

NOVEL ISO-QUINOLINE DERIVATIVES AS ORALLY AVAILABLE SELECTIVE HPV E7 DEGRADER THERAPEUTICS



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Introduction

The high-risk Human Papilloma Virus (HPV) encoded E7 protein is generally considered as an addictive oncoprotein for HPV-induced neoplastic and malignant disease. There is strong preclinical evidence that E7 is essential for the induction, progression, and maintenance of HPV-caused tumors. Loss of function of high-risk E7 was reported to lead to senescence and eventually to apoptosis in various in vitro experiments, and to tumor regression in surrogate experimental animal models. Strikingly, high-risk E7 genes are known to be most stable from an evolutionary standpoint and thus significantly less mutated than genes of any other HPV-encoded RNA or protein. Accumulating evidence suggests that loss of E7 function is a promising therapeutic strategy for HPV-positive cancers. Therefore, we are positing that there is sufficient scientific confidence that a molecule able to introduce a loss of function phenotype of E7 should represent a novel therapeutic option for an unmet medical need, and that the use of suitable E7 therapeutics goes far beyond cervical cancer. We elected to strive for oral applicable small-molecule-degrader drugs by their ease of use, also given the trending increase of incidences for HPV-induced disease in low resource and remote areas. Initial target indications could be advanced cervical cancer and advanced HPV-positive head and neck cancer.

A selective high-throughput screen based on in silico models, and comprehensive structure-activity-relationship (SAR) studies brought about robust drug candidate molecules that induce the degradation of E7 originating from HPV16 and HPV18 (representing the most aggressive forms of HPV) in a monospecific, proteasome-dependent fashion. The degradation of E7 differs significantly from the standard PROTAC technology. Lead compounds were selected with favorable drug-like properties from varying 2,3,4-substituted isoquinolin-1-ones. Strong and fast E7 protein degrader-activities are obtained in various HPV-driven tumor cells in vitro, and at low to sub μM levels in xenograft mouse tumor models in vivo. Drug-like properties, such as a favorable pharmacokinetics, and the pharmacodynamics of E7 protein degradation correlating with a dose-dependent tumor growth inhibition were obtained.

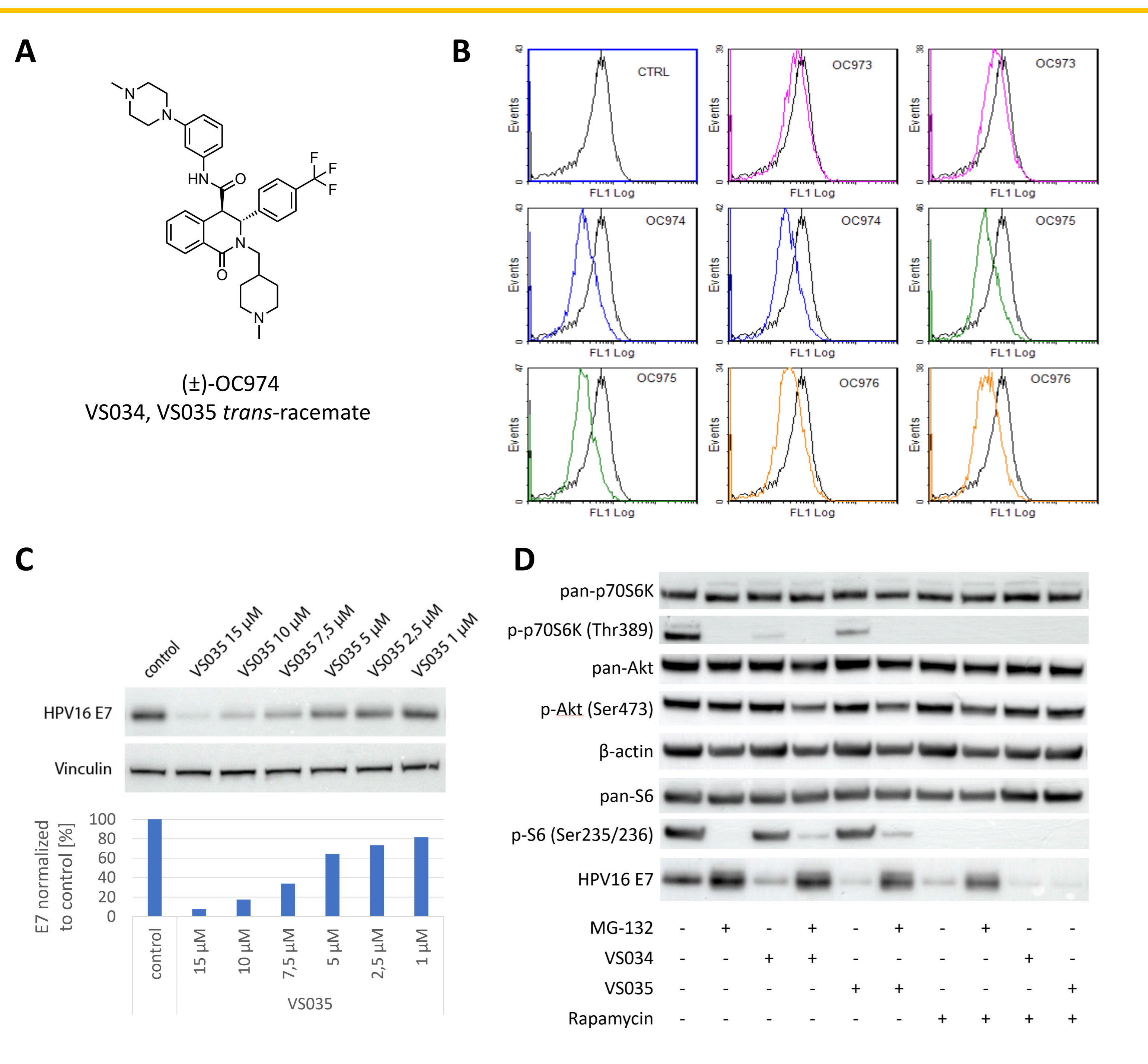


Figure 1: A) The chemical structure of the lead candidate for the development of selective E7 monospecific degraders is shown. The trans-enantiomers were synthesized for further structure-activity studies. B) Shows an example of the E7 degrader screen used for the isolation of the lead candidate OC974. For this, high resolution molecular modeling studies revealed structural ensembles of E7 used for docking studies employing in-house in silico small molecule libraries. In silico hits were chemically synthesized and assessed in an alpha screen with flow cytometry and highly specific E7 mAbs. A shift in E7 signal was compared to the translation inhibitor cycloheximide (CHX). Histograms show degradation of E7 after 6 hours of incubation of HPV16 positive CaSki cervical cancer cells. A shift of the signal to the left indicates degradation of E7. Antibody intensity as a measure of E7 protein level is shown in logarithmic scale. C) Dose range study, analyzing cell extracts from CaSki cells after 6 hours of incubation with the stereoisomer VS035 from OC974 in a western blot read out is shown. D) Western blot of the two enantiomers VS035 and VS034 from cell extracts as described in C). Antibodies used were mAb 16E7, pan p70S6 kinase, p(T389) p70S6 kinase, pan Akt, p(S473) Akt, β -actin, pan S6 ribosomal protein, p(S235/236) S6. Treatments were done with 10 μM VS034 and VS035, 10 μM proteasome inhibitor MG132, 0.2 μM Rapamycin combined as indicated.

