# I. INTRODUCTION

## 1.1 Background

Glioblastoma multiforme (GBM) is the most aggressive and prevalent malignant brain tumor in adults, arising from glial cells and typically developing within the cerebral hemispheres, including the frontal, temporal, and parietal lobes (Sipos et al., 2025). Despite decades of clinical and translational research, the median overall survival of patients diagnosed with GBM remains dismal at approximately 12 to 15 months, even when maximal therapeutic intervention is applied (Kanderi et al., 2022). The current standard of care, which comprises surgical resection followed by concurrent radiotherapy and chemotherapy with temozolomide (TMZ), has failed to substantially alter long-term outcomes (Mehta et al., 2017). A central impediment to therapeutic progress is the near-universal development of TMZ resistance, with over 50% of patients exhibiting primary non-response to the agent and approximately 90% experiencing tumor recurrence within two years of initial treatment (Pu et al., 2025; Singh et al., 2020).

A promising yet relatively underexplored molecular target in glioblastoma is the ORAI1 calcium channel, a principal mediator of store-operated calcium entry (SOCE) through the calcium release-activated calcium (CRAC) channel pathway (Pan & Ma, 2014). Seminal work by Motiani et al. (2013) have demonstrated that GBM-derived cell lines, including GBM1, GBM8, and U251, exhibit significantly elevated SOCE activity compared to non-malignant human primary astrocytes (HPA), suggesting a functional dysregulation of calcium signaling in GBM progression. Importantly, this enhanced SOCE activity has been attributed predominantly to transcriptional overexpression of ORAI1 rather than broad activation of the STIM1/ORAI1 signaling axis, as STIM1 expression remains relatively unchanged across these models (Collins et al., 2013; Hammad et al., 2023). STIM1 functions as an endoplasmic reticulum $Ca^{2+}$ sensor that detects intracellular $Ca^{2+}$ store depletion which then activates ORAI1 channels at the plasma membrane to initiate SOCE; therefore, the observed enhancement in calcium influx appears to arise mainly from increased ORAI1 channel availability rather than altered STIM1-mediated signaling capacity (Lu et al., 2025; Woo et al., n.d.). Furthermore, increased ORAI1 expression has been associated with poor clinical prognosis, higher World Health Organization (WHO) tumor grade, and elevated plasma biomarker levels in patients with grade III–IV gliomas (Zhang et al., 2025). Collectively, these findings highlight ORAI1 as a biologically and clinically relevant therapeutic target whose aberrant activity may contribute to the aggressive and treatment-resistant phenotype of GBM.

The mechanistic basis linking ORAI1-mediated calcium signaling to GBM pathogenesis has been progressively elucidated. Elevated intracellular calcium influx through ORAI1/STIM1 channel complexes activates proline-rich tyrosine kinase 2 (Pyk2), which undergoes autophosphorylation at

tyrosine residue 402 (Lunz et al., 2019; Lysechko et al., 2010). Phosphorylated Pyk2 recruits and activates Src kinase, initiating downstream signaling pathways that promote extracellular matrix (ECM) degradation and tumor invasion (Kucheryavykh & Yuriy, 2025). Simultaneously, Pyk2 facilitates AKT activation, driving multiple pro-survival and pro-proliferative mechanisms (Aswathanarayan et al., 2026). Activated AKT suppresses apoptosis through inhibition of BAD, caspase-9, and FOXO-mediated pro-apoptotic transcription, while promoting protein synthesis via mTORC1 activation (Valdés-Rives et al., 2017). AKT additionally phosphorylates MDM2, enhancing p53 degradation and thereby suppressing p53-dependent transcription of apoptotic mediators including BAX, PUMA, and NOXA (Tat et al., 2025). Furthermore, AKT-mediated inactivation of GSK-3β increases Cyclin D1 expression and accelerates cell cycle progression, accompanied by reduced p21/p27-mediated cell cycle inhibition (Atkins et al., 2012). AKT signaling also enhances MMP-2 and MMP-9 expression through NF-κB and AP-1 activation, while stabilization of HIF-1α further promotes invasion and angiogenesis (Zheng et al., 2025). Collectively, these interconnected pathways link ORAI1-mediated calcium signaling to sustained proliferation, suppression of apoptosis, and enhanced invasiveness in glioblastoma multiforme.

Despite this mechanistic rationale, existing ORAI1-targeting strategies, including the benchmark compound Synta66, a well-characterized inhibitor of SOCE and the ORAI1 pore, have demonstrated effective blockade of calcium release-activated calcium (CRAC) channel activity without producing direct anti-tumor cytotoxicity in GBM cell models (Waldherr et al., 2020). Limitations associated with current inhibitors include ORAI isoform selectivity concerns and insufficient bioavailability data, highlighting the need for next-generation compounds with improved pharmacological profiles (Liardo et al., 2024). In this context, a series of twenty structural derivatives of Synta66, each bearing discrete chemical modifications intended to modulate potency, selectivity, membrane permeability, and pharmacokinetic behavior, have been developed for preliminary evaluation.

A critical pharmacological limitation of Synta66 that further motivates the development of structural derivatives resides in its isoform-selective inhibitory profile across the three mammalian ORAI channel paralogues. Investigations employing ORAI1/2/3-triple knockout cell systems have conclusively demonstrated that while Synta66 abrogates ORAI1 channel function with high potency, it paradoxically potentiates ORAI2 activity and exerts negligible inhibitory effect on ORAI3 (Zhang et al., 2020). This divergent pharmacological profile is mechanistically attributable to discrete amino acid differences within the extracellular binding region across ORAI isoforms; specifically, the substitution of histidine 113 (H113) in the ORAI1 loop1 binding site with a tyrosine residue in ORAI2, which fundamentally alters the binding geometry and molecular interactions available to the Synta66 pharmacophore (Waldherr et al., 2020). The consequence of this isoform selectivity in the oncological context is pharmacologically consequential: selective blockade of ORAI1-mediated calcium influx by Synta66 may

inadvertently potentiate ORAI2-dependent calcium entry, creating a compensatory survival pathway through which GBM cells may sustain intracellular calcium homeostasis and downstream pro-survival signaling despite ORAI1 inhibition. Given that heteromeric ORAI1-ORAI2 channel complexes constitute an appreciable proportion of native CRAC channels in GBM cells, Synta66-mediated stimulation of ORAI2 could functionally offset the intended therapeutic effect of ORAI1 blockade, contributing to the absence of cytotoxic activity observed in GBM models (Tiffner et al., 2021).

This isoform selectivity gap therefore constitutes a primary pharmacological rationale for the development of structurally modified Synta66 derivatives with broadened ORAI inhibitory profiles. A derivative capable of simultaneously inhibiting ORAI1 and suppressing or neutralizing ORAI2 activity would eliminate the compensatory calcium entry pathway and substantially increase the probability of achieving therapeutically meaningful intracellular calcium depletion and consequent disruption of AKT-mediated survival signaling in GBM. The novelty of the Synta66 derivative library investigated in the present study derives precisely from this therapeutic ambition: by introducing targeted modifications to the three principal pharmacophoric regions of the Synta66 scaffold, including the dimethoxyphenyl hydrophobic anchor, the central amide linker, and the fluorinated heteroaromatic ring, it may be possible to generate analogues with altered binding geometry at the ORAI extracellular interface that confer broader isoform inhibitory coverage encompassing both ORAI1 and ORAI2. The twenty derivatives evaluated in the present study, designated MPT1B783 through MPT1B802, each incorporate one or more discrete structural alterations to these pharmacophoric elements, rendering this library not merely an incremental modification of a parent scaffold but a systematic probe of the structural determinants governing ORAI isoform selectivity, GBM cytotoxicity, and pharmacokinetic optimization within this compound class. To date, no published study has evaluated the cytotoxic potential of Synta66-derived structural analogues in multi-cell-line GBM models inclusive of TMZ-resistant variants, establishing the present investigation as a novel contribution to the pharmacological development of ORAI-targeted GBM therapeutics.

**1.2 Objective**

The primary objective of this study was to conduct a preliminary cytotoxic screen of twenty Synta66-derived compounds (MPT1B783–MPT1B802) against the A172 GBM cell line, using Synta66 as a reference benchmark, with the overarching aim of identifying promising lead derivatives for subsequent validation and optimization. Secondary objectives included the determination of the half-maximal inhibitory concentration ($IC_{50}$) for the most active compound and the evaluation of its time-dependent cytotoxic activity across a panel of GBM cell lines, encompassing both parental and drug-resistant variants.

### 1.3 Hypothesis

It was hypothesized that among the twenty Synta66 derivatives evaluated, at least one compound would demonstrate superior cytotoxic activity against A172 cells relative to the Synta66 comparator at a fixed screening concentration of 10 μM, and that the identified lead compound would retain significant anti-proliferative activity against TMZ-resistant GBM cell lines, consistent with a mechanism independent of conventional chemotherapy resistance pathways.