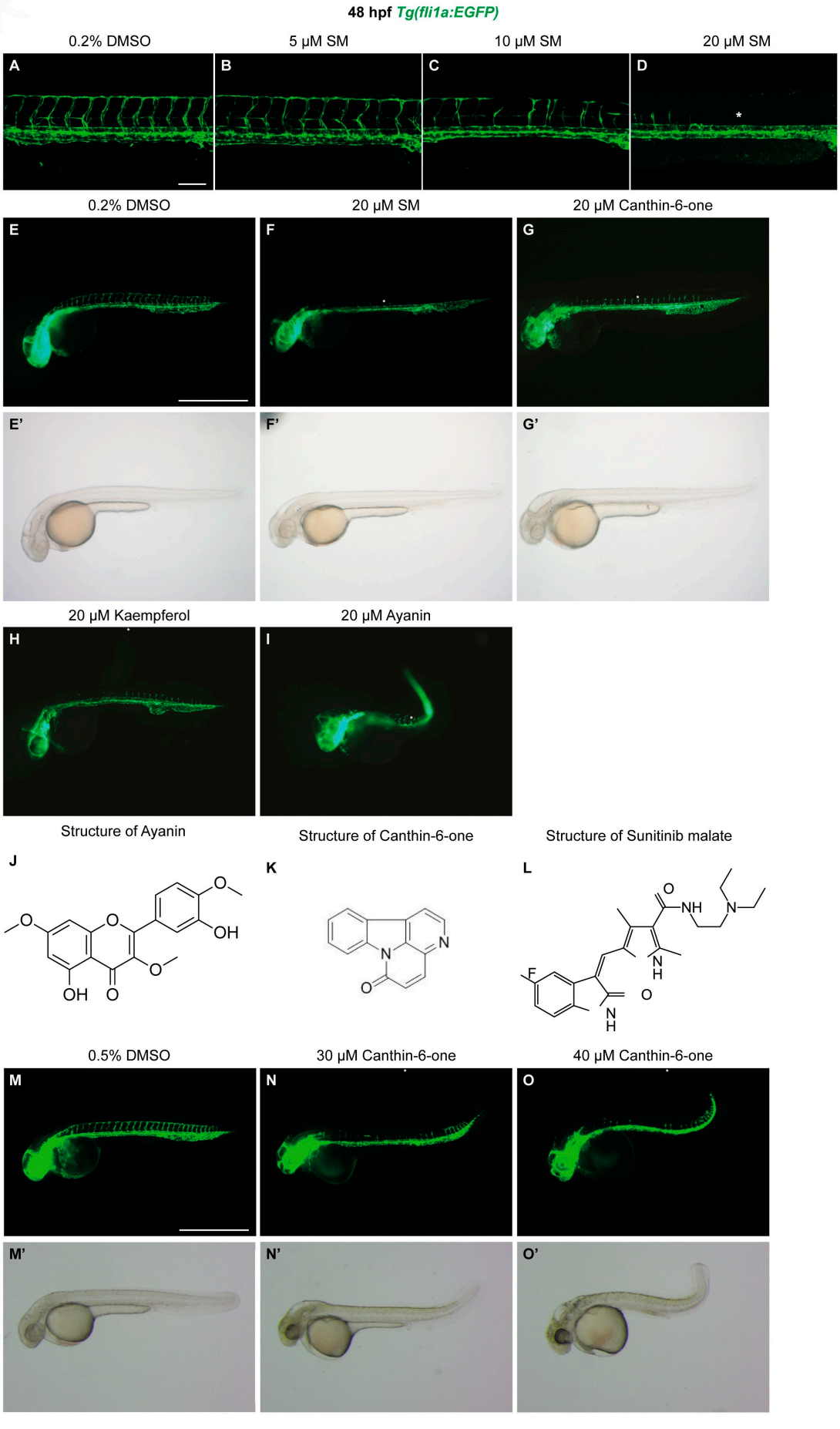
48 hpf Tg(fli1a:EGFP)
0.2% DMSO
5 μM SM
10 μM SM
20 μM SM
A
B
C
D
0.2% DMSO
20 μM SM
20 μM Canthin-6-one
E
F
G
E'
F'
G'
20 μM Kaempferol
20 μM Ayanin
H
I
Structure of Ayanin
Structure of Canthin-6-one
Structure of Sunitinib malate
J
K
L
O
OH
OH
O
N
N
O
NH
N
N
H
F
O
N
H
0.5% DMSO
30 μM Canthin-6-one
40 μM Canthin-6-one
M
N
O
M'
N'
O'

