Selective Antiproliferative Activity of an Oleandrigenin-Derived Androstane in Prostate Cancer Cells

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Supplementary Information

Tab. S1 Chemical names of tested cardiac glycoside derivatives

Compound No.	Chemical name
1	Oleandrigenin
2	3-*O*-Acetyl oleandrigenin
3	16β-Acetoxy-17β-(Furyl-3)-5β,14β-androstan-3β,14-diol
4	17β-(Furyl-3)-5β,14β-androstan-3β,14,16β-triol
5	17β-(Furyl-3)-3β-tertbutyldimethylsilyloxy-5β,14β-androstan-14,16β-diol
6	3-*O*-Methyl-5α-oleandrigenin
7	16β-Acetoxy-17β-(5H-furan-2-on-3-yl)-3β-methoxy-5α-androst-14-ene
8	17β-(5H-Furan-2-on-3-yl)-3β-methoxy-5α-androstan-14,16-diene
9	16β-Acetoxy-14,15β-epoxy-3β-methoxy-5α-card-22-enolide
10	16β-Acetoxy-14,15α-epoxy-17β-(5H-furan-2-on-3-yl)-3β-methoxy-5α,14α-androstane

Tab. S2 List of primary (A) and secondary (B) antibodies used in experiments.

A. Primary antibodies

Target	Clone	Type	Host	Dilution	Manufacturer
AR	N-20	polyclonal	rabbit	1:200/1:50*	Santa Cruz Biotechnology
pAR (Ser81)	–	monoclonal	rabbit	1:500	Sigma-Aldrich
Bcl-2	100	monoclonal	mouse	1:500	Biogenex
Bcl-X_L	H-62	monoclonal	rabbit	1:100	Novocastra
Bid	B-3	monoclonal	mouse	1:100	Santa Cruz Biotechnology
β-actin	C4	monoclonal	mouse	1:1000	Santa Cruz Biotechnology
Caspase-3	Asp175	polyclonal	rabbit	1:1000	Cell Signaling Technology
CDK4	DCS-31	monoclonal	mouse	1:200	Santa Cruz Biotechnology
Cyclin A	H432	polyclonal	rabbit	1:200	Santa Cruz Biotechnology
Cyclin B1	GNS1	monoclonal	mouse	1:1000	Cell Signaling Technology
Cyclin D_1	72-13G	monoclonal	rabbit	1:100	Santa Cruz Biotechnology
Cyclin E	HE12	monoclonal	mouse	1:200	Santa Cruz Biotechnology
ER-α	D-12	monoclonal	mouse	1:200/1:50*	Novocastra
ER-β	H-150	monoclonal	rabbit	1:100/1:50*	Santa Cruz Biotechnology
MCM-7	141.7	monoclonal	mouse	1:500	Sigma-Aldrich
Nkx3.1	D2Y1A	monoclonal	rabbit	1:500	Cell Signaling Technology
p16	JC8	monoclonal	mouse	1:100	Santa Cruz Biotechnology
p21	12D1	monoclonal	rabbit	1:1000	Cell Signaling Technology
p53	DO1	monoclonal	mouse	1:500	Invitrogen
PSA/KLK3	D6B1	monoclonal	rabbit	1:250	Cell Signaling Technology
Rb	4H1	monoclonal	mouse	1:1000	Cell Signaling Technology
pRb (S807/811)	S807/811	polyclonal	rabbit	1:1000	Cell Signaling Technology
Src	-	monoclonal	mouse	1:1000	Cell Signaling Technology

* dilution for immunofluorescence

B. Secondary antibodies

Target	Description	Dilution	Manufacturer
HRP-conjugated goat anti-mouse IgG	Goat anti-mouse IgG (H+L), HRP conjugated	1:6000	Cell Signaling Technology
HRP-conjugated goat anti-rabbit IgG	Goat anti-rabbit IgG (H+L), HRP conjugated	1:6000	Cell Signaling Technology
Alexa Fluor 594-conjugated goat anti-mouse IgG	Goat anti-mouse IgG (H+L), Alexa Fluor 594 conjugated	1:1000	Thermo Fisher Scientific (Life Technologies)

Target	Description	Dilution	Manufacturer
Alexa Fluor 488-conjugated goat anti-rabbit IgG	Goat anti-rabbit IgG (H+L), Alexa Fluor 488 conjugated	1:1000	Thermo Fisher Scientific (Life Technologies)

Fig. S1 Structures of tested cardiac glycoside derivatives.

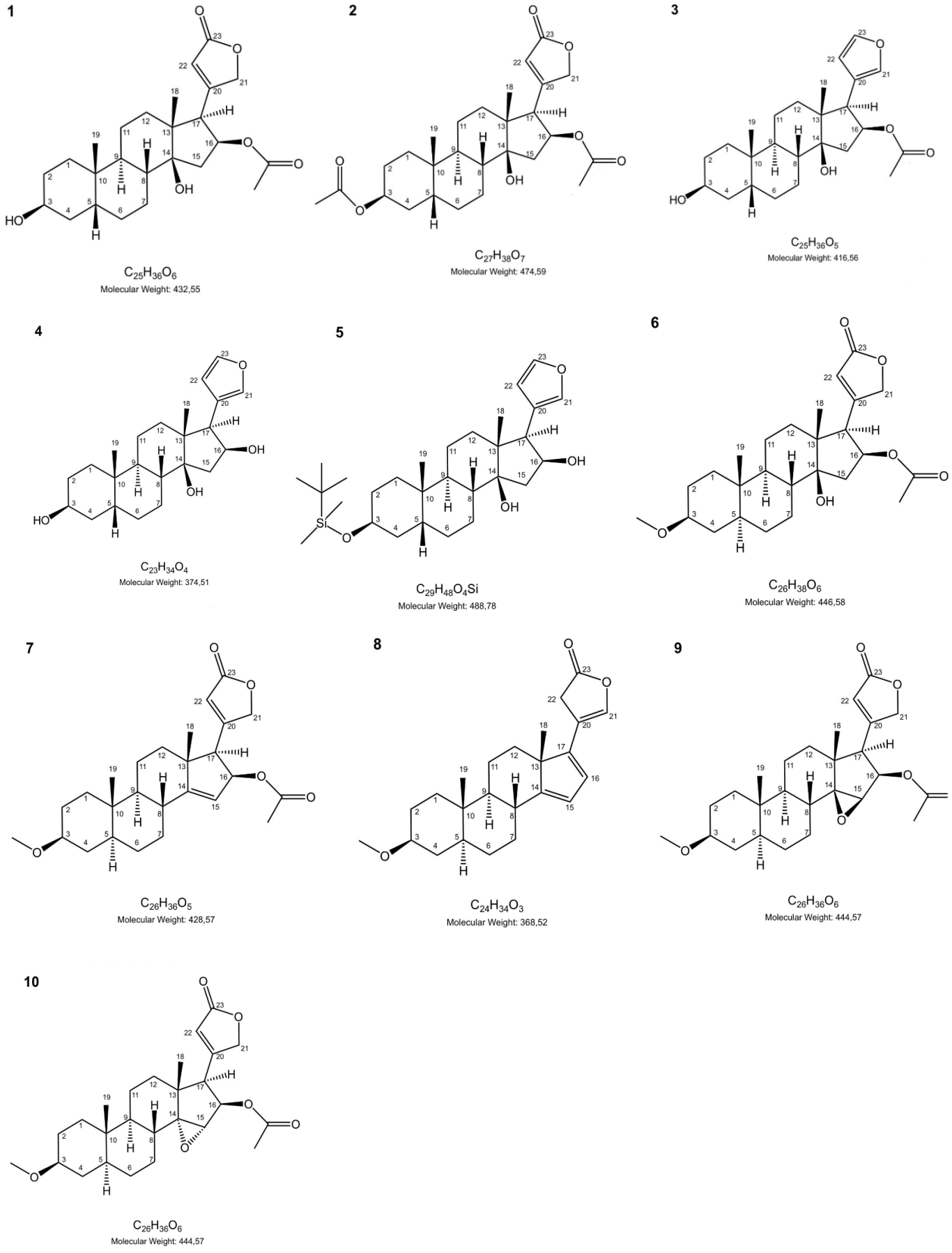


Fig. S2 Representative bright-field images of untreated control and compound **10**-treated RWPE-1, DU-145 and LNCaP cells after 24 and 48 h of treatment at the IC_{50} concentration. Magnification, 200×.

