

Title: m3DinAI Drug Quest: A Machine Learning-Driven Platform for High-Throughput Screening of antitumoral agents in 3D Cancer Models

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The use of 3D spheroids that mimic tumor microenvironment has advanced significantly the cancer drug discovery field. However, characterizing and quantifying phenotypic alterations in spheroids treated with drugs remains a challenge. **m3DinAI Drug Quest** is a machine learning-based tool designed to analyze the cytotoxic effects of antitumoral agents in 3D spheroids via high throughput screening (HTS) assays. **m3DinAI Drug Quest** analyzes and quantifies **phenotypic changes** in high content images in an automated mode. This automation markedly improves both the speed and accuracy of compound screening, while minimizing manual labor and human bias.

In this study, m3DinAI was employed to analyze the cytotoxic effects of 2,400 microbial **natural products** from MEDINA's library on spheroids of triple negative breast cancer (TNBC) cell lines. Spheroids were made using HCC1806 cells and the NanoShuttle technology (Greiner Bio-One) in 384-well plates. The HTS assay resulted in 60 hit extracts that induced distinct phenotypic changes compared to untreated controls.

m3DinAI Drug Quest demonstrates significant potential as a tool for cancer **drug discovery** using **complex models**. By improving accuracy, reducing manual workload, and minimizing bias, this platform accelerates the identification of **promising drug candidates, which is of special relevance for TNBC**, which is the most aggressive type of breast cancer and it lacks targeted therapy.