Figure S1. Canthin-6-one, kaempferol, and ayanin at 20 μM inhibit developmental angiogenesis in zebrafish. (**A–D**) Lateral confocal images of 48 hpf *Tg(fli1a:EGFP)* embryos treated with 0.2% DMSO (n = 16, **A**) or sunitinib malate (SM) at either 5 μM (n = 17, **B**), 10 μM (n = 15, **C**), or 20 μM (n=20, **D**). Only SM at 20 μM completely inhibits ISV development. (**E–I**) Lateral fluorescent (**E–I**) and trans-light images (**E'–G'**) of 48 hpf *Tg(fli1a:EGFP)* embryos treated with either 0.2% DMSO (**E,E'**), 20 μM sunitinib malate (SM, **F,F'**), 20 μM canthin-6-one (**G,G'**), 20 μM kaempferol (**H**), or 20 μM ayanin (**I**). White asterisks indicate lack of intersegmental vessels (ISVs). (**J–L**) Structure of ayanin (**J**), canthin-6-one (**K**), or SM (**L**). (**M–O'**) Lateral fluorescent (**M-O**) and trans-light images (**M'–O'**) of 48 hpf *Tg(fli1a:EGFP)* embryos treated with 0.5% DMSO (n = 20, **M,M'**) or canthin-6-one at either 30 μM (n=17, N,N') or 40 μM (n=18, O,O'). Scale Bars: 100 μm for (A) and 1 mm for (E) and (M).

