# Oxidative modifications as potential drug attenuators

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## MOTIVATION

The tumor microenvironment (TME) consist of various cell types, including tumor, stromal and immune cells. Inflammation is frequently observed in the TME, being associated with production and release of reactive oxygen species (ROS). However, it is unclear to which extent ROS can modify the activity of antitumor drugs.

To this end, we here screened a library of more than 400 experimental and clinically relevant pharmaceutical compounds employed as anticancer drugs. After selecting those compounds with high efficacy in A375 melanoma cells, we then compared the efficacy of pristine drugs with those heavily oxidized by various ROS produced by an atmospheric pressure argon plasma jet (kINPen).

Highly oxidation-sensitive drugs will be analyzed by mass spectrometry to identify chemical structures associated with enhanced oxidation sensitivity, which may corroborate reduced drug action in inflamed TME.

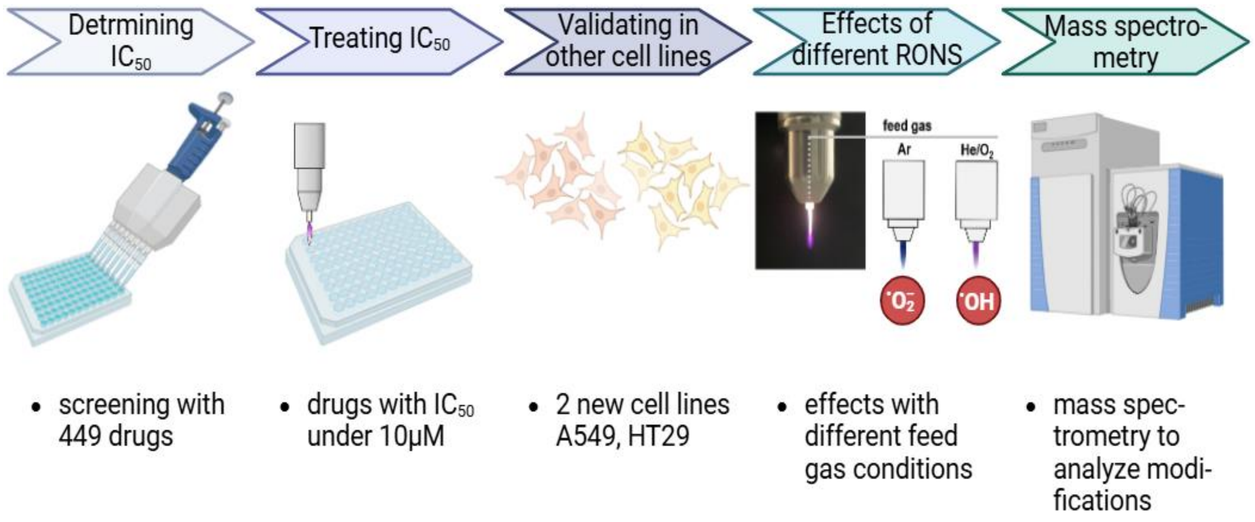
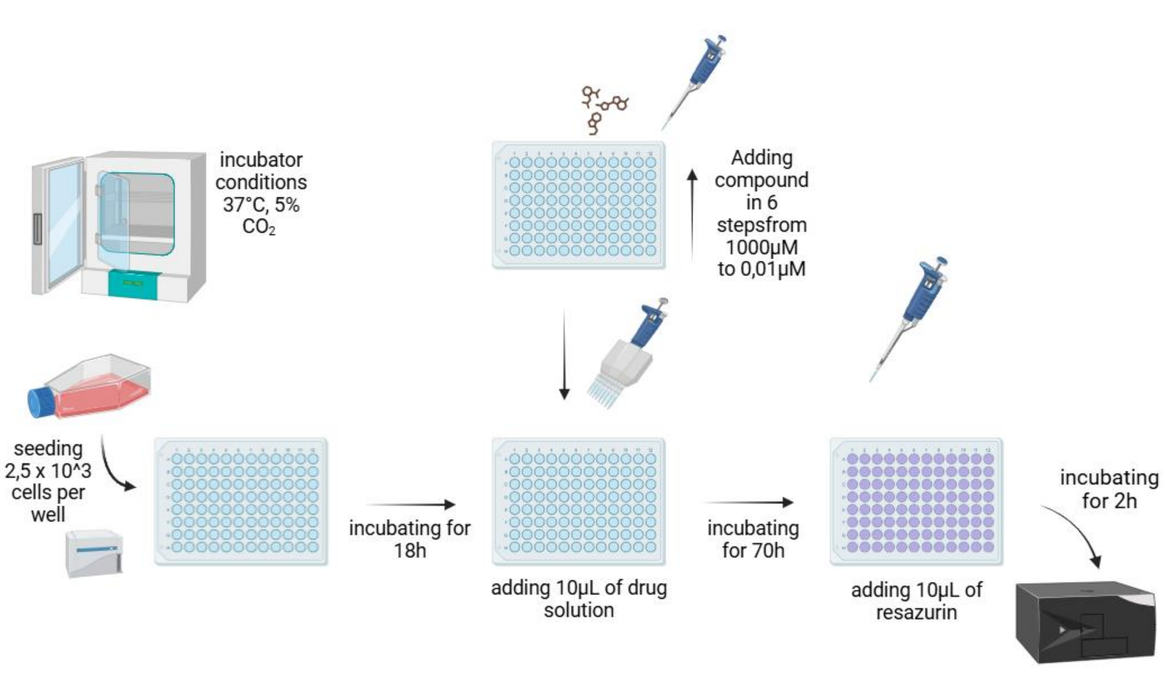



