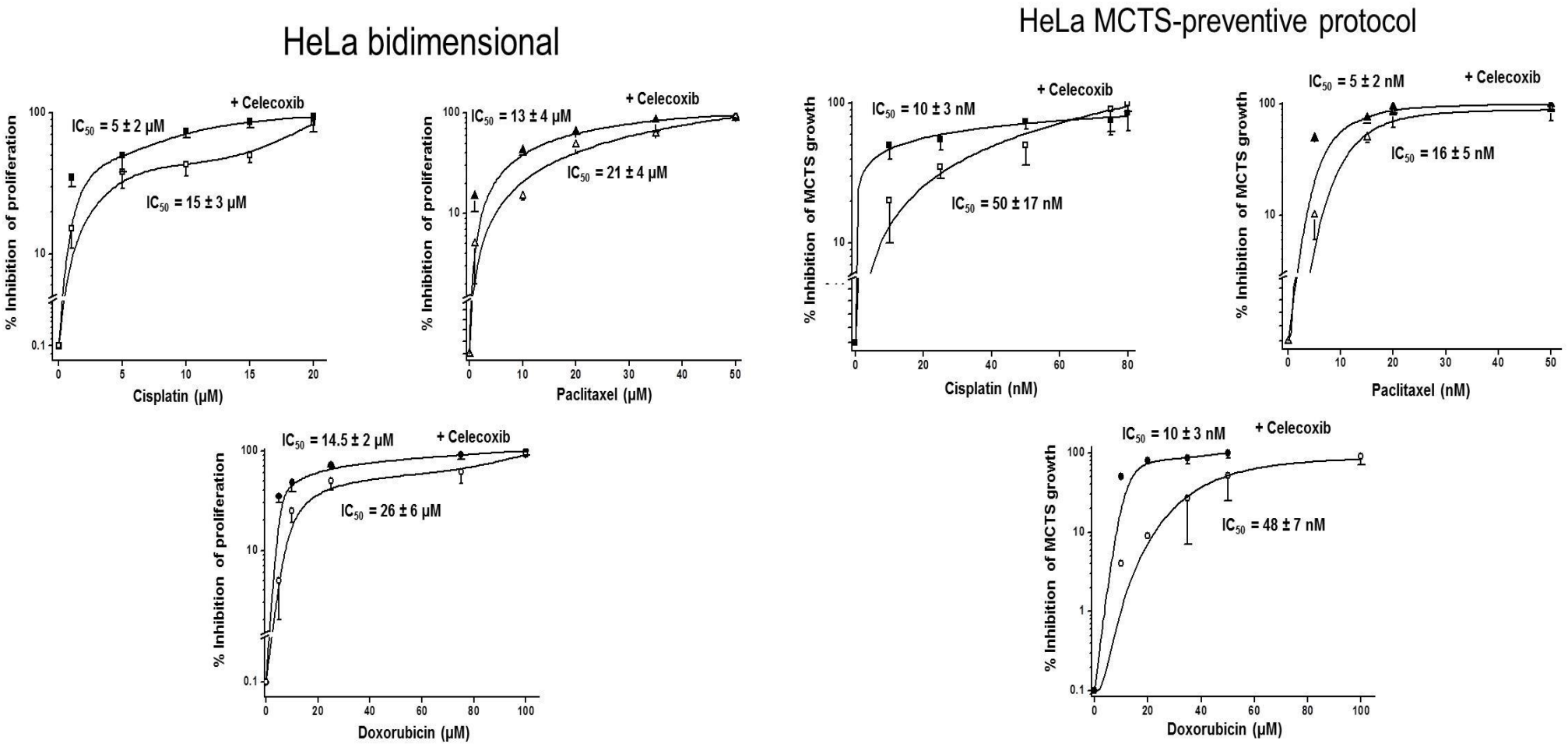
CI was calculated as outlined in the Material and Methods section. The data shown represent the mean ± S.D. of at least three different independent bidimensional cultures (n=3).