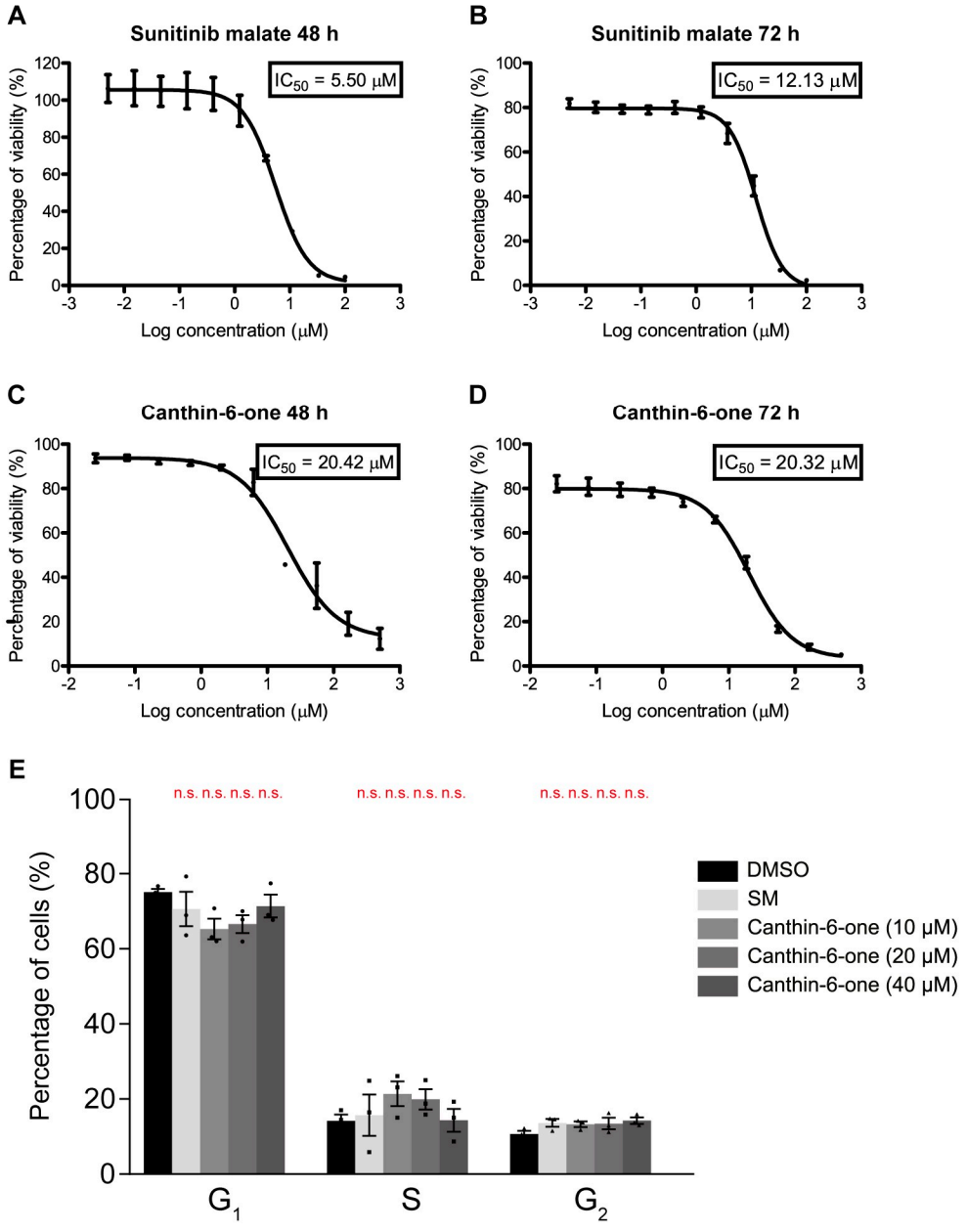

Figure S2. Canthin-6-one impairs HUVEC viability and proliferation. (**A-D**) Cell viability plot of HUVECs treated with either sunitinib malate (SM, A,B) or canthin-6-one (**C,D**) at 0.05 to 1000 μM for 48 h (**A,C**) or 72 h (**B,D**, n = 3). (**E**) Bar chart showing percent-age of HUVECs treated with either DMSO, SM, or canthin-6-one at indicated concentrations in G0/G1, S, or G2/M phase (n = 3). While not significant, canthin-6-one treatment at all concentrations showed a marginal increase of percentage of HUVECs in G2/M phase. Statistical test: Kruskal-Wallis test was conducted for graph **(E)**. $p > 0.05$ (not significant, n.s.).

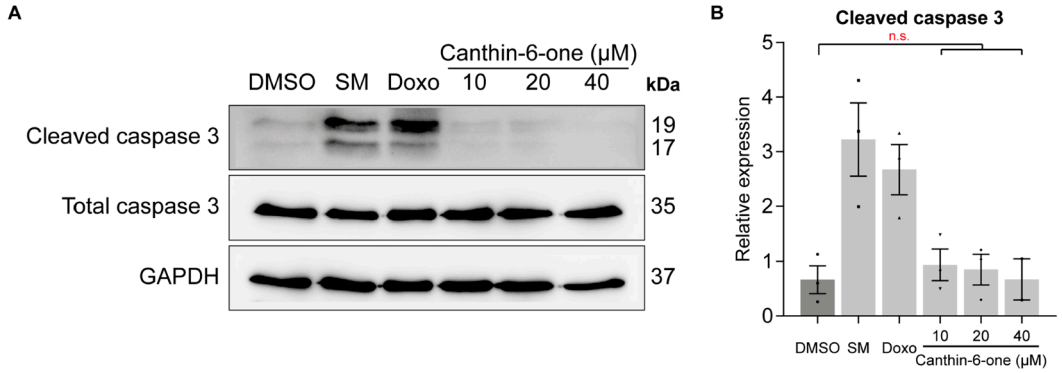

Figure S3. Canthin-6-one does induce endothelial cell apoptosis. (**A**) Western blot analysis of lysates isolated from HUVECs treated with either 0.2% DMSO, 10 μM sunitinib malate (SM), 500 nM doxorubicin, or canthin-6-one at indicated concentrations for 1 h (n = 3). Protein levels of cleaved caspase 3, total caspase 3, and GAPDH were assessed. The full-length blots are presented in Figure S4. (**B**) Densitometry analysis of cleaved caspase 3 protein levels from western blot analysis in image (**A**). Cleaved caspase 3 protein level was normalised to total caspase 3 protein level. Statistical test: Kruskal-Wallis test was conducted for graph **(B)**. $p > 0.05$ (not signifi-cant, n.s.).

