Coumarin-Palladium(II) Complex Acts as a Potent and Non-Toxic Anticancer Agent against Pancreatic Carcinoma Cells

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Abstract: Pancreatic carcinoma still represents one of the most lethal malignant diseases in the world although some progress has been made in treating the disease in the past decades. Current multiagent treatment options have improved the overall survival of patients, however, more effective treatment strategies are still needed. In this paper we have characterized the anticancer potential of coumarin-palladium(II) complex (C1) against pancreatic carcinoma cells. Cells viability, colony formation and migratory potential of pancreatic carcinoma cells were assessed *in vitro*, followed by evaluation of apoptosis induction and *in vivo* testing on zebrafish. Presented results showed remarkable reduction in pancreatic carcinoma cells growth *both in vitro* and *in vivo*, being effective at micromolar concentrations (0.5 μM). Treatments induced apoptosis, increased BAX/BCL-2 ratio and suppressed the expression of *SOX9* and *SOX18*, genes shown to be significantly up-regulated in pancreatic ductal adenocarcinoma. Importantly, treatments of the zebrafish-pancreatic adenocarcinoma xenografts resulted in significant reduction in tumor mass, without provoking any adverse toxic effects including hepatotoxicity. Presented results indicate the great potential of the tested compound and the perspective of its further development towards pancreatic cancer therapy.