Figure S1


HeLa bidimensional
MCTS
Preventive Protocol
Curative Protocol
% Inhibition of growth
% Inhibition of growth
Cisplatin (nM)
Cisplatin (µM)
20 84 -2 0 98 94
15 50 9 -20 85 87
10 43 -14 -5 77 74
5 38 -3 5 46 50
1 15 -3 -3 22 35
0 0 0 0 2 6
0 1 2.5 5 10
80 85 -2 98 20 94
75 75 -4 98 35 87
50 74 -3 71 45 74
25 55 -7 68 10 50
10 50 -45 47 75 35
0 0 0 2 1.5 4
0 0.1 0.4 0.7 1
Celecoxib (nM)
300 80 99 -5 100
200 74 75 -2 99
100 64 65 -3 94
10 45 36 0 99
5 10 15 -1 98
0 0 4 5 7
0 2 4 8
Celecoxib (µ M)
% BTA
% BTA
% BTA
Cisplatin (µM)
Cisplatin (nM)
Cisplatin (µM)
20 0 -82 -84 12 10
15 0 -41 -30 33 31
10 0 -98 -38 32 25
5 0 -41 -33 21 6
1 0 -18 -18 5 14
0 0 0 0 0 0
0 1 2.5 5 10
Celecoxib (µM)
80 0 -87 11 -66 5
75 0 -79 21 -41 8
50 0 -77 19 -30 20
25 0 -62 41 -46 -9
10 0 -95 35 23 -20
0 0 0 0 0 0
0 0.1 0.4 0.7 1
Celecoxib (nM)
300 0 15 -90 13
200 0 -3 -51 18
100 0 -14 -71 23
10 0 -13 -50 82
5 0 1 -16 86
0 0 0 0 0
0 2 4 8
Celecoxib (µM)

FigureS1. Representative drug matrixes showing the effect of cisplatin *plus* celecoxib on HeLa cell cultures. Upper panel: the percentages of cell proliferation inhibition from single drug used are shown in white boxes; for drug combinations, these are shown in gray boxes. Lower panel: Bliss-type Additivism (BTA) percentage values, light gray boxes indicate an infra-additive effect; dark gray boxes indicate a supra-additive effect.

Figure S2


HeLa bidimensional
HeLa MCTS-preventive protocol
+ Celecoxib
IC50 = 5 ± 2 µM
IC50 = 15 ± 3 µM
% Inhibition of proliferation
Cisplatin (µM)
IC50 = 13 ± 4 µM
IC50 = 21 ± 4 µM
Paclitaxel (µM)
IC50 = 14.5 ± 2 µM
IC50 = 26 ± 6 µM
Doxorubicin (µM)
IC50 = 10 ± 3 nM
IC50 = 50 ± 17 nM
% Inhibition of MCTS growth
Cisplatin (nM)
IC50 = 5 ± 2 nM
IC50 = 16 ± 5 nM
Paclitaxel (nM)
IC50 = 10 ± 3 nM
IC50 = 48 ± 7 nM
Doxorubicin (µM)

