Chapter 1

Introduction

1.1 Background

Glioblastoma multiforme stands as one of the most formidable challenges in contemporary oncology, characterized by its near-universal therapeutic resistance, rapid infiltrative growth pattern, and a biochemical landscape marked by the convergence of multiple dysregulated oncogenic signaling pathways (Pouyan et al., 2025; Singh et al., 2025). Despite incremental advances in understanding its molecular underpinnings, the fundamental drivers of GBM progression and treatment evasion remain incompletely characterized, necessitating the continued investigation of alternative and novel molecular targets (Durga & Pusapati, 2025). Calcium signaling, and in particular the SOCE pathway mediated through ORAI1-STIM1 interaction, has emerged as a mechanistic axis of considerable interest in this context, given its demonstrable roles in regulating cell cycle progression, apoptotic susceptibility, and transcriptional reprogramming (Motiani et al., 2013).

Among the pharmacological tools developed to interrogate this axis, Synta66 has been the most extensively characterized small-molecule ORAI1 antagonist. Synta66 engages the extracellular loops connecting transmembrane domains 1 and 3 of the ORAI1 pore, physically occluding calcium permeation without disrupting STIM1-dependent channel gating (Waldherr et al., 2020). While this mechanism is sufficient to abolish CRAC current in electrophysiological assays, Synta66 has consistently failed to reproduce this inhibitory efficacy as a direct anti-tumor cytotoxic agent when applied to GBM cell models. Three specific limitations underlie this translational gap. First, Synta66 does not achieve meaningful anti-tumor cytotoxicity in GBM cell lines despite effectively silencing CRAC current, implying that channel blockade alone is insufficient to trigger cell death in a tumor context sustained by redundant survival signaling (Liardo et al., 2024). Second, Synta66 exhibits incomplete selectivity across the three ORAI isoforms; pharmacological profiling indicates that ORAI1, ORAI2, and ORAI3 possess distinguishable channel properties and are not uniformly antagonized by

first-generation pyrazole compounds, raising the possibility that ORAI2- or ORAI3-mediated calcium entry persists as a compensatory route even when ORAI1 is blocked (Zhang et al., 2020). Third, the bioavailability and pharmacokinetic profile of Synta66 remains poorly characterized, with no systematic data addressing membrane permeability, metabolic stability, or in vivo exposure, constraining its translational development beyond an in vitro pharmacological probe (Liardo et al., 2024). Collectively, these limitations establish Synta66 as a mechanistically informative but therapeutically insufficient scaffold, and they define the specific problem space that motivates the derivatization strategy pursued in this thesis.

These limitations collectively motivate the central premise of the present thesis: rather than using the parent compound Synta66, this study investigates a chemically modified derivative designed to overcome the pharmacological shortcomings of the original scaffold. Medicinal chemistry principles establish that discrete structural modifications to lipophilic and polar regions of pyrazole-based CRAC channel inhibitors can yield substantial gains in inhibitory potency and can alter isoform selectivity profiles by several fold relative to the parent compound (Yonetoku et al., 2006; Zhang et al., 2020). Guided by this precedent, twenty structurally distinct Synta66 derivatives, designated MPT1B783 through MPT1B802, were synthesized and screened by MTT viability assay against A172 glioblastoma cells across 24-hour and 72-hour exposure windows. This preliminary screening was undertaken specifically to identify, from among the twenty candidates, the single derivative demonstrating the most pronounced reduction in A172 cell viability, on the reasoning that the compound producing the greatest loss of viability would represent the derivative most likely to have acquired direct cytotoxic activity absent from the parent Synta66 scaffold. Among the twenty derivatives evaluated, MPT786 produced the greatest reduction in A172 cell viability at both time points, with a determined half-maximal inhibitory concentration (IC50) of 9.020 microM. It is on this basis, rather than on the basis of a screen conducted across the full spectrum of GBM cell lines, that MPT786 was selected as the lead compound for downstream transcriptomic characterization; the present thesis therefore

reports the greatest induction of cytotoxicity specifically within A172 cells, and this scope is maintained consistently throughout the study design.

The novelty of the present investigation is therefore twofold. First, unlike prior studies that have evaluated Synta66 itself, this work characterizes a rationally selected structural derivative that has been empirically demonstrated to possess direct anti-GBM cytotoxic activity, a property that the parent compound lacks. Since chemical modification of the Synta66 scaffold is understood to alter isoform-selectivity determinants located at the ORAI channel pore, MPT786 and its derivatives are of specific interest for their theoretical capacity to more completely occlude the ORAI2 isoform in addition to ORAI1, thereby closing the compensatory calcium-entry route that may otherwise be exploited by GBM cells to evade ORAI1-selective inhibition. The transcriptomic characterization of MPT786 undertaken in this thesis therefore represents the first mechanistic step toward validating whether structural modification of Synta66 confers not only enhanced cytotoxic potency but also a more complete blockade of CRAC-mediated survival signaling in GBM.