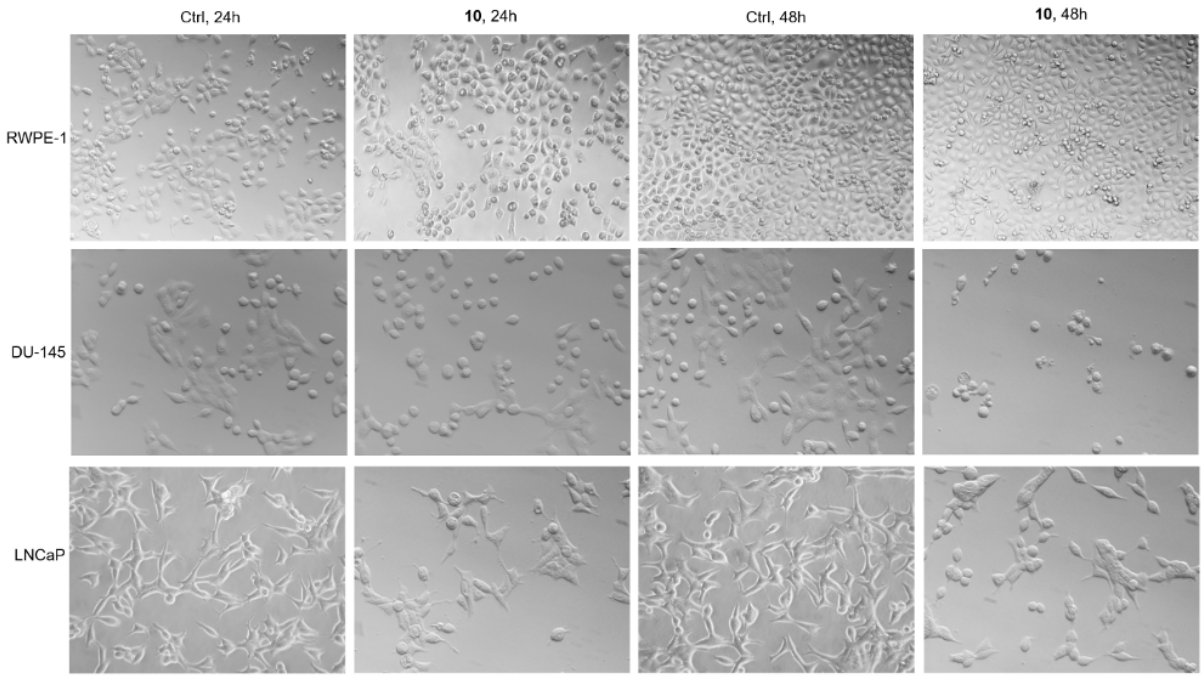
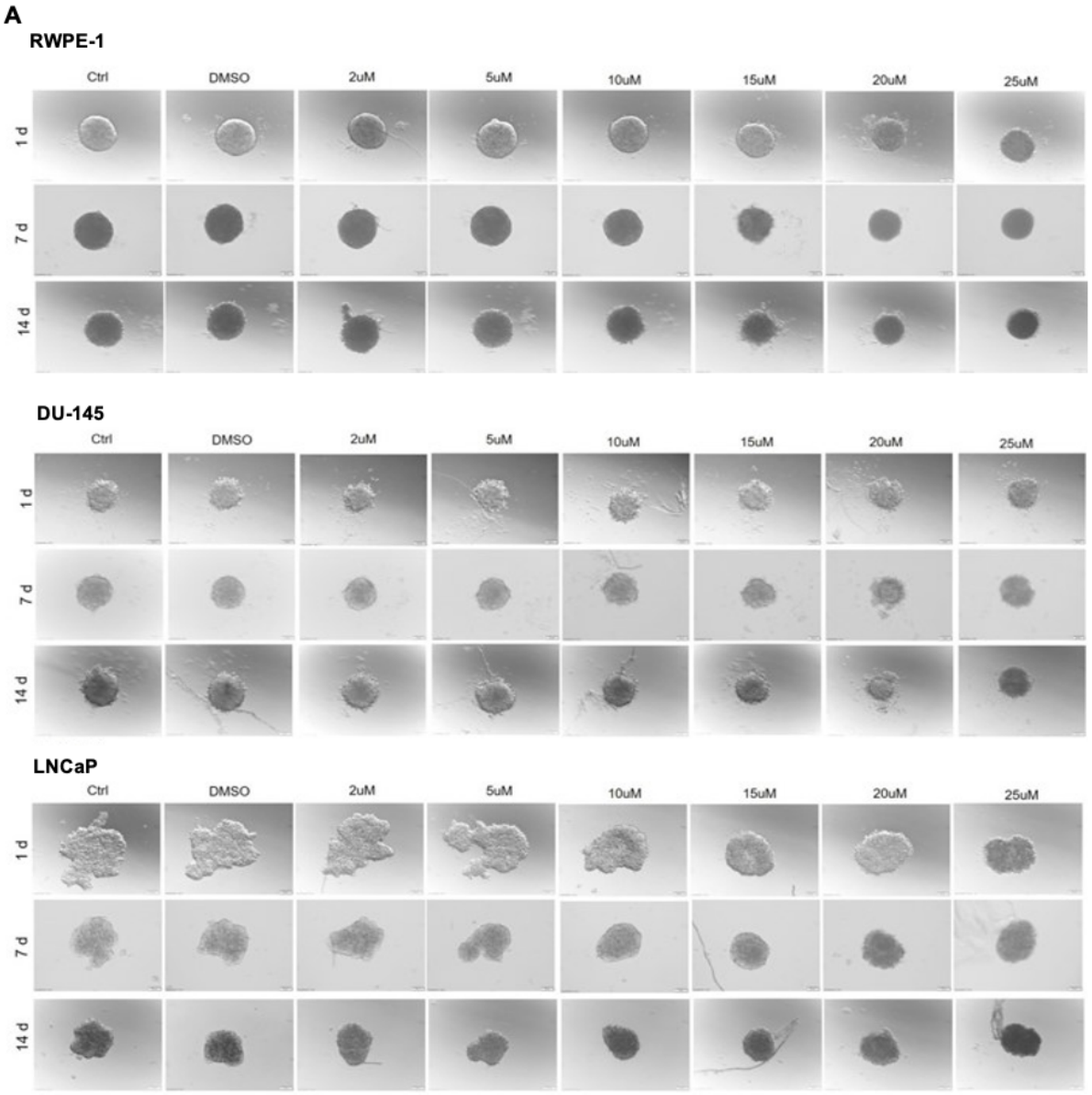