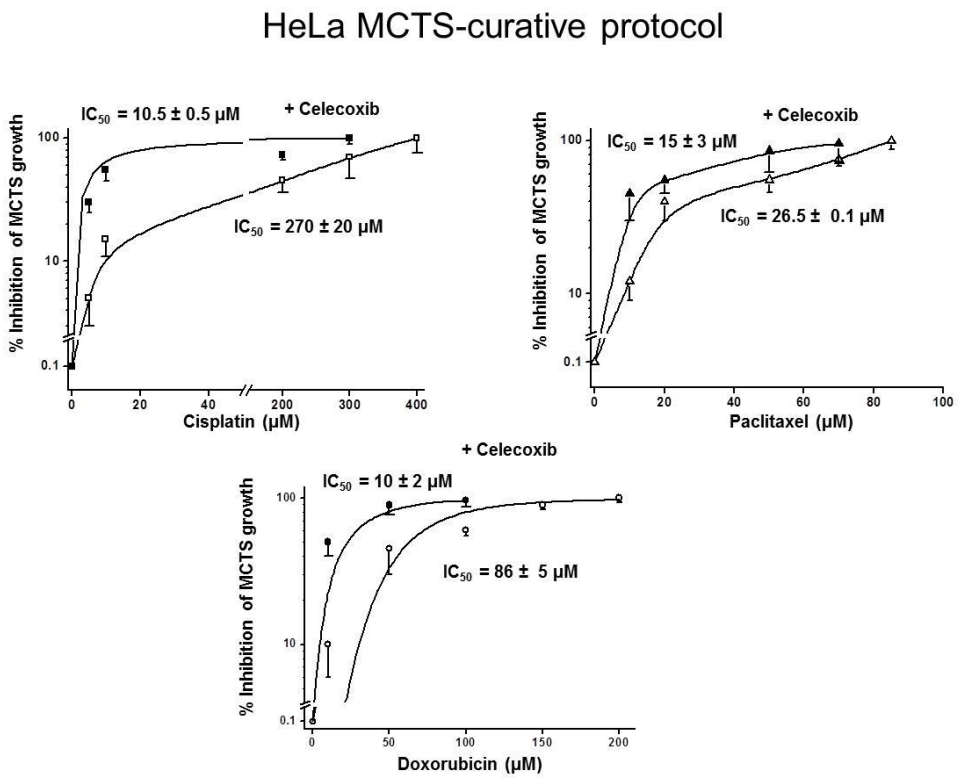


Figure S2. Logarithmic dose-response curve shows the effect of cisplatin, paclitaxel and doxorubicin on HeLa bidimensional cultures (n=3) and MCTS under preventive and curative protocols (n= 30 MCTS), with the presence of celecoxib. For bidimensional cultures, celecoxib was added at 5 -10 µM. For MCTS, celecoxib was added at 0.4-1 nM or 2-6 µM in preventive or curative protocols, respectively.

