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N. **Title:**

OC28 1,5-DICAFFEOYLQUINIC ACID, FROM ONOPORDON ILLIRICUM, BLOCKS EBOV REPLICATION COUNTERACTING THE IFN-B PRODUCTION INHIBITION BY THE VP35 EBOLA VIRUS PROTEIN

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Abstract:

Background: Ebola virus (EBOV) is one of the deadliest infective agents and plays a lead role as the etiological agent of Ebola Virus Disease (EVD). The high lethality of EVD is linked to the ability of EBOV to efficiently bypass the host's innate antiviral response through the activity of the multifunctional viral protein VP35. This protein is a polymerase cofactor that is essential for EBOV replication and also binds the non-self 5'-ppp dsRNA synthesized as product of viral replication and transcription, hiding the RNA from the cellular receptor, preventing RIG-I activation, and inhibiting the IFN-β production.

EBOV VP35 contains an N-terminal domain and a C-terminal RNA-binding domain (RBD). Investigations on the VP35 RBD led to the identification of small molecules that can block EBOV replication disrupting the interaction between VP35 and viral Nucleoprotein, blocking its polymerase co-factor function. But, no molecule able to inhibit EBOV replication by blocking VP35-dsRNA interaction and IFN-β suppression has been identified yet.

Methods: With the aim to screen for inhibitors of ds-RNA/VP35 interaction, we established a novel and robust fluorescence-based rVP35-RNA interaction assay and a miniaturized gene reporter assay that measures IFN-β induction by viral dsRNA and its inhibition by VP35 expression.

Results: We hence screened a small library of natural extracts. Among them, the butanolic fraction of Onopordum Illyricum showed to inhibit the VP35-dsRNA interaction (IC50 26.9 ± 3.3 μg/ml) and subverted EBOV VP35 inhibition of the IFN-β at 1 μg/ml (p value = 0.0018), but was not able to boost IFN-β production in absence of VP35. Considering the promising results, the main extract components where isolated and tested. Of note, 1,5-dicaffeoylquinic acid inhibited dsRNA-VP35 binding with an IC50 value of 8.5 μM. Similarly, 1,5-dicaffeoylquinic acid was able to revert the EBOV VP35 inhibition of at 1 μM concentration (p value = 0.0022) but was not able to induce an IFN production by itself. In order to evaluate the potential effect against EBOV replication, we selected an Ebola virus isolated from a clinical sample derived from the latest Ebola virus outbreak in West Africa. After assessing, by MTT assay, that no significant cytotoxicity was observed, the compound was combined with viral suspensions and inoculated on target cells. 1,5-dicaffeoylquinic acid was able to inhibit EBOV infection with EC50 value of 9.1 μM.

Conclusions: Overall, our data indicate that 1,5-dicaffeoylquinic acid is a powerful inhibitor of EBOV replication targeting the VP35 IFN production inhibitory effect. The development of 1,5-dicaffeoylquinic acid derivatives with more potent anti-VP35 activity will be a good starting point to move further steps in the discovery of an effective anti-EVD therapeutic strategy.