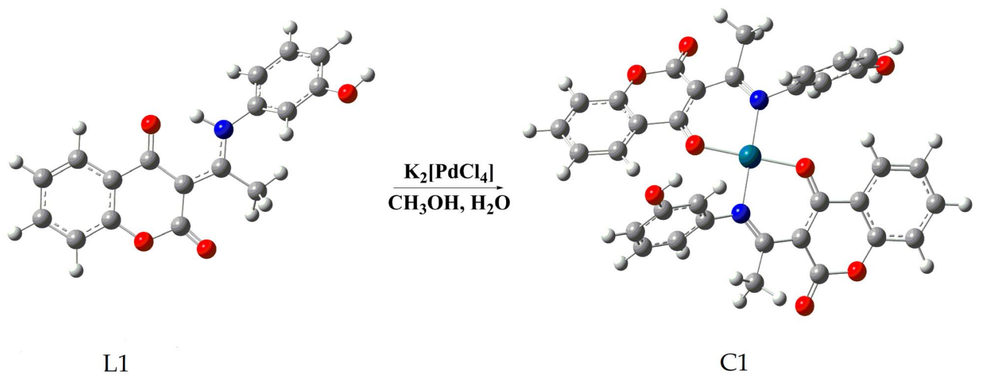
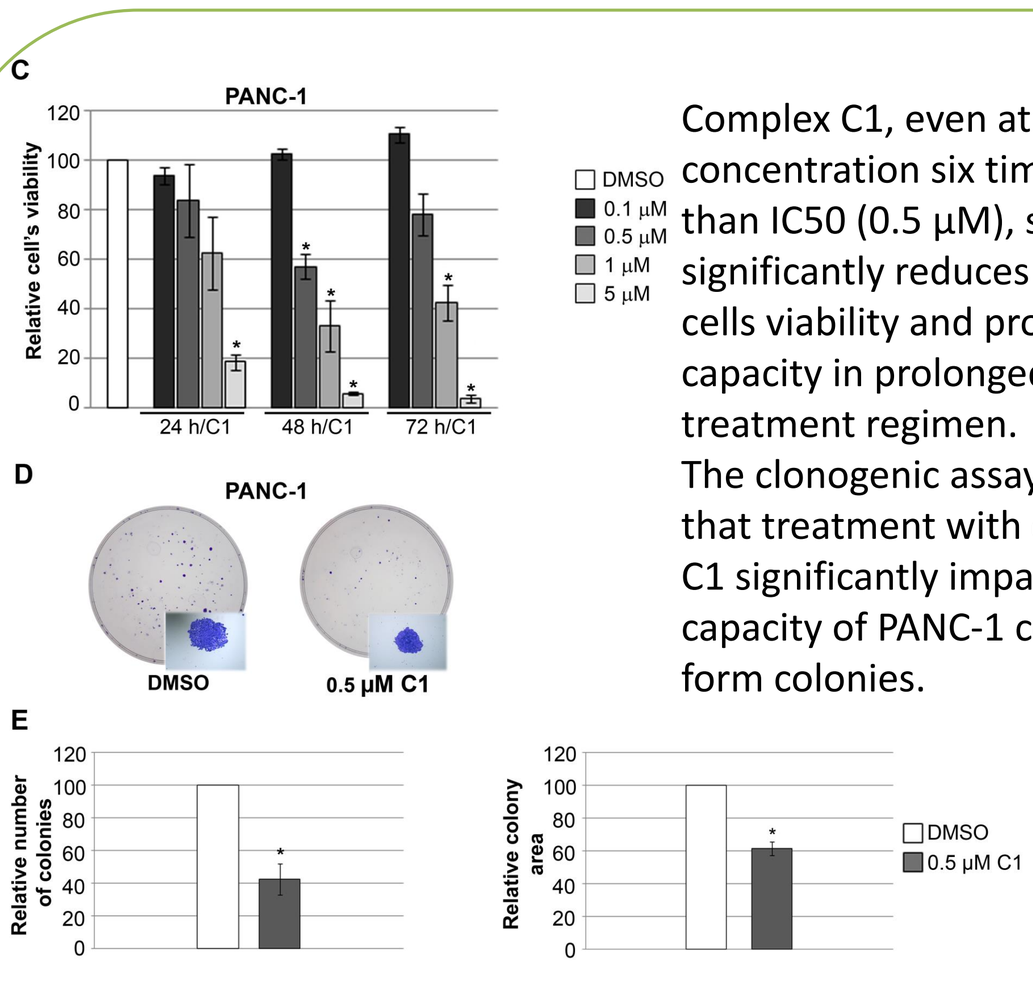
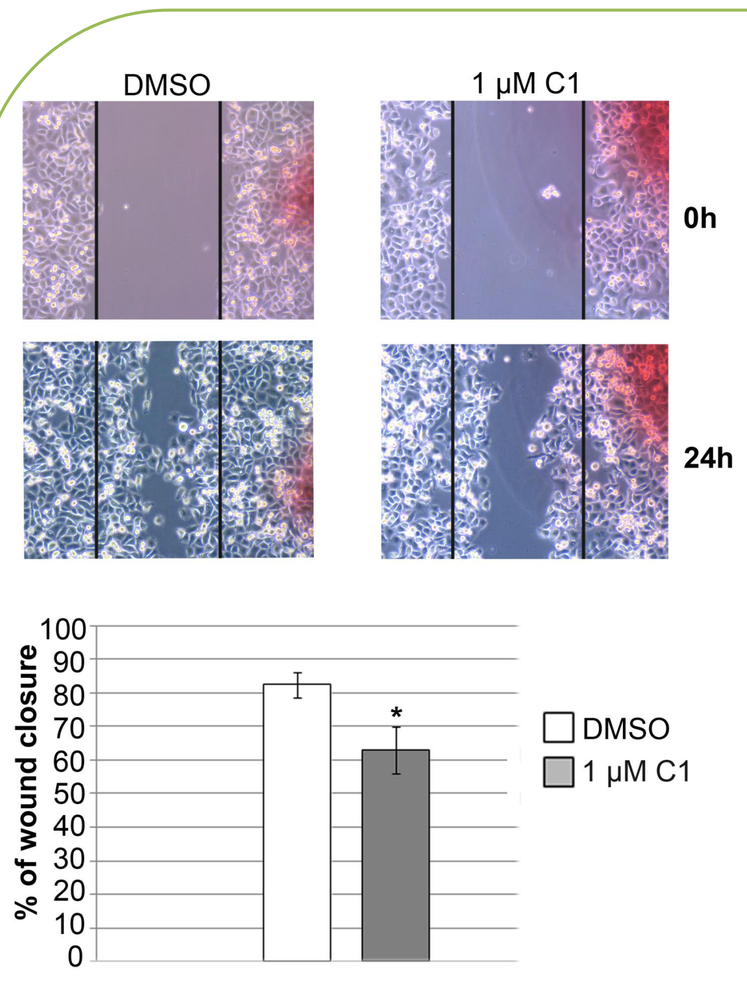


Figure 1. Schematic representation of the general procedure for the synthesis of complex Bis (3-(1-((3-hydroxyphenyl) amino)ethylidene) chroman-2,4-dione-palladium(II) complex (C1)



Complex C1, even at a concentration six times lower than IC50 (0.5 μM), still significantly reduces PANC-1 cells viability and proliferative capacity in prolonged treatment regimen.
The clonogenic assay revealed that treatment with complex C1 significantly impaired the capacity of PANC-1 cells to form colonies.

Figure 2. Effects of complex C1 on pancreatic carcinoma cells viability and potential to form colonies



We tested the migratory potential of PANC-1 cells upon treatment with complex C1, during 24 h. Results showed that treatment with complex C1 reduced the ability of PANC-1 cells to close the scratched area.