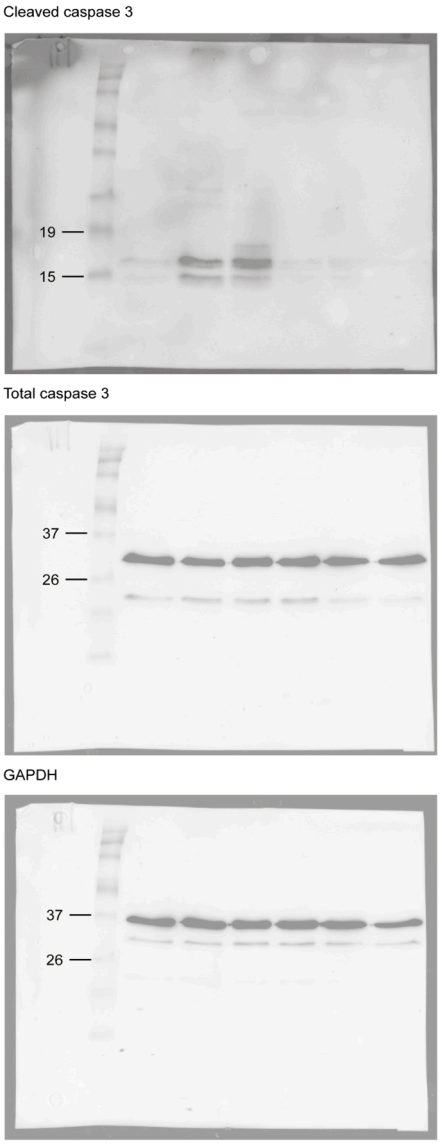

Figure S4. Original western blot images of Figure S3A. Images were taken using the Bio-Rad ChemiDoc Imaging system (Bio-Rad, CA, USA). Original western blot images were processed using the Fiji image processing software.

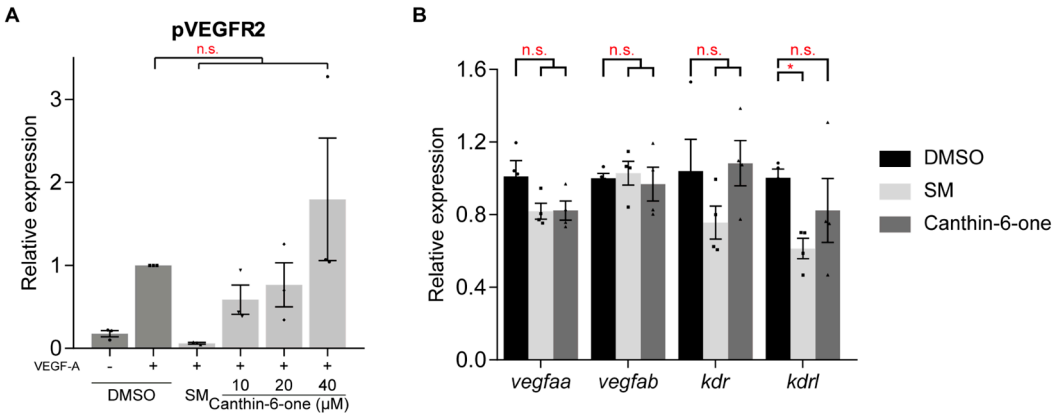

Figure S5. Canthin-6-one does not inhibit VEGFA-induced phosphorylation of VEGFR2 in HUVECs and *vegfa*/*vegfr2* expression in zebrafish. (**A**) Densitometry analysis of pVEGFR2 protein levels from western blot analysis in Figure 3E. pVEGFR2 protein level was normalised to total VEGFR2 protein level. (**B**) qPCR analysis of expression levels of *vegfaa*, *vegfab*, *kdr*, or *kdrl* in 48 hpf zebrafish embryos treated with either 0.2% DMSO, 20 μM SM, or 20 μM canthin-6-one from 16 hpf (n = 3). All data were normalised to *actb1* mRNA level. Statistical test: Kruskal-Wallis test was conducted for graphs (**A**,**B**). $p \leq 0.05$ (*), $p > 0.05$ (not significant, n.s.).