**Fig. 1.** Project scheme and major steps to identify oxidation-sensitive chemical drugs in anticancer drugs.

## 1. $IC_{50}$ DETERMINATION OF 449 COMPOUNDS



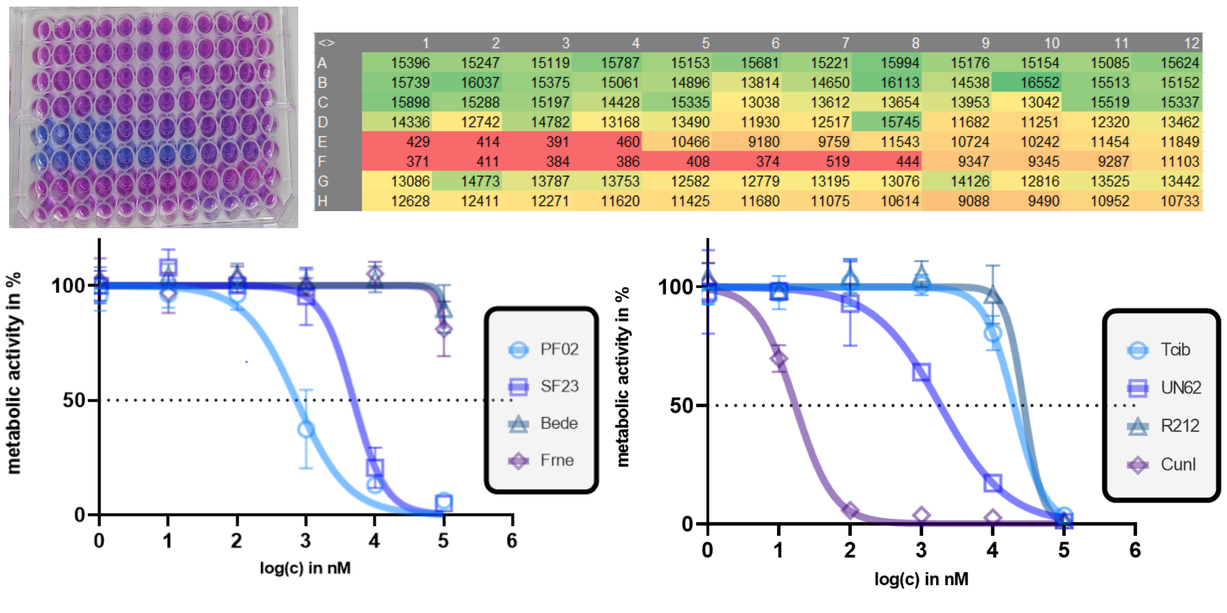
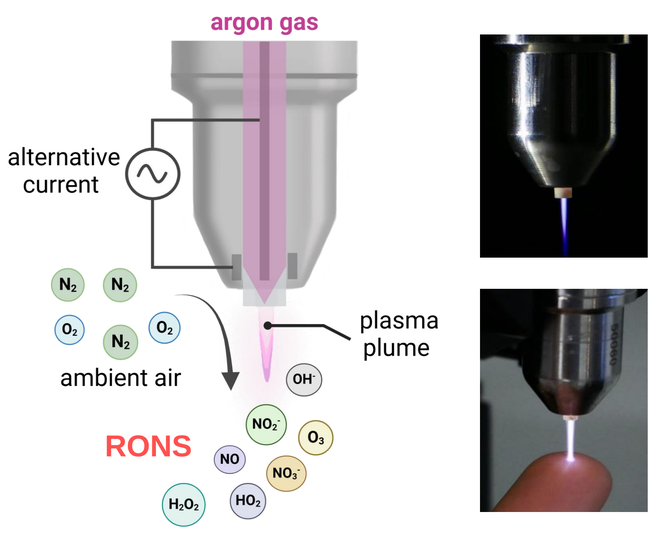




**Fig. 2.** Workflow for the first step (left): first, we tested a library of 449 compounds on their inhibitory concentration 50 ($IC_{50}$) on the metabolic activity of A375 melanoma cells. Example tested plate in the upper left; in the upright an example data in excel. Two representative graphs (bottom) on log-transformed concentrations and pharmacological reduction of metabolic activity.