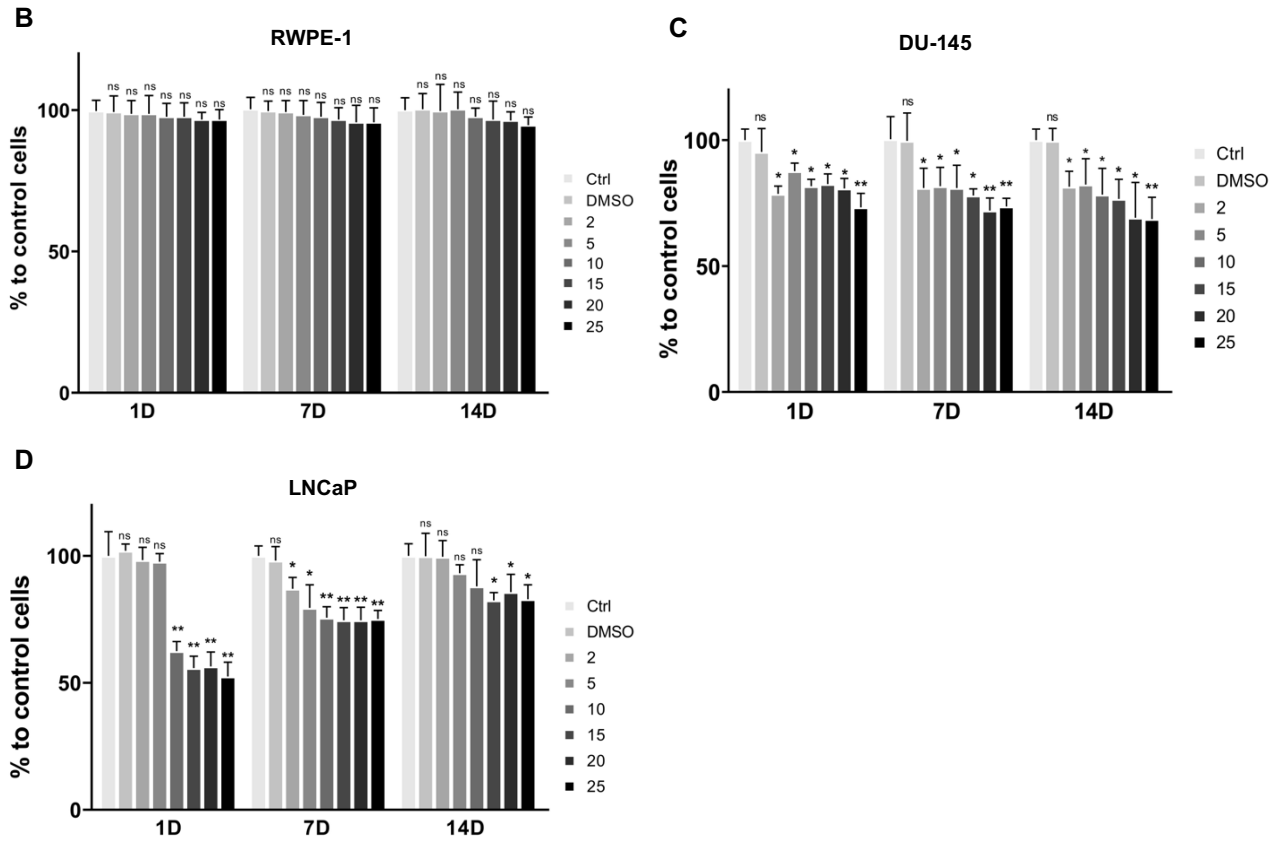