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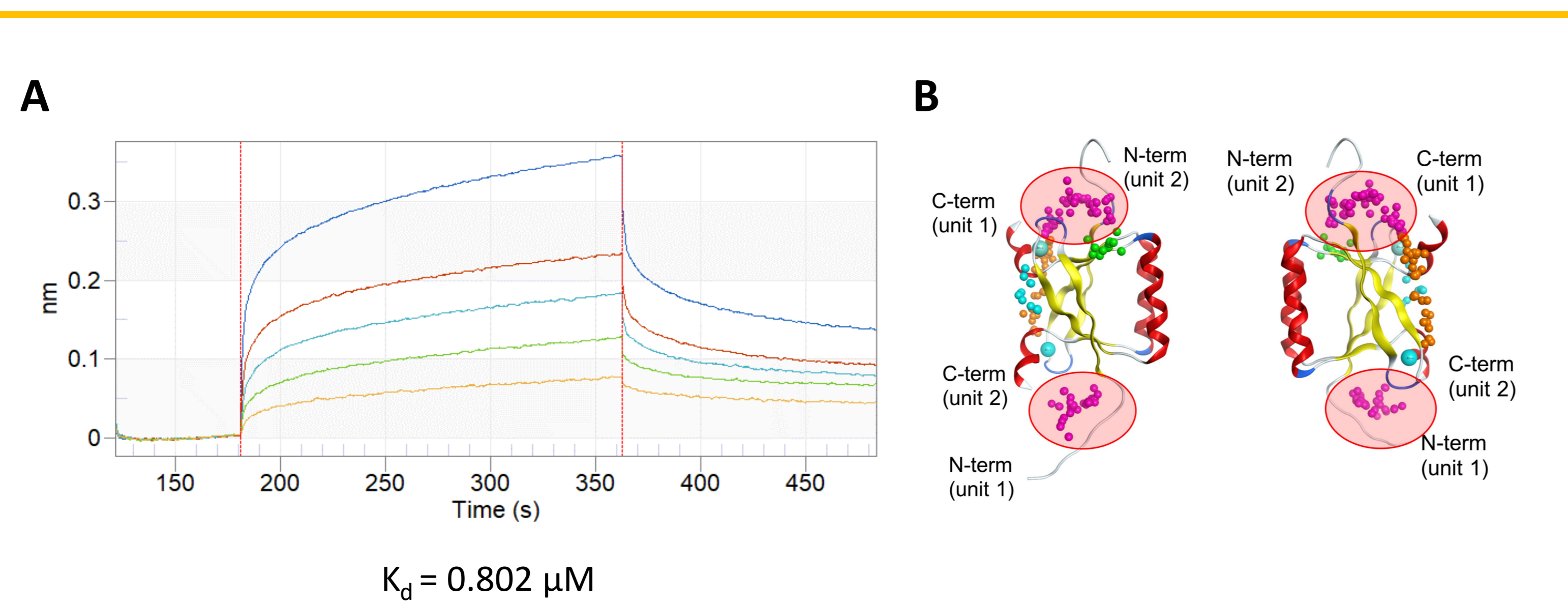


Figure 2: A) Biolayer interferometry experiment of bound biotinylated VS035 and highly purified HPV16 E7 proteins, in the presence of unspecific protein, shows a strong dose-dependent binding signal for HPV16 E7 with a calculated dissociation constant - K_d of 0.802 μM . Calculations were based on the molecular structural predictions shown in B). B) A low-resolution structure of the E7 dimer is shown in complex with VS035; binding sites in the HPV16 E7 CR1/CR2 for VS035 are highlighted.

Conclusion

Using molecular dynamics modeling, SAR studies and directed degradation assays, it was possible to isolate small molecule lead candidates for oral HPV16 E7 protein degraders. The molecules resemble first in class, proprietary, New Chemical Entity (NCE) degrader drug candidates for HPV-induced disease. The isolated molecules do not need a covalently coupled E3-ubiquitin ligase for function and show advantageous PK-PD properties.

It is anticipated that based on the presented results it is of high interest to follow these molecules into the clinics towards a development of oral HPV tumor-therapeutics.