Figure S3

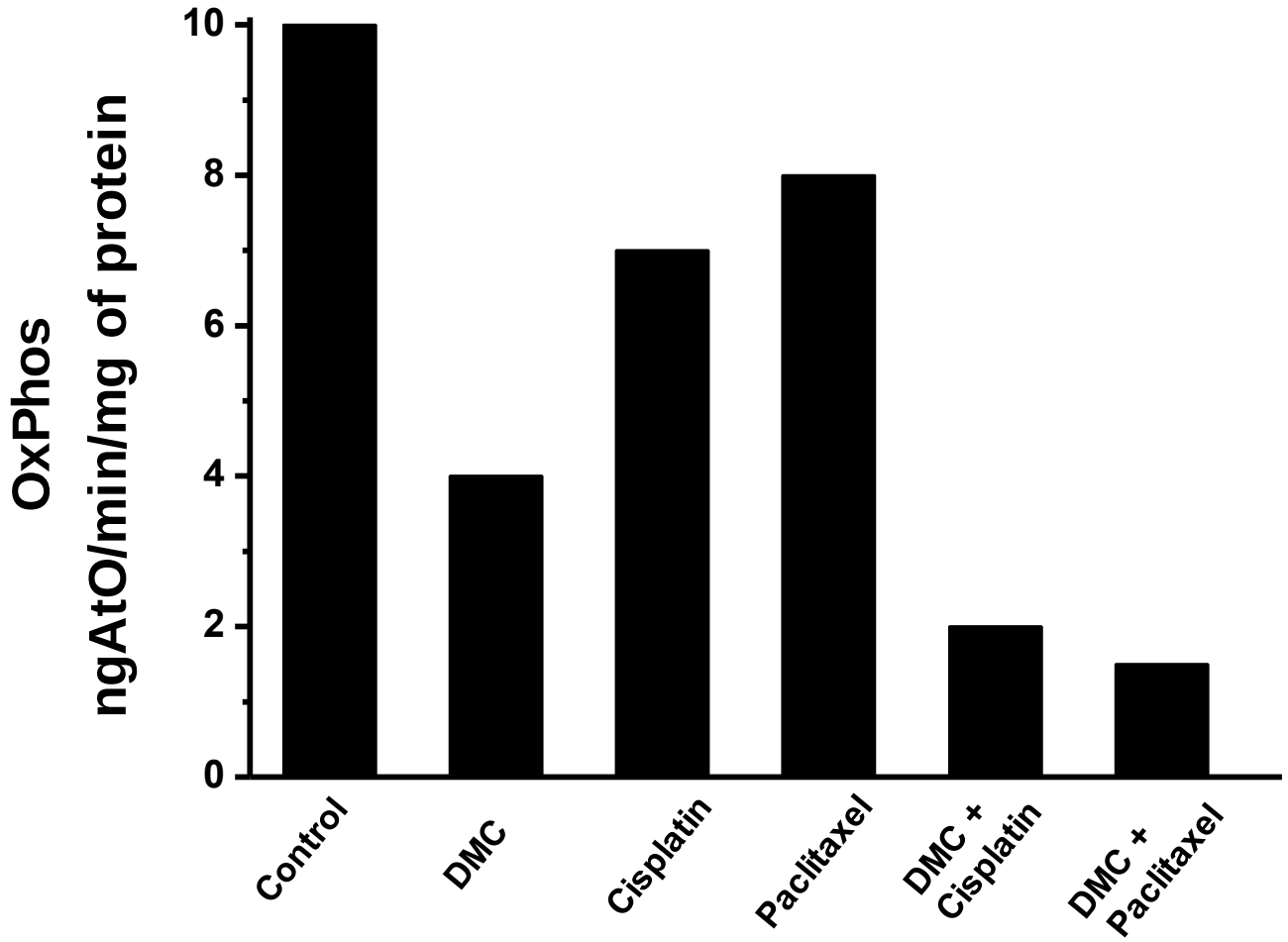


Figure S3. Effect of DMC (15 µM), paclitaxel (20 µM) and cisplatin (5 µM) alone or in combination, on OxPhos fluxes, after 24 h exposure in HeLa cells. n=1; control (no added drugs).