Fig. S3 Time-dependent effect (1–14 days) of compound **10** (2–25 μM) on the cellular integrity of RWPE-1, DU-145 and LNCaP spheroids. (**A**) Representative bright-field images of untreated control and compound **10**-treated spheroids after 1, 7 and 14 days of treatment at all tested concentrations (2–25 μM). Magnification, 100×. Quantification of spheroid area in (**B**) RWPE-1, (**C**) DU-145 and (**D**) LNCaP cells. Data are expressed relative to untreated controls. Asterisks indicate significant differences compared with the corresponding control (*) $p < 0.05$, (**) $p < 0.01$.




B
RWPE-1
% to control cells
100
50
0
1D
7D
14D
Ctrl
DMSO
2
5
10
15
20
25
C
DU-145
% to control cells
100
50
0
1D
7D
14D
Ctrl
DMSO
2
5
10
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20
25
D
LNCaP
% to control cells
100
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Fig. S4 Complete immunofluorescence images of nuclear steroid receptors in DU-145 and LNCaP cells treated with compound **10** at the IC_{50} concentration for 24 h. ER-α and ER-β are shown for DU-145 cells, whereas AR, ER-α and ER-β are shown for LNCaP cells. Individual fluorescence channels (green), DAPI (blue), and the corresponding merged images are shown for each treatment condition. Magnification, 400×.