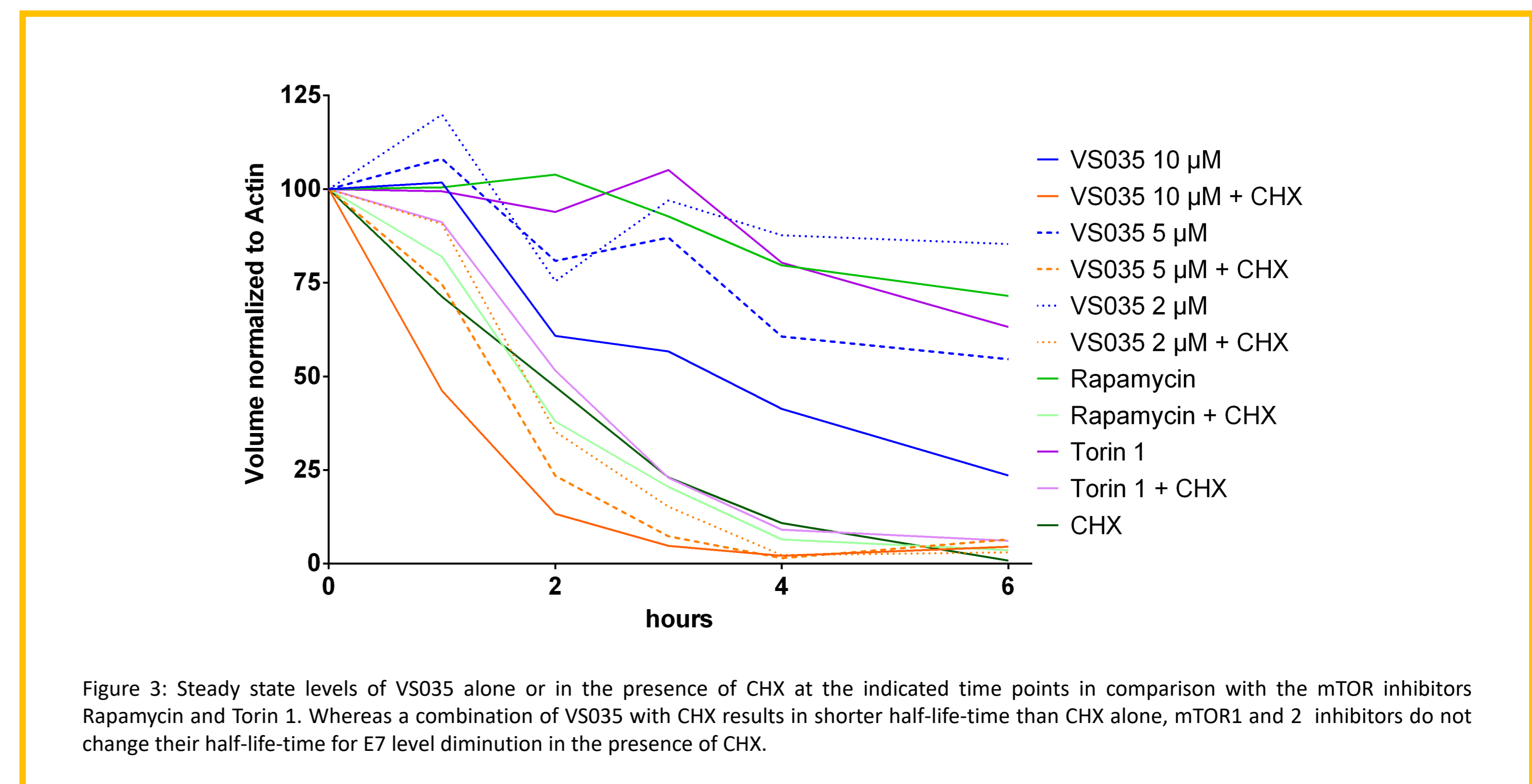


Figure 3: Steady state levels of VS035 alone or in the presence of CHX at the indicated time points in comparison with the mTOR inhibitors Rapamycin and Torin 1. Whereas a combination of VS035 with CHX results in shorter half-life-time than CHX alone, mTOR1 and 2 inhibitors do not change their half-life-time for E7 level diminution in the presence of CHX.