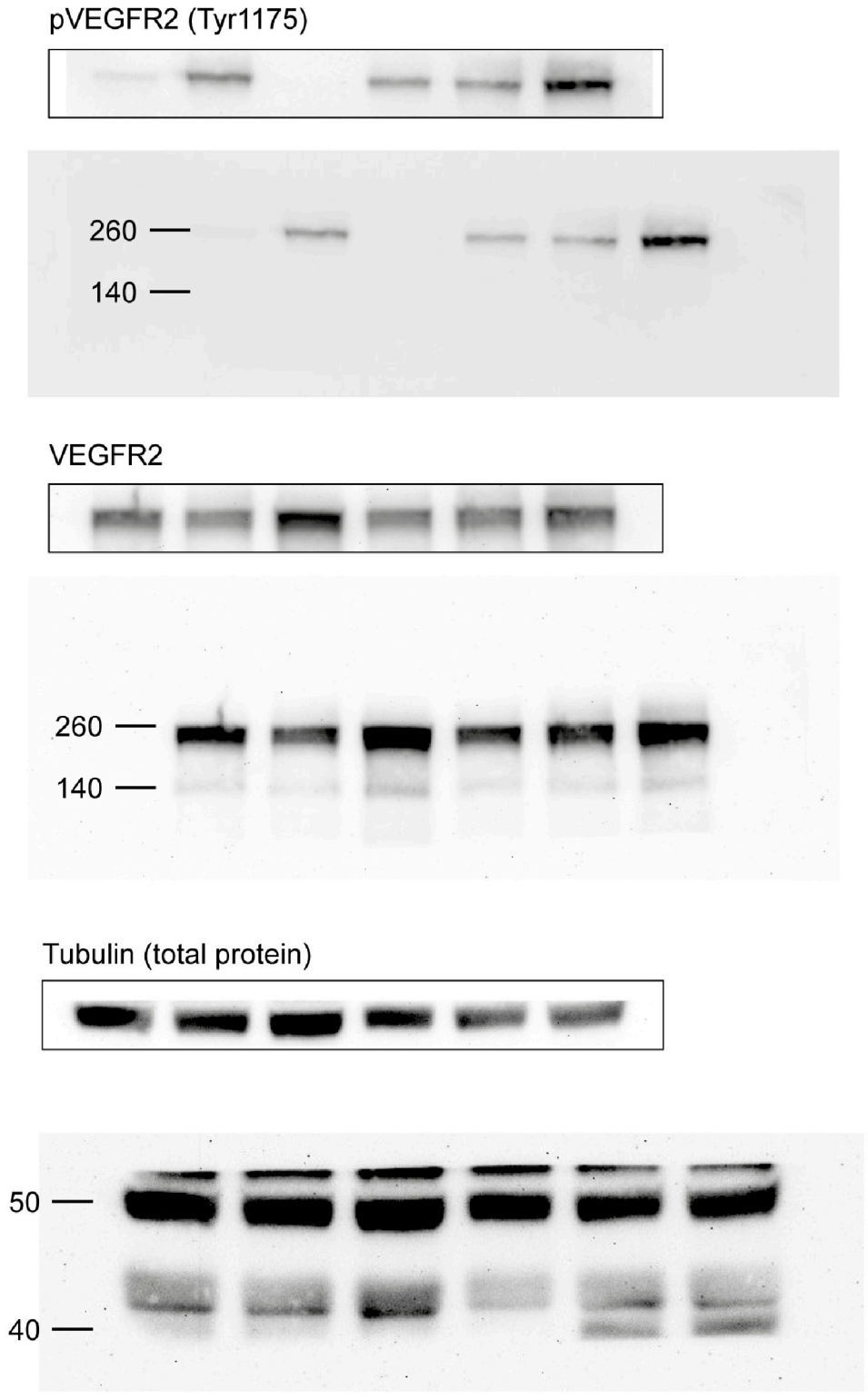

Figure S6. Original western blot images of Figure 3E. Images were taken using the Chemilmager™ imaging system (Alpha Innotech, CA, USA). Original western blot images were processed using the Fiji image processing software.