Figure S4

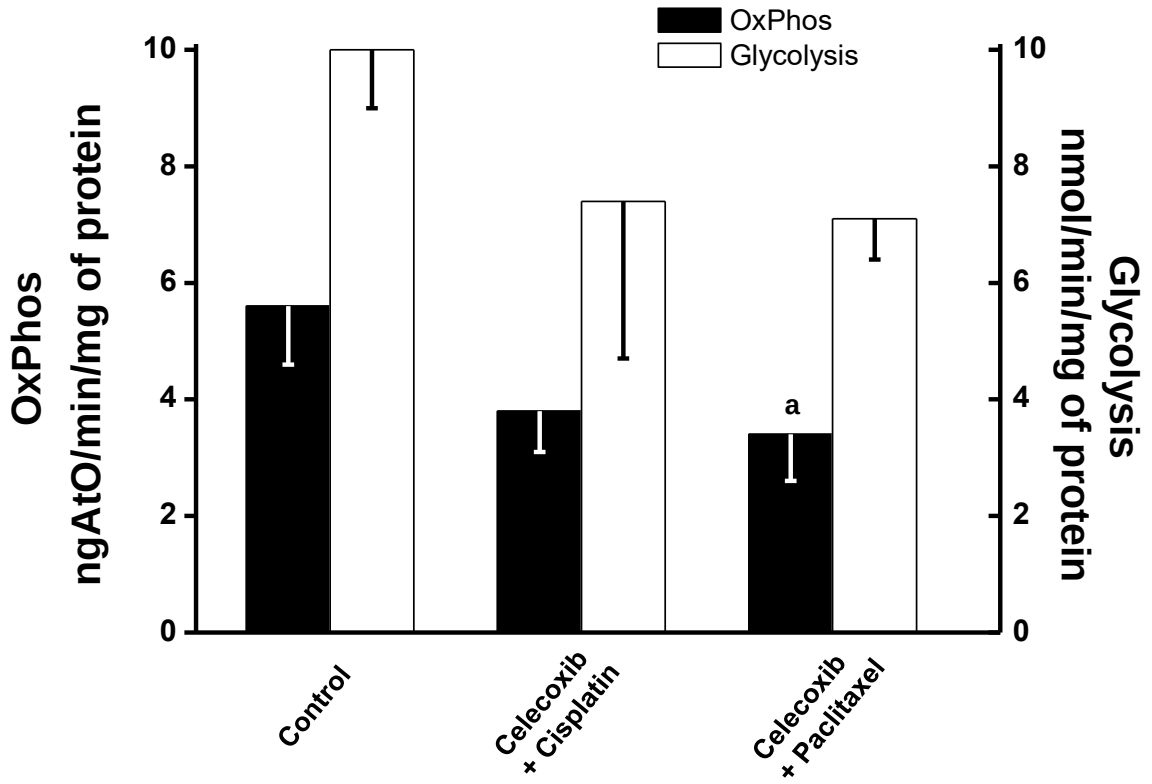


Figure S4. Effect of celecoxib (5 µM), paclitaxel (15 µM) and cisplatin (2 µM) added in combination on OxPhos and glycolysis fluxes, after 24 h exposure in mouse 3T3 fibroblast. The data show the mean ± S.D. of at least three different preparations. [a]P< 0.05 *vs.* control (no added drugs).

FIGURE S5.

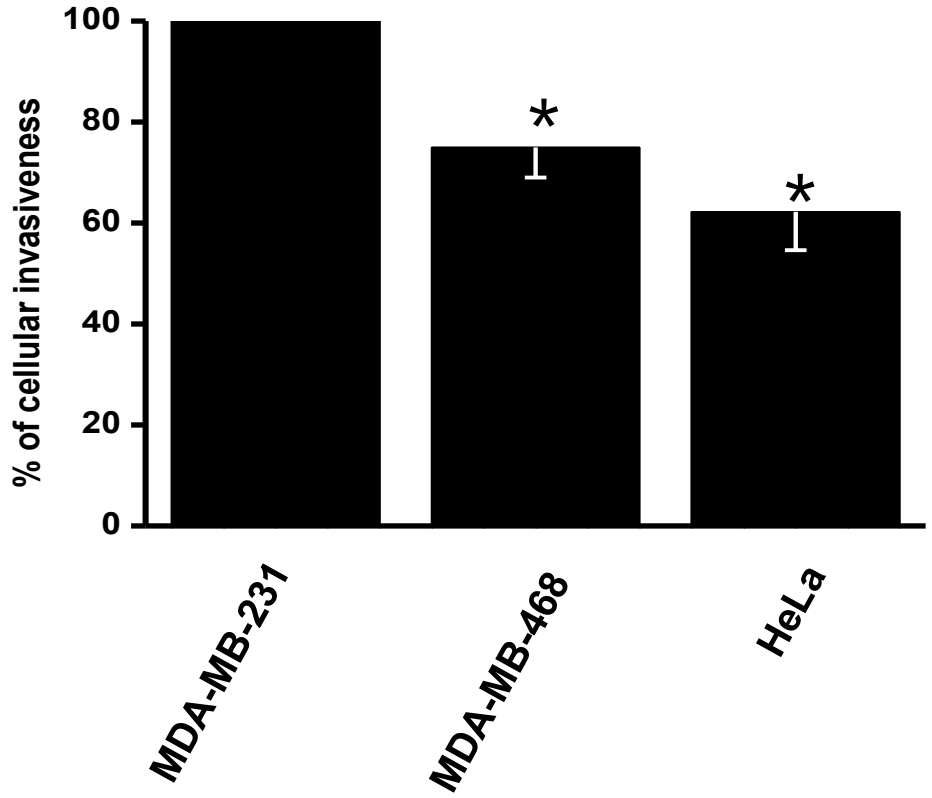


Figure S5. Invasiveness potential of different metastatic cancer cell lines. n= 3, *P<0.05 *vs.* MDA-MB-231.

