Supplementary Material

Artemisinin-Daumone Hybrid Inhibits Cancer Cell-Mediated Osteolysis by Targeting Cancer Cells and Osteoclasts

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Table S1. Altered gene expression of transcription factors in MDA-MB-231 breast cancer cells treated with ARTD (5 µM)

Position	Gene symbol	Fold change	Position	Gene symbol	Fold change
A01	AR	-1.69	D10	MYB	0.00
A02	ARNT	0.08	D11	MYC	1.66
A03	ATF1	1.54	D12	MYF5	1.34
A04	ATF2	1.17	E01	MYOD1	1.34
A05	**ATF3**	**22.75***	E02	NFAT5	-1.17
A06	ATF4	3.16	E03	NFATC1	1.29
A07	CEBPA	1.76	E04	NFATC2	-1.82
A08	CEBPB	2.62	E05	NFATC3	0.03
A09	CEBPG	4.00	E06	NFATC4	-1.19
A10	CREB1	1.34	E07	NFKB1	1.05
A11	CREBBP	1.22	E08	NFYB	-1.50
A12	CTNNB1	-1.45	E09	NR3C1	1.11
B01	DR1	1.67	E10	PAX6	-0.07
B02	**E2F1**	**-5.19***	E11	POU2AF1	-0.53
B03	E2F6	1.66	E12	PPARA	-0.02
B04	EGR1	8.56	F01	PPARG	2.05
B05	ELK1	0.16	F02	RB1	-1.11
B06	ESR1	1.29	F03	REL	-1.21
B07	ETS1	3.38	F04	RELA	1.42
B08	ETS2	-1.79	F05	RELB	2.29
B09	FOS	-0.47	F06	SMAD1	1.80
B10	FOXA2	0.10	F07	SMAD4	1.28
B11	FOXG1	-1.73	F08	SMAD5	1.37
B12	FOXO1	-0.01	F09	SMAD9	-4.16
C01	GATA1	1.41	F10	SP1	-0.09
C02	GATA2	-2.42	F11	SP3	0.00
C03	GATA3	2.34	F12	STAT1	1.96
C04	GTF2B	0.23	G01	STAT2	0.89
C05	GTF2F1	1.06	G02	STAT3	-1.28
C06	HAND1	0.03	G03	STAT4	1.23
C07	HAND2	-2.69	G04	STAT5A	1.68
C08	HDAC1	-1.29	G05	STAT5B	1.57
C09	HIF1A	-0.02	G06	STAT6	1.07
C10	HNF1A	-2.07	G07	TBP	1.28
C11	**HNF4A**	**-21.17***	G08	TCF7L2	1.24
C12	HOXA5	1.41	G09	TFAP2A	-1.90
D01	HSF1	1.73	G10	TGIF1	1.35
D02	ID1	-1.89	G11	TP53	0.10
D03	IRF1	2.53	G12	YY1	0.03
D04	JUN	3.50	H01	ACTB	1.05
D05	JUNB	1.61	H02	B2M	1.35
D06	JUND	1.20	H03	GAPDH	0.00
D07	MAX	1.44	H04	HPRT1	-1.48
D08	MEF2A	1.68	H05	RPLP0	0.05
D09	MEF2C	1.69			

The gene expression levels are indicated as fold change (* P <0.05, fold change > 4).

Table S2. Altered gene expression of transcription factors in A549 lung cancer cells treated

with ARTD (5 μM)

Position	Gene symbol	Fold change	Position	Gene symbol	Fold change
A01	AR	-1.61	D10	MYB	-1.41
A02	ARNT	-0.06	D11	MYC	1.63
A03	ATF1	1.37	D12	MYF5	1.33
A04	ATF2	1.56	E01	MYOD1	1.17
A05	**ATF3**	**8.47***	E02	NFAT5	-1.25
A06	ATF4	3.61	E03	NFATC1	1.15
A07	CEBPA	1.58	E04	NFATC2	-1.67
A08	CEBPB	2.25	E05	NFATC3	1.10
A09	CEBPG	4.51	E06	NFATC4	-1.25
A10	CREB1	1.26	E07	NFKB1	-1.05
A11	CREBBP	-1.13	E08	NFYB	-1.50
A12	CTNNB1	-1.80	E09	NR3C1	-1.28
B01	DR1	1.55	E10	PAX6	2.08
B02	**E2F1**	**-7.27***	E11	POU2AF1	-0.88
B03	E2F6	1.08	E12	PPARA	-0.05
B04	EGR1	7.83	F01	PPARG	1.51
B05	ELK1	0.03	F02	RB1	-1.32
B06	ESR1	1.17	F03	REL	-1.42
B07	ETS1	1.99	F04	RELA	1.22
B08	ETS2	-2.15	F05	RELB	2.58
B09	FOS	-1.47	F06	SMAD1	1.37
B10	FOXA2	-1.10	F07	SMAD4	1.18
B11	FOXG1	1.36	F08	SMAD5	1.16
B12	FOXO1	-1.35	F09	SMAD9	1.35
C01	GATA1	-0.22	F10	SP1	-0.07
C02	GATA2	-1.66	F11	SP3	-1.10
C03	GATA3	2.43	F12	STAT1	1.71
C04	GTF2B	1.16	G01	STAT2	1.76
C05	GTF2F1	0.11	G02	STAT3	-1.59
C06	HAND1	1.59	G03	STAT4	1.41
C07	HAND2	-1.23	G04	STAT5A	2.30
C08	HDAC1	-1.74	G05	STAT5B	1.52
C09	HIF1A	-1.09	G06	STAT6	-0.02
C10	HNF1A	-1.60	G07	TBP	1.26
C11	**HNF4A**	**-5.00***	G08	TCF7L2	-1.10
C12	HOXA5	0.01	G09	TFAP2A	-1.26
D01	HSF1	1.45	G10	TGIF1	1.24
D02	ID1	-1.30	G11	TP53	1.45
D03	IRF1	0.16	G12	YY1	0.04
D04	JUN	2.97	H01	ACTB	-1.23
D05	JUNB	1.28	H02	B2M	1.23
D06	JUND	0.05	H03	GAPDH	-1.07
D07	MAX	1.27	H04	HPRT1	-1.24
D08	MEF2A	1.14	H05	RPLP0	1.33
D09	MEF2C	1.17			