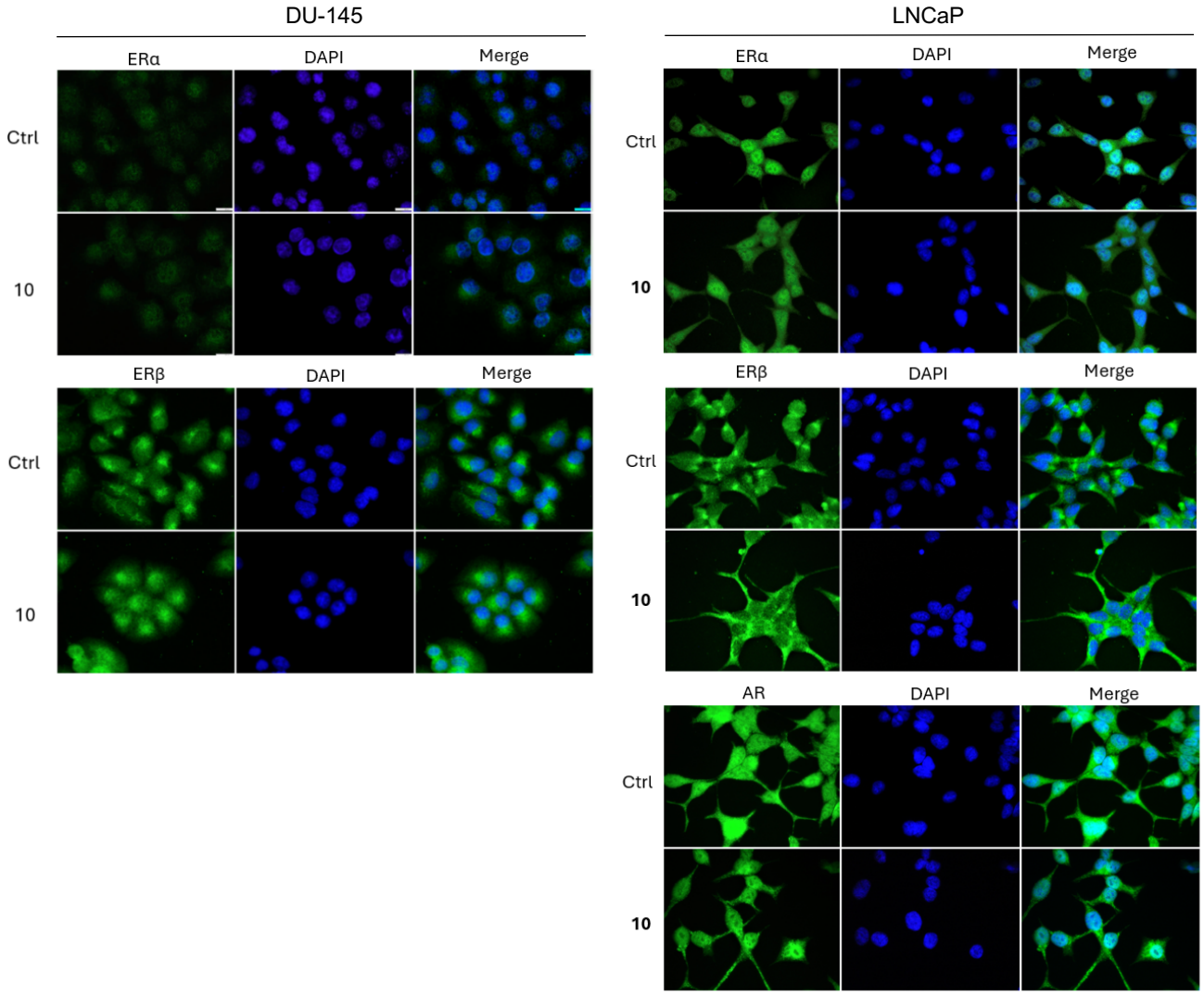
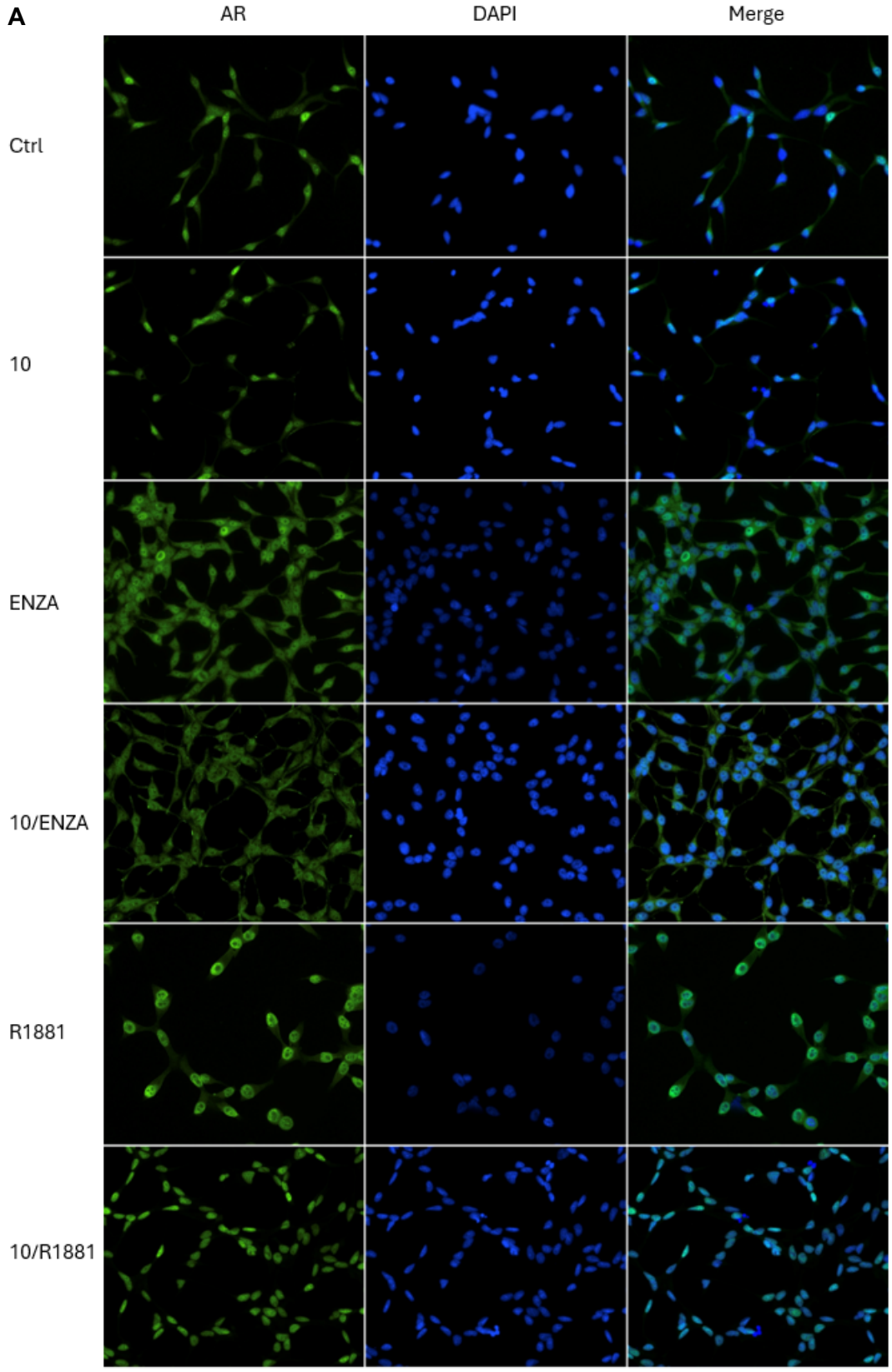
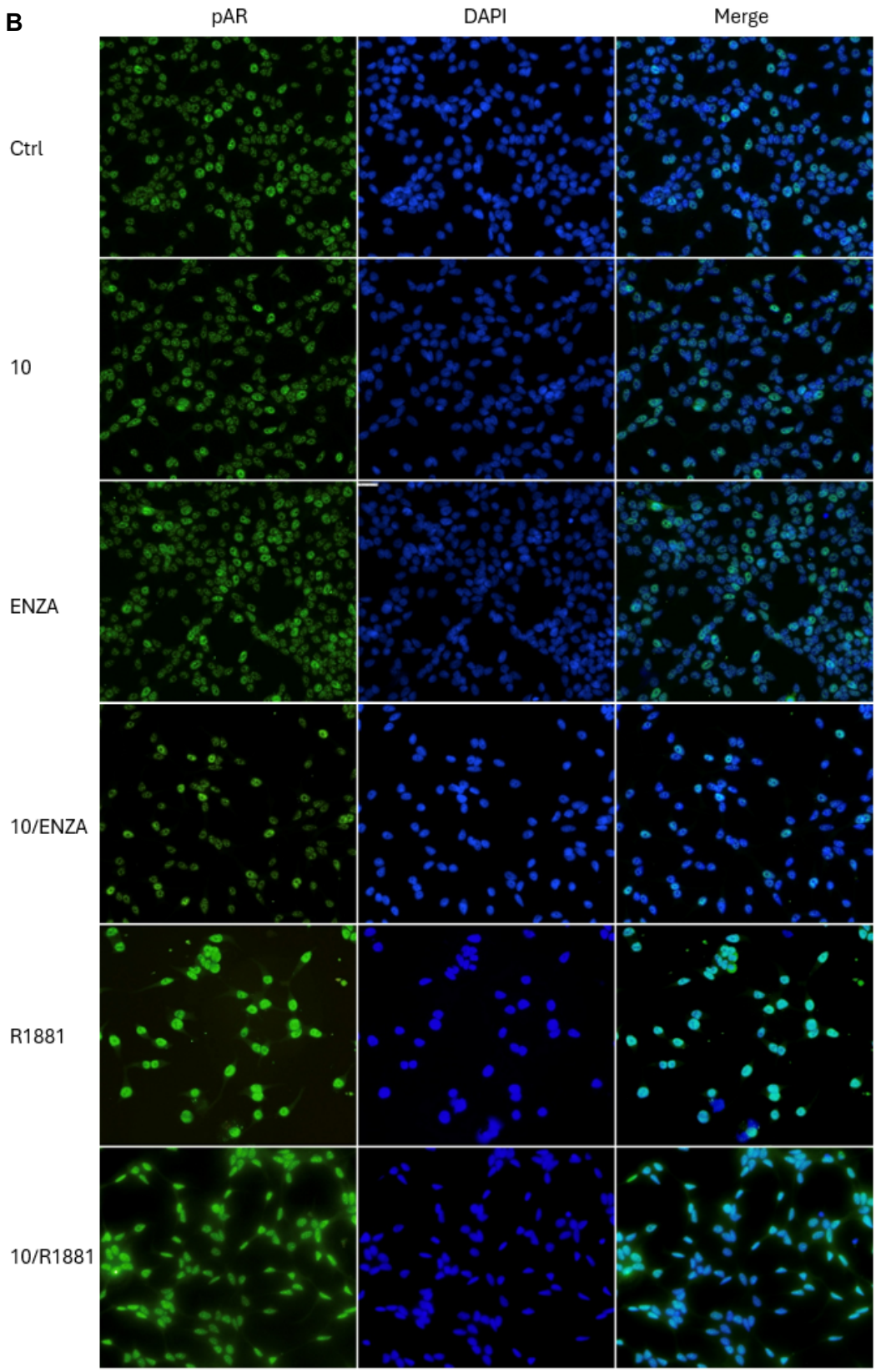


Fig. S5 Complete immunofluorescence images of AR and phosphorylated AR (Ser81) in LNCaP cells treated with compound **10** at the IC_{50} concentration, R1881 (1 nM), enzalutamide (1 μM), or their combinations for 24 h in charcoal-stripped serum medium. Individual fluorescence channels for AR or pAR (Ser81) (green), DAPI (blue), and the corresponding merged images are shown for each treatment condition. Magnification, 200×.




B
pAR
DAPI
Merge
Ctrl
10
ENZA
10/ENZA
R1881
10/R1881