Although the clinical correlation components of this study draw on transcriptomic and survival datasets encompassing GBM broadly (via the CGGA mRNAseq_325 cohort spanning WHO grades II-IV) and, where indicated, pan-cancer expression comparisons (via GEPIA2), the experimental (wet-laboratory) component of this thesis was deliberately restricted to a single, molecularly well-defined cell line, A172, rather than a panel of GBM lines. This decision requires explicit justification given that the downstream clinical datasets are not similarly restricted.

A172 cells possess a genomic and molecular profile that is uniquely suited to isolating the mechanistic question under investigation, namely, whether ORAI1 inhibition reactivates p53-dependent apoptotic signaling via relief of AKT-driven MDM2 activity. Three features of the A172 molecular profile are of central importance. First, A172 cells retain a wild-type TP53 allele, confirmed by sequencing across exons 2 through 11, ensuring that the p53 protein retains an intact DNA-binding and transactivation domain and is therefore structurally capable of engaging its canonical

transcriptional targets should upstream suppressive signaling be relieved (Hernández Borrero & El-Deiry, 2021; Zhu et al., 2021). Second, and critically, A172 cells retain wild-type PTEN function, in direct contrast to the commonly used U87MG line, which harbors homozygous PTEN deletion and consequently exhibits constitutive, calcium-independent hyperactivation of the PI3K/AKT axis (Georgescu, 2010; Rajaa et al., 2013). In a PTEN-null background, AKT activity would remain elevated irrespective of ORAI1 pharmacological blockade, such that any observed p53 reactivation could not be unambiguously attributed to ORAI1 inhibition. The intact PTEN status of A172 cells therefore removes this confound and permits a substantially cleaner mechanistic readout of the ORAI1-AKT-MDM2-p53 axis. Third, A172 cells possess a verified, STR-authenticated genomic identity, addressing a documented and persistent misidentification problem in the U87MG lineage (Bhatt et al., 2010), and are classified within the clinically aggressive mesenchymal GBM transcriptional subtype, and additionally display well-characterized endogenous SOCE and ORAI1 channel activity, confirming that the pharmacological target of interest is functionally expressed and experimentally accessible in this model.

It is acknowledged that this wild-type TP53, wild-type PTEN profile does not represent the majority of clinical GBM, in which TP53 mutation or functional p53 inactivation (via MDM2 amplification, CDKN2A deletion, or PTEN loss) is common (Zhang et al., 2018). The A172 model was therefore selected not to be representative of all GBM molecular subtypes, but rather to provide unambiguous proof-of-concept that ORAI1 inhibition can, in a background where the p53 apoptotic machinery is structurally intact and not confounded by PTEN-null AKT hyperactivation, produce measurable p53 pathway reactivation at the transcriptomic level. The subsequent extension of the resulting gene signature to the CGGA mRNAseq_325 cohort, which is unselected for TP53 or PTEN status and spans the full histological grade spectrum, was undertaken precisely to determine whether the mechanistic signal identified under these controlled experimental conditions in A172 cells retains prognostic and biological relevance across the molecularly heterogeneous GBM population encountered in clinical practice.

1.2 Objective

To investigate the molecular mechanism underlying the anticancer activity of an ORAI1/CRAC inhibitor in A172 cells by characterizing transcriptional response of glioblastoma cells to ORAI1/CRAC inhibitor treatment, identifying genes from previous RNA sequencing datasets, and evaluating their clinical and prognostic significance through bioinformatic and survival analyzes.

1.3 Hypothesis

MPT786, a novel Synta66-derived ORAI1/CRAC channel inhibitor, will induce a transcriptional response in A172 human glioblastoma cells characterized by the upregulation of genes involved in DNA damage checkpoint signaling, cell cycle arrest, and apoptosis. ORAI1 inhibition will allow transcriptional activation of pro-apoptotic effectors. The resulting transcriptional program will be clinically relevant, with ORAI1-correlated pathway genes exhibiting significant positive associations with adverse overall survival in glioma patient cohorts, consistent with the pathogenic role of chronically elevated SOCE in sustaining GBM proliferation and therapy resistance.