Figure S6

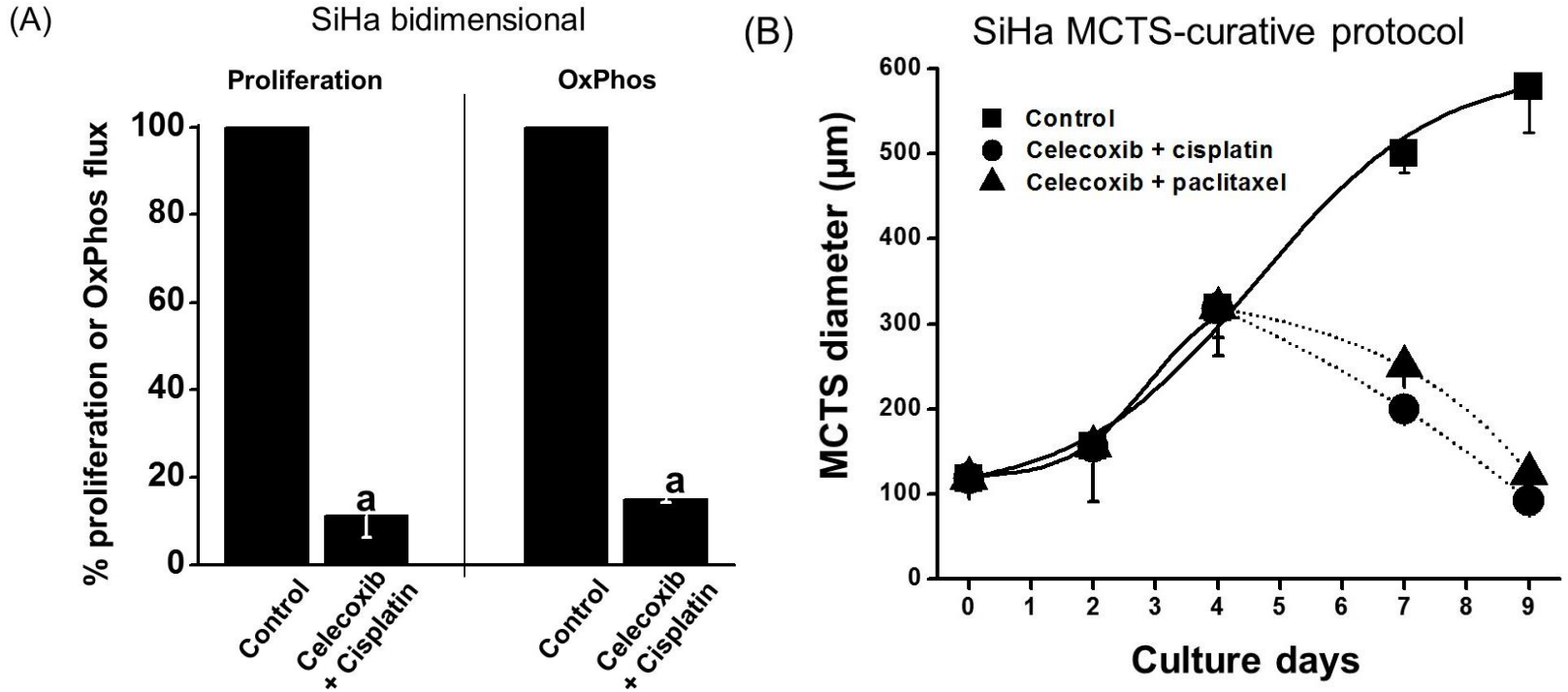
