

Cardiac Glycosides in Glioblastoma: From Na⁺/K⁺-ATPase Inhibition to Divergent Stress Pathways

Svara Venkatesan

Cardiac glycosides, a class of steroid natural products historically used to treat heart failure, have re-emerged as candidate anticancer agents in glioblastoma (GBM), the most aggressive primary brain tumor in adults. All of them share one starting point: high-affinity binding to a conserved pocket on the Na⁺/K⁺-ATPase (NKA) ion pump, which blocks the pump's normal cation exchange cycle (Kanai et al., 2021; Laursen et al., 2015). This review traces what happens after that shared event and argues that the pathway then splits into two only partially overlapping downstream branches: an endoplasmic reticulum (ER) stress response driven through PERK–eIF2 α –CHOP signaling, best documented for bufalin in glioma cell lines (Shen et al., 2014), and a suppression of hypoxia-inducible factor 1- α (HIF-1 α), best documented for digoxin and digitoxin (Zhang et al., 2008; Lee et al., 2017). A complication runs through both branches: the same ER-stress response can trigger protective autophagy that delays rather than causes cell death, so the outcome may depend on a stress "threshold" that has not yet been quantified for any single glycoside in a single GBM line (Shen et al., 2014; Yan et al., 2015). Translating either branch into a usable GBM therapy is complicated by a real concentration gap: the doses shown to suppress HIF-1 α or trigger ER stress in vitro generally exceed doses reached safely in humans, with digitoxin a partial exception (Lee et al., 2017; Kurzeder et al., 2025). The review closes by proposing molecular docking, using existing crystal structures of glycoside-bound NKA (PDB 7DDH, 4RET, 4RES) and the PERK kinase domain (PDB 3QD2), as a way to generate hypotheses about why different glycosides favor different branches, not to confirm them.

Introduction

Glioblastoma (GBM) is the most common and most lethal primary malignant brain tumor in adults, with median survival under two years even with maximal surgery, radiation, and temozolomide (TMZ) chemotherapy. Resistance to TMZ, poor blood–brain barrier (BBB) penetration by most chemotherapeutics, and the tumor's reliance on stress-adaptive survival pathways all limit what current therapy can do. This has motivated interest in drug repurposing: testing agents already approved for other conditions against GBM, on the logic that their human safety and pharmacokinetic profiles are already partly known.

Cardiac glycosides (also called cardiotonic steroids, CTS) are one such class. Digoxin, digitoxin, ouabain, bufalin, and related compounds have been prescribed for heart failure and arrhythmia for decades, and all of them inhibit the same target: the Na⁺/K⁺-ATPase (NKA) ion pump. Over the past two decades, a separate cancer-biology literature has reported that these same compounds are selectively cytotoxic to a range of tumor types, including glioma, at concentrations that leave normal cells comparatively unaffected. What is less settled is why: NKA inhibition sits upstream of several plausible downstream mechanisms, and the published literature on cardiac glycosides in GBM is scattered across biochemistry, cell biology, and clinical pharmacology papers that rarely cite each other directly.

This review tries to assemble that literature into a single causal picture. Section 1 lays out the shared initiating event, the structural basis of CTS binding to NKA, and its relevance to GBM specifically. Section 2 argues that the pathway then splits into two downstream stress

responses, an ER-stress/unfolded protein response (UPR) branch and a HIF-1 α suppression branch, and compares the evidence for each. Section 3 introduces a complication that cuts across both branches: the same stress responses can activate protective autophagy that shields tumor cells from death rather than causing it, suggesting a dose- or duration-dependent threshold separating a survival response from a lethