**Preliminary screening of selected compounds for suppressing lactate and superoxide (SOX) formation in four tumorigenic cell lines.**

First, all selected compounds (33) were tested for the suppression of lactate and SOX by 4 tumorigenic cell lines. In the experiment, tumorigenic cell lines were inoculated with 50 µM of the respective potential inhibitor at an initial concentration of 1x106 cells and incubated for 36 hours. The concentrations of extracellular lactate and mitochondrial SOX were determined as described in the Materials and Methods. The statistically significant difference between untreated cells (DMSO control vehicle) and cells treated with each inhibitor was determined.

**Jurkat cells** showed the lowest value of significant difference in lactate suppression compared to untreated cells with cmpd No. 9, while cpmds No. 3, 23, and 30 showed higher values. Similar results were obtained for the suppression of SOX formation, with cmpds No. 30 show the most significant difference, while cmpds No. 3, and 9 were less effective. No significant differences were observed between vehicles and cells treated with other compounds for either the lactate or SOX generations (**Fig. 2**).

In **Caco-2 cells**, the most striking statistical differences were found in the suppression of lactate by cmpds No. 3, 9, 23, 30, while the strongest suppression of SOX formation was by cmpds No. 3, 9, 30 and 32. No significant differences were observed in either lactate or SOX formation between the vehicle and cells treated with other compounds (**Fig. S1**).

Lactate formation was also significantly suppressed in **COLO 829 cells** when treated with cmpds No. 3, 9, 29 and 30, however cmpd No. 9 showed the most significant differences. In SOX suppression between vehicle and treated cells the cmpds No. 3, 9, 23, and 30 showed significant differences. No significant differences were observed between vehicles and cells treated with other compounds for either lactate or SOX generations (**Fig. S2**).

Significant differences in lactate suppression in **MDA-MB-231 cells** between vehicle and cells treated with cmpds No. 3, 9, or 30 were observed, with cmpds No. 9 and 30 showing stronger differences. Cells treated with cmpd No. 30 also showed the lowest P values for suppression of SOX formation, however, slightly reduced but still significant abilities to suppress SOX formation were observed in the presence of cmpds No. 3 or 9. No significant differences were observed in either lactate or SOX formation between the vehicle and cells treated with other compounds (**Fig. S3**).

Of the 33 compounds selected by screening, cmpds No. 3, 9, 23, 29, 30, 31, and 32 (marked in red in Figure 3) exhibited the ability to strongly suppress lactate or superoxide formation in at least one tumorigenic cell line (**Table 1**).

The assigned numbers of the inhibitors, their structures, molecular weights, and supplier codes are listed in **Table S1**.

**Table 1:** A list of small-molecule inhibitors reducing human PFK1 iso-enzyme activities.

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| **Compound No.** | **IUPAC name** |
| 3 | 2-[(5-benzo[1,3]dioxol-5-yl-1,3,4-oxadiazol-2-yl)sulfanyl]-N-isoxazol-3-yl-acetamide |
| 9 | 3-methoxy-6-(3-{1-[(5-methyl-1,2,4-oxadiazol-3-yl)methyl]-1H-pyrazol-3-yl}phenyl)pyridazine |
| 23 | N-[5-(methanesulfonamido)-1,3,4-thiadiazol-2-yl]-6,7,8,9-tetrahydro-5H-carbazole-3-carboxamide |
| 29 | 3-(4-chlorophenyl)sulfonyl-N-(5-isoxazol-5-yl-1,3,4-oxadiazol-2-yl)propenamide |
| 30 | 4-[3-(5-amino-1,3,4-oxadiazol-2-yl)isoxazol-5-yl]phenol |
| 31 | (2R)-N-(5-isoxazol-5-yl-1,3,4-oxadiazol-2-yl)-2,3-dihydro-1,4-benzodioxine-2-carboxamide |
| 32 | 3-(benzenesulfonyl)-N-(5-isoxazol-5-yl-1,3,4-oxadiazol-2-yl)propenamide |

However, cmpds No. 3 and 9 were able to strongly inhibit unwanted metabolic activities in all tumorigenic cells tested. Similarly, cmpd No. 30 could also be considered a strong inhibitor, as a slightly lower level of SOX suppression was only observed in COLO 829 cells.