SUMMARY

Gliomas represent one of the most challenging malignancies of the central nervous system, characterized by extensive cellular heterogeneity, high proliferative rates and invasiveness, and an ability to evade host immune surveillance. Despite advances in surgery, radiotherapy, and chemotherapeutic regimens, current treatment options frequently provide only modest clinical benefits and are accompanied by substantial systemic toxicities. These limitations underscore an urgent need for novel therapeutic agents capable of simultaneously targeting multiple oncogenic processes while maintaining acceptable safety profiles. In this study, we developed and implemented an integrative drug-screening framework to systematically evaluate the anti-glioma potential of two candidate compounds, dihydroartemisinin (DHA) and naphthalimide imidazole boron complex (NImBC), and have attempted to decipher their underlying mechanisms of action. By integrating the *in vitro* and *in vivo* outputs, the work aimed to establish a robust therapeutic strategy that bridges molecular insights with organism-level outcomes.

Initial *in vitro* analysis using C6 glioma cell lines revealed that DHA markedly reduces glioma cell viability in a dose-dependent manner. Flow cytometric analyses demonstrated that the compound induces cell-cycle arrest at G0/G1 (lower dose) and G2/M phases (higher dose), suggesting interference with key regulatory checkpoints. This was accompanied by a significant modulation of reactive oxygen species (ROS) dynamics, with elevated ROS levels correlating with cytotoxicity. Apoptotic profiling further confirmed the induction of apoptotic cell death, supported biochemically by increased caspase-3 activation. To investigate whether these cellular responses translated into organismal benefit, DHA was tested across multiple *Drosophila* glioma models harbouring distinct oncogenic drivers, offering valuable insights into the signalling circuits engaged by the drug. The drug not only induced tumour regression but also restored disrupted cellular architecture. Importantly, it enabled successful adult emergence in otherwise lethal glioma genotypes, highlighting its ability to rescue whole-

organismal viability. Our observation of DHA-induced ROS modulation in mutant brains further supports the concept of a critical ROS threshold, wherein further ROS elevation preferentially drives tumour cells into cytotoxicity while remaining within a tolerable range for the non-tumourous tissues, such as in wild-type and parent controls. The therapeutic effects of DHA were further validated in mammalian glioma models, where it induced maximum regression of subcutaneous tumours and significantly delayed progression of orthotopic tumours. The treated cohort showed more favourable clinical scores and a trend towards improved survival, consistent with the reduced disease burden observed following treatment. Further, the utilised dosage did not induce discernible toxicity in major organs, as verified by histological and biochemical analyses. Mechanistic investigations revealed that DHA suppresses AKT and mTOR activation, suggesting potential modulation of downstream proliferative and survival pathways in glioma. Additionally, reduced STAT3 phosphorylation in the treated samples are indicative of the influence of the drug on the tumour-promoting signalling networks.

Using the drug-discovery platform, we investigated two organometallic candidates of boron (NImBC) and ruthenium (FcRuC) and identified NImBC as a promising lead. The drug exerted robust cytotoxic effects *in vitro* that were accompanied by induction of apoptotic death. Its activity was particularly evident in *Drosophila* glioma brains, where the compound modulated ROS levels, restored the ring-like arrangement of the proliferating neuroblasts in the brain lobes, and rescued the otherwise lethal phenotype across multiple mutant backgrounds, demonstrating functional recovery at the level of neural tissue architecture. Collectively, our findings establish a multi-tiered pipeline for preclinical drug assessment and uncover mechanistic insights into the therapeutic potential of DHA and NImBC, highlighting the importance of further evaluating these compounds for improving strategies for better patient survival outcomes.

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