***Dose-dependent inhibition of lactate formation***

The lactate formation, growth yield, and survival under normal and inhibitory conditions of four tumorigenic cell lines were investigated. Initially, dose-dependent effects of selected inhibitors (cmpds No. 9 and 30) to suppress lactate formation were tested. Cells were grown in the presence of vehicle or inhibitors at different concentrations (20, 40, 60, and 80 µM) and lactate levels in the supernatant were determined sequentially.

We observed that **Jurkat cells** in the presence of a vehicle started secreting lactate immediately after the start of incubation and continued to produce lactate until the end of the experiment at 72 hours. In contrast, cells treated with cmpds No. 9 and 30 produced no or only small amounts of lactate in the first 24 hours regardless of the concentration of inhibitors in the medium but began to produce lactate in a dose-dependent manner as the incubation progressed. A somewhat stronger suppression of lactate formation was observed in the cells treated with cmpd No. 30 (**Fig. 5A**).

To investigate possible cytostatic effects of the inhibitors, the total cell number of Jurkat cells treated with vehicles of different concentrations of inhibitors was determined immediately after the end of incubation. No significant differences in the total number of cells were observed, regardless of the vehicle or the different concentrations of inhibitors in the media (**Fig. 5B**).

The potential cytotoxicity of the inhibitors was also assessed. At the end of the incubation, the average percentage of dead cells was determined in the presence of a vehicle and a 40 µM concentration of each inhibitor. No significant negative effects on Jurkat cell survival were observed in the presence of cmpds No. 9 (3.74±0.3 % dead cells) and No. 30 (4.04±0.4 % dead cells), which corresponds to the number of dead cells in the medium with vehicle (3.76±0.35 %) (**Fig. 5C**).

When **Caco-2** **cells** were tested with cmpds No. 9 and 30, a dose-dependent reduction in lactate accumulation was again observed. However, in the medium with different concentrations of cmpd No. 9, lactate excretion was greatly reduced until about 24 hours after growth, followed by a dose-dependent increase in lactate formation in the late hours. Similar results were obtained in the medium with cmpd No. 30. In the media with vehicle or both inhibitors, a similar total number of cells was determined at the end of incubation, indicating that the inhibitors had no negative effects on cell growth (**S4A Fig**.). No cytotoxic effects of the inhibitors were observed either (**S4B Fig**.). The average percentage of dead cells in the control and in the presence of the different concentrations of inhibitors was as follows: Vehicle (3.84±0.54%), cmpd 9 (3.33±0.14%), and cmpd 30 (4.32±0.51%) (**S4C Fig**.).

In general, similar results of suppression of lactate formation were also obtained with **COLO 829** **cells**. Clear dose-dependent effects of suppressed lactate formation were observed with cmpd No. 9, while cmpd No. 30 proved to be slightly less efficient, as the concentration of 40 µM reduced lactate formation by only about half. Again, both inhibitors were more effective only in the early phase of incubation (**S5A Fig**.). No significant differences in the total cell number compared to the control were observed due to the different concentrations of the inhibitors in the medium (**S5B Fig**.). The average percentage of dead COLO 829 cells in the presence of a vehicle and the presence of inhibitors was as follows: Vehicle (4.4±0.46%), cmpd No. 9 (3.65±0.78%) and cmpd 30 (4.27±0.49) (**S5C Fig**.).

Although the dose-dependent effect of the two inhibitors tested was also observed in the **MDA-MB-231 cells**, it was again only fully evident in the early phases of inoculation, while the weaker inhibition was more evident in the late hours of growth. In the early stages of growth, strong inhibition was observed by all concentrations, especially by cmpd No. 30. However, after about 24 hours, lactate accumulation resumed, initially at the lowest concentration (20 µM) and then gradually decreased in a dose-dependent manner (**S6A Fig**.). No significant differences in total cell number were observed due to the different concentrations of inhibitors in the medium (**S6B Fig**.). The average percentage of dead MDA-MB-231 cells in the control and the presence of the inhibitors was as follows: Vehicle (4.28±0.78%), cmpd No. 9 (6.59±0.29%), and cmpd No. 30 (3.4±0.21%) (**S6C Fig**.).