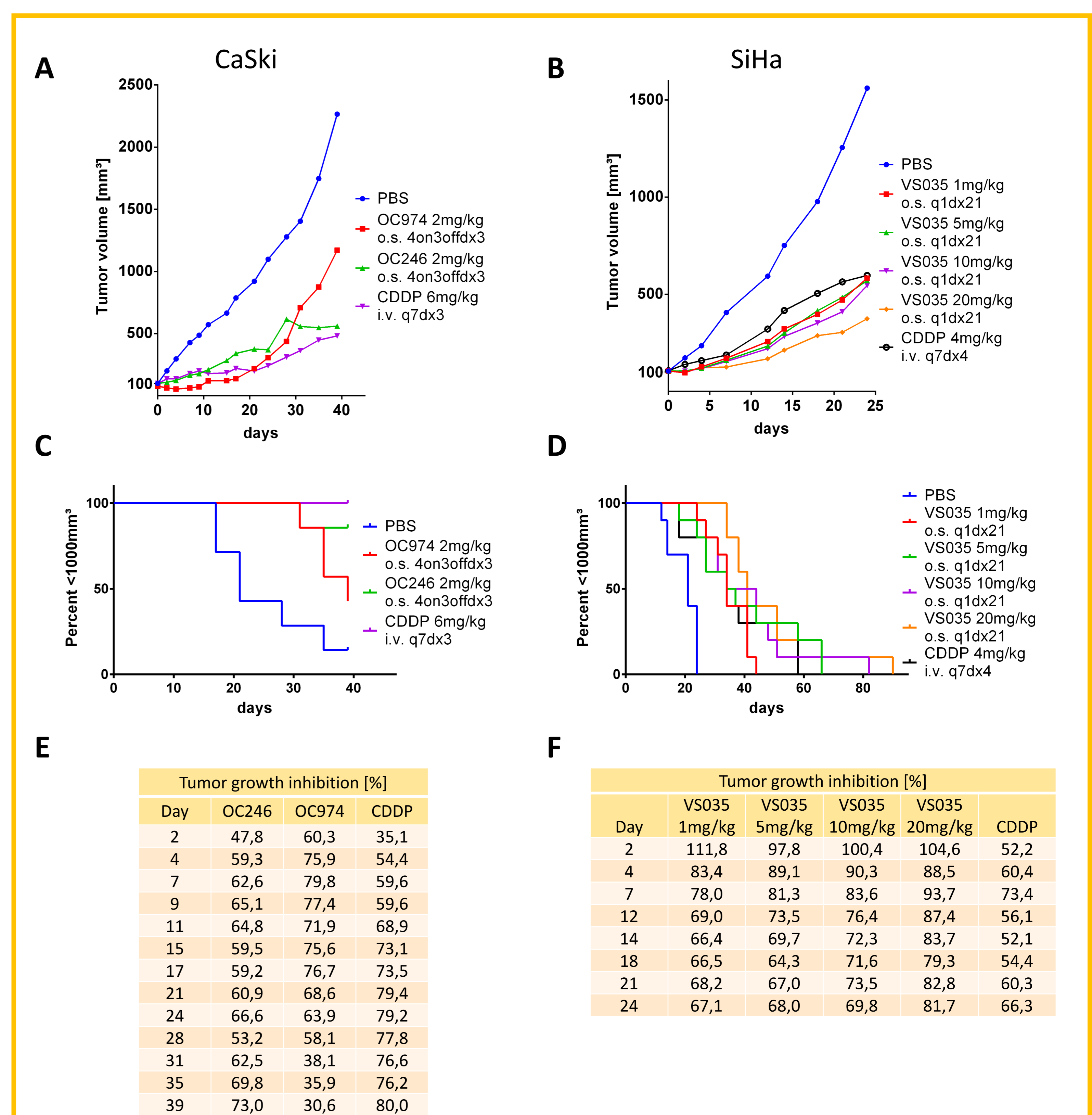


Figure 4: The anti-tumor effect of different iso-quinoline derivative E7 degraders (OC246, OC974, VS035) was analyzed in xenograft models. A) Xenograft experiment in nude mice with CaSki cervical cancer cells after oral treatment of the mice (n = 10/group) daily once for 4 days on 3 days off for 3 cycles and cisplatin (CDDP) by i.v. injection at day 0, 7, 14, starting tumor-size was 100 mm³. OC246 B) Xenograft for SiHa cells same as in A) except that oral treatment was daily for 21 days. Oral treatments in both xenograft models show strong anti-tumor activity after treatment with OC974 or VS035 in cervical cancer xenograft experiments. C) + D) show corresponding Kaplan Meyer calculations for survival as calculated for a 1000 mm³ endpoint. E) + F) calculated Tumor growth inhibition as $(1 - (\text{mean volume increase of treated tumors}) / (\text{mean volume increase of control tumors})) \times 100\%$