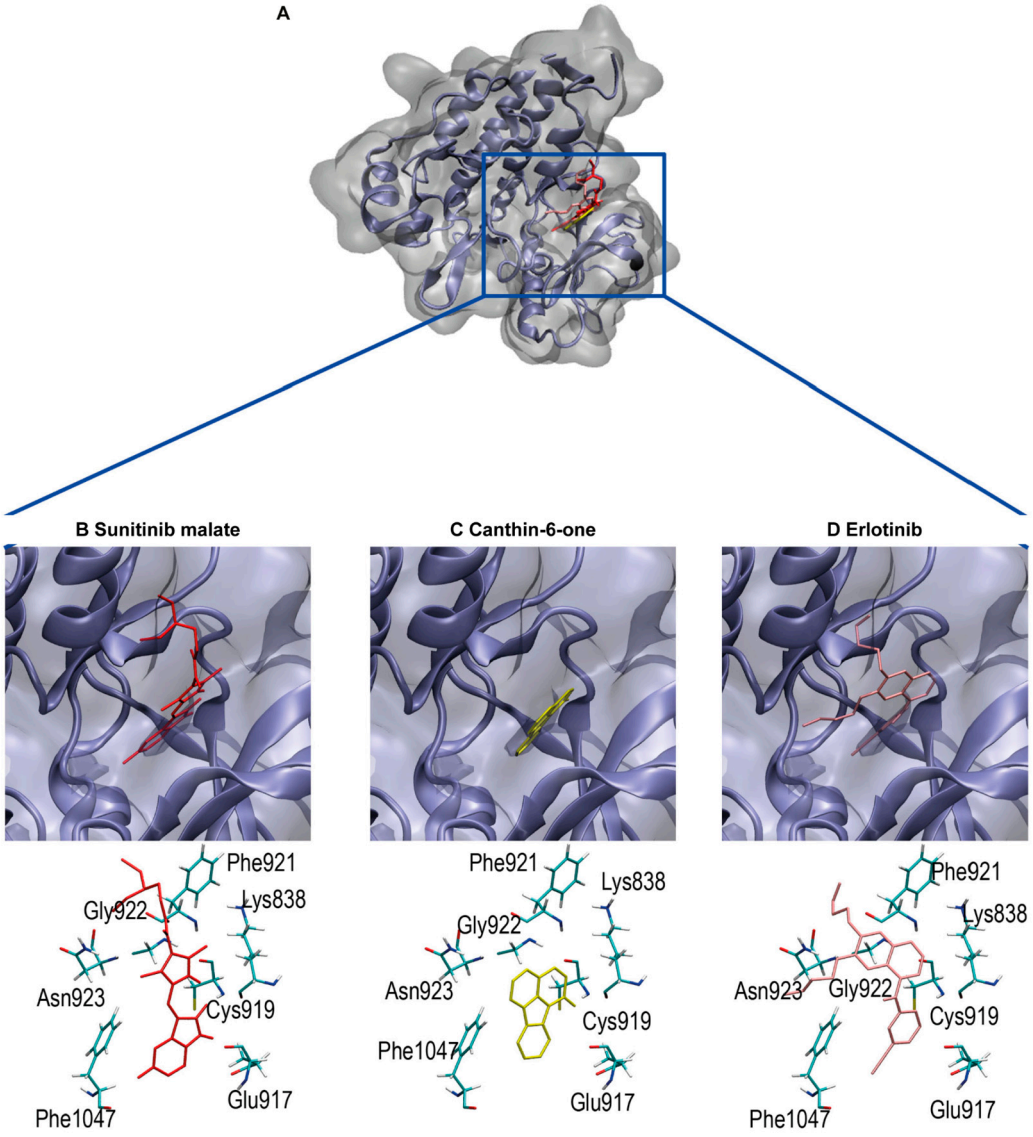

Figure S7. Canthin-6-one does not bind efficiently with VEGFR2. (**A-D**) Potential interacting residues at the binding sites of VEGFR2 (**A**) with sunitinib malate (SM, red, **B**), canthin-6-one (yellow, **C**), or erlotinib (pink, **D**).