Figure 3. Effect of complex C1 on PANC-1 cell's migratory potential

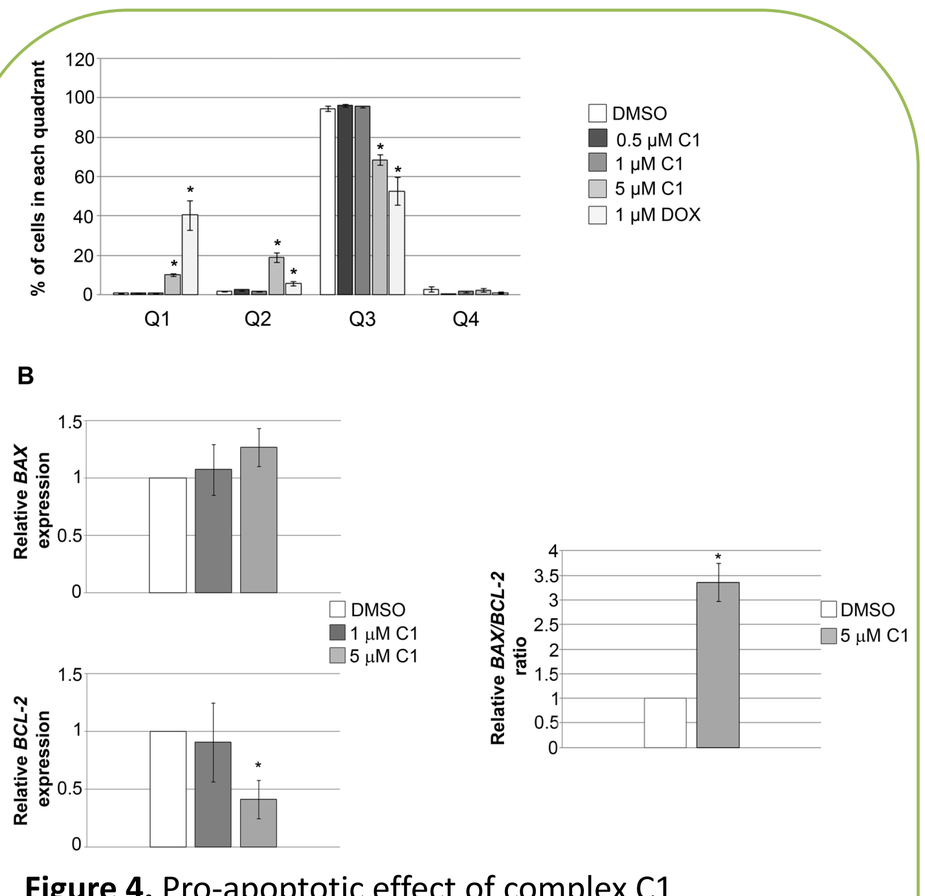
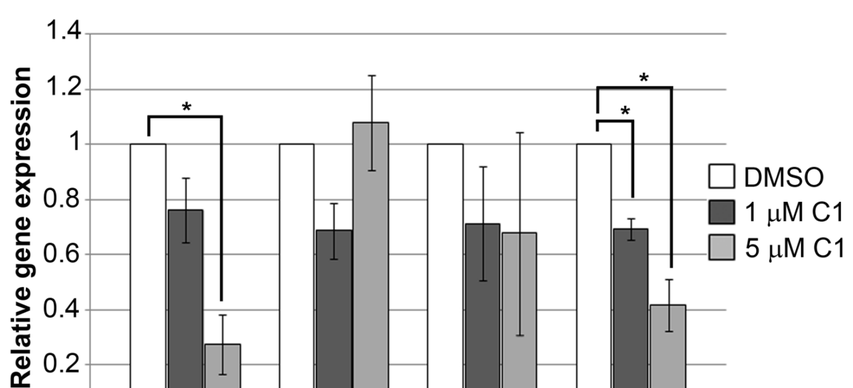


Figure 4. Pro-apoptotic effect of complex C1 on PANC-1 cells.

Complex C1 is able to induce cell death in PANC-1 cells partially by inducing apoptosis, whereas doxorubicin in the same experimental conditions exerts pro-necrotic activity. We observed apoptosis that is, at least in part, governed by increase in *BAX* and decrease in *BCL-2* expression in PANC-1 p53 mutated background.



Complex C1 successfully reduces *SOX9* and *SOX18* expression, showing the potential of complex C1 to target expression of these genes previously shown to be significantly up-regulated in PDAC samples.

Figure 5. Effect of complex C1 on selected SOX genes expression

Complex C1 was very effective in reducing tumor growth in zebrafish xenograft model and, importantly, showing no signs of hepatotoxicity in zebrafish, marking this compound as non-toxic and a safe agent, with promising anticancer activity.

Figure 6. Anticancer activity of complex C1 against human PANC-1 cells in zebrafish and *in vivo* hepatotoxicity