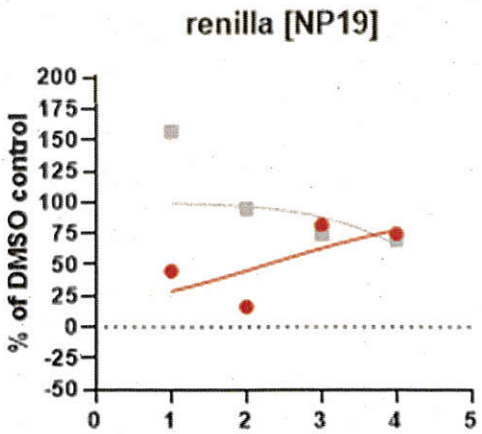
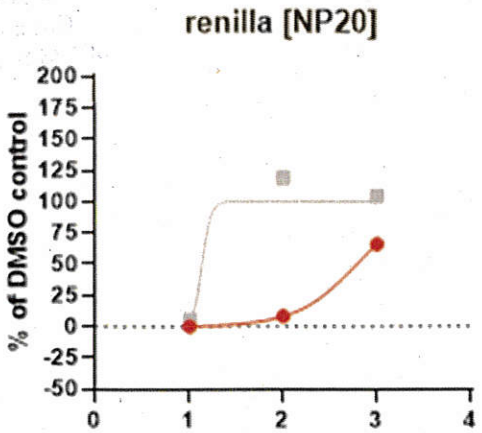
Report on the activity of Proteflazidum against human coronavirus CoV229E.

The activity test has been performed in the laboratory of Prof. Thomas Pietschmann, TWINCORE, Hannover Medical School, Department of Experimental Virology. The samples of Proteflazid have been sent from the ukrainian company ECOPHARM to the Department of Pharmaceutical Biotechnology of the Saarland University. The samples for testing have been evaporated and solved in DMSO in the below mentioned concentrations. The samples have been analyzed on the high-resolution LC MS to ensure their stability after evaporation. The activity test has been performed blindly within many other natural products and extracts. A Dual Luciferase Reporter Gene Assay was used to estimate virus replication within cell line and the toxicity of the tested compound to human cells. The RLU (relative light units) were measured for the renilla luciferase assay (depicting the CoV229E replication) as well as for the firefly luciferase assay (showing the viability of the cells). The grey dots/line in the graphs represent the viability of the cells and the red dots/line represent the replication of the CoV229E virus. The RLU (relative light units) values were normalized to the DMSO control and the background of the non-infected cells was substracted (CoV229E infection in the presence of DMSO =100%).

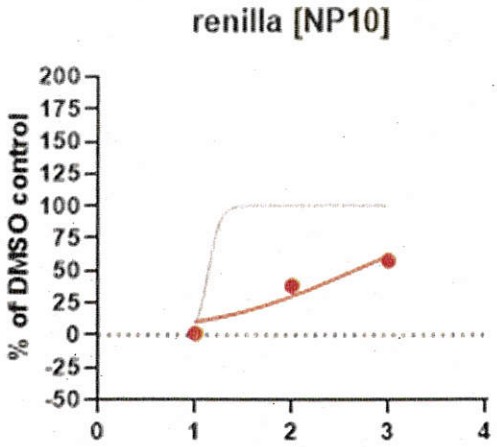
Several different concentrations have been tested and the highest concentration is toxic to the cells but for the middle concentration, F-Luc (firefly luminescence) counts (= viability) are still high (over 100%) whereas R-Luc (Renilla luminescence) counts (= CoV229E) are nearly at 0% of control (at least for NP20). In addition, already the lowest concentration decreases R-luc counts down to appr. 50 % with viability over 100%. It can be interesting to repeat the experiment with Proteflazidum and try even lower concentrations to be able to determine IC50 values. In addition, it would be very useful to perform the fractionations and to identify the active fraction.



1 – 0.0003 mg/ml
2 – 0.00003 mg/ml
3 – 0.000003 mg/ml
Proteflazidium concentrations tested



1 – 0.0053 mg/ml
2 – 0.001 mg/ml
3 – 0.0001 mg/ml
Proteflazidium concentrations tested



1 – 0.0053 mg/ml
2 – 0.001 mg/ml
3 – 0.0001 mg/ml
Proteflazidium concentrations tested

We used the Dual-Glo Luciferase Assay System from Promega in this assay.

Saarbrücken, 02 Sptember 2020.

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