## 2. GAS PLASMA-MEDIATED DRUG OXIDATION





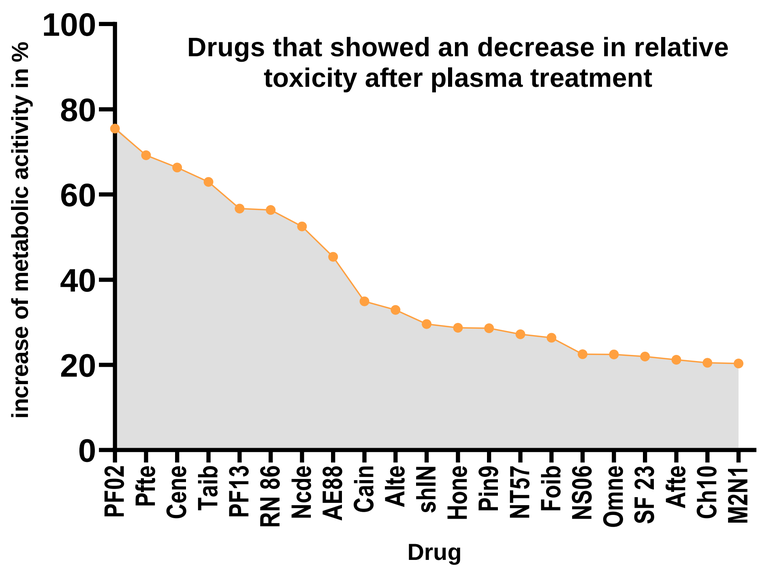
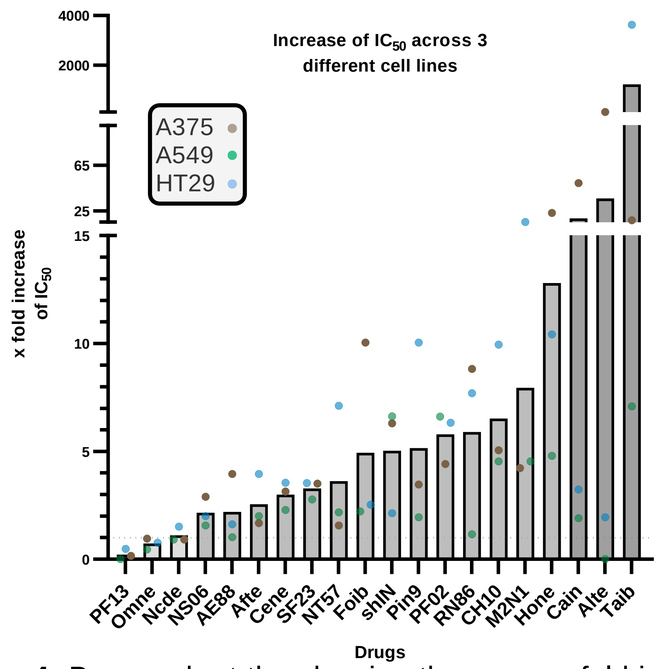




**Fig. 3:** Schematic picture showing the principle function of the kINPen (left). Two live pictures of the working kINPen and how it conducts (middle). Waterfall plot that showcases relative difference of the the metabolic activity at their respective $IC_{50}$ (right).

## 3. VALIDATING THE RESULTS IN OTHER CELL LINES




| Name | Mean of $IC_{50}$ increase | Range of $IC_{50}$ increase |
|---|---|---|
| Cene | 2,99 | 2,29 - 3,55 |
| SF23 | 3,27 | 2,77 3,53 |
| Foib | 4,93 | 2,22 - 10,04 |
| shIN | 5,02 | 2,14 - 6,63 |
| PF02 | 5,79 | 4,42 - 6,61 |
| CH10 | 6,51 | 4,54 - 9,95 |
| M2N1 | 7,94 | 4,23 - 15,07 |
| Hone | 12,80 | 4,79 - 23,19 |

**Fig. 4:** Bar graph at the showing the mean x fold increase of the $IC_{50}$ over all the cell lines A375, A549, HT29 (left). Table of the top 8 drugs with feasible results that will be taken for further investigation (right).

## CONCLUSION & OUTLOOK

The experiments until now showed a big variation in sensitiveness to the oxidation process, but it overall favored the inactivation of drugs. The variation not only occurred between drugs but also between the same drug in different cell lines. Nonetheless 8 promising candidates were selected for further investigation steps.

Besides the mass spectrometry and testing different gas compositions, to get a different ROS line up, we also planning a variety of different biological assays. With that we will get a in depth look on the biological effects of our final selected drugs.

plasmatis Plasma plus Cell

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