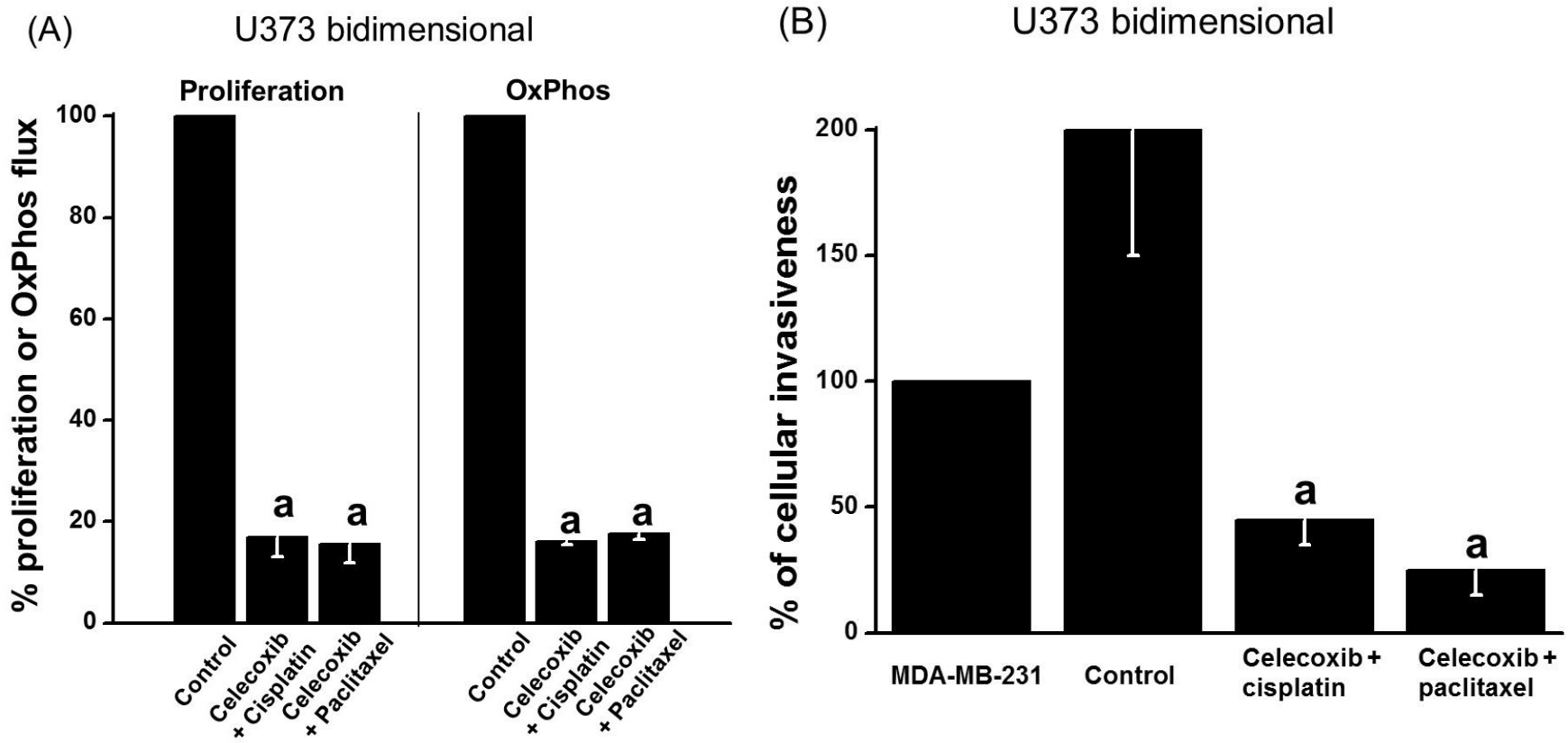