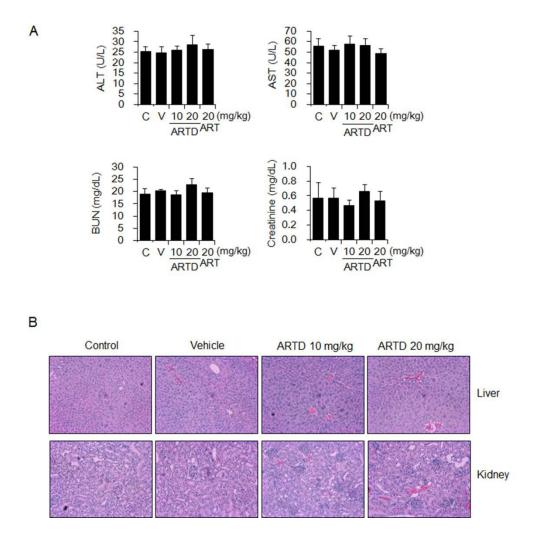The gene expression levels are indicated as fold change (* P <0.05, fold change > 4).

Fig. S1. The effects of ARTD on normal tissues of mice injected with MDA-MB-231 or A549 cells. (A) Serum levels of tissue damage-related biochemical parameters. Serum levels of ALT and AST, which are indicators of liver damage, and BUN and creatinine, which are indicators of kidney damage, were quantified in mice injected with MDA-MB-231 cells and treated with artemisinin (ART) and ARTD as shown in Figure 1, using their respective commercial assay kits. Data are expressed as the mean ± SEM. (B) Histological images of tissue damage. Liver and kidney tissues were collected from mice injected with A549 cells and treated with ARTD as shown in Fig. 2. The tissues were fixed in 10% buffered formalin and the sections were stained with H&E. (Original magnification, x100).
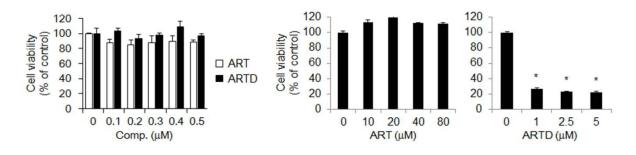
Fig. S2. The effects of ARTD on the viability of BMMs. BMMs (5×10^4 cells/well) in 96-well plates were treated with artemisinin (ART) or ARTD at the indicated concentrations in the presence of M-CSF (30 ng/mL) for 5 days. The cell viability was detected with a MTT assay. Data are expressed as the mean ± SEM. *$P < 0.05$ *versus* untreated cells
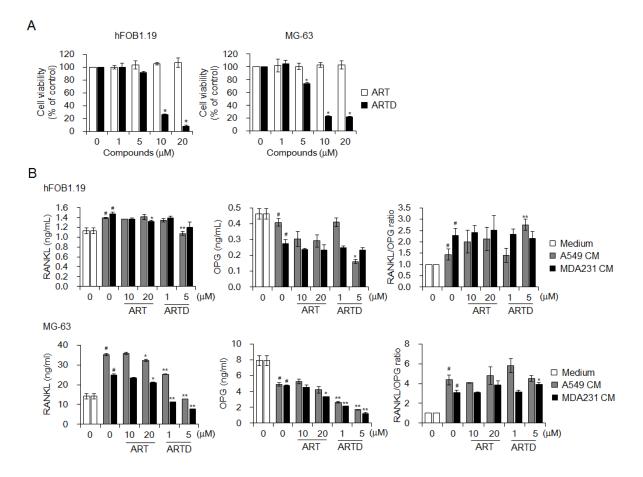
Fig. S3. The effect of ARTD on the levels of RANKL and OPG secreted from human osteoblastic hFOB1.19 and osteosarcoma MG-63 cells. (A) hFOB1.19 or MG-63 cells were incubated in RPMI 1640 or DMEM with ART and ARTD at the indicated concentrations for 24 h. Cell viability was measured with an MTT assay. Data are expressed as mean ± SEM. *P < 0.01 *versus* untreated cells. (B) A549 or MDA-MB-231 (MDA231) cells (2 x 10^6 cells/100 mm dish) were incubated in RPMI 1640 or DMEM containing 10% FBS for 24 h. Culture media were changed to serum-free DMEM/F12 and the cells were cultured for 24 h. The culture medium was collected and centrifuged at 500 ×g for 5 min. The supernatant was collected as the conditioned medium (CM). hFOB1.19 or MG-63 cells (1 × 10^5 cells/well) were cultured in 96-well plates with 10% FBS-DMEM/F12 for 24 h and then treated with 70% CM of A549 or MDA-MB-231 cells without or with ART or ARTD. Twenty-four hours later, RANKL and OPG levels in cultured media were measured using the commercially available

RANKL and OPG ELISA kits, respectively. Data are expressed as mean ± SEM. [#]P<0.05 *versus* cultured media, [*]P<0.05, [**]P<0.01 *versus* cultured media with A549 CM or MDA-MB-231 CM alone.