Figure S6. (A) Effect of celecoxib (3 µM) and cisplatin (1 µM) added in combination on cell proliferation and OxPhos flux, after 24 h exposure in SiHa bidimensional cultures. Control cell proliferation (100%) corresponded to 34 x 10^3 cells after 48 h culture. OxPhos flux control (100%) corresponded to 10 ± 1.5 ng At O/min/mg of protein. The data shown represent the mean ± S.D. of at least three different preparations for bidimensional culture. $^{a}P < 0.05$ *vs.* control (no added drugs).
(B) Effect of celecoxib (2 µM), cisplatin (1 µM) and paclitaxel (10 µM) added in combination on SiHa MCTS growth using curative protocol (n= 5 spheroids)

Figure S7


(A)
U373 bidimensional
Proliferation
OxPhos
% proliferation or OxPhos flux
100
80
60
40
20
0
a
a
a
a
Control
Celecoxib + Cisplatin
Celecoxib + Paclitaxel
Control
Celecoxib + Cisplatin
Celecoxib + Paclitaxel
(B)
U373 bidimensional
% of cellular invasiveness
200
150
100
50
0
a
a
MDA-MB-231
Control
Celecoxib + cisplatin
Celecoxib + paclitaxel

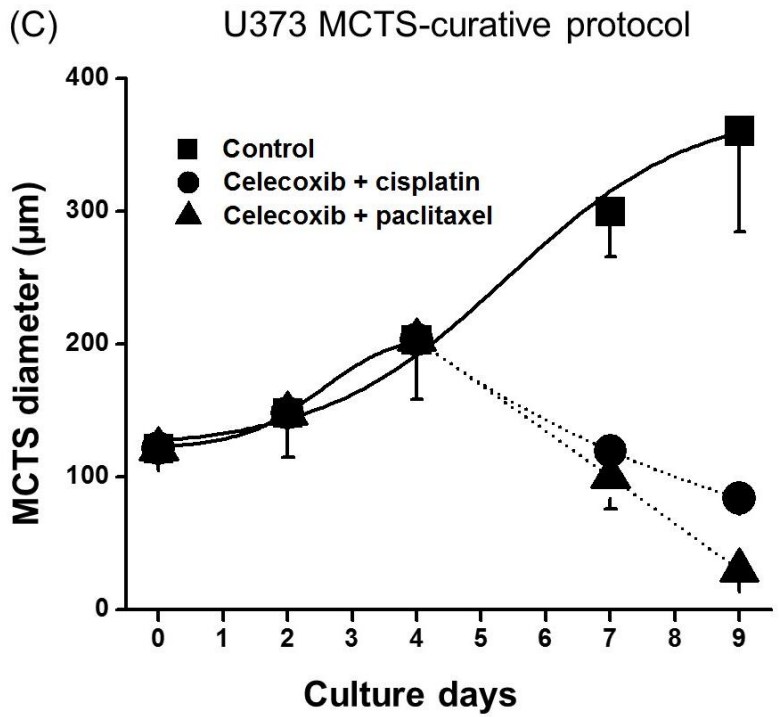


Fig S7. Effect of celecoxib (5 µM), cisplatin (2 µM) and paclitaxel (15 µM) added in combination on (A) cell proliferation, OxPhos flux and (B) invasiveness after 24 h exposure in U373 bidimensional cultures. Control cell proliferation (100%) corresponded to 48 x 10^3 cells. OxPhos flux control (100%) corresponded to 13 ± 5 ng At O/min/mg of protein. The data show the mean ± S.D. of at least three different preparations for bidimensional culture. $^{a}P < 0.05$ *vs.* control (no added drugs). (C) Effect of celecoxib (4-5 µM), cisplatin (1 µM) and paclitaxel (25 µM) added in combination on U373 MCTS growth using curative protocol (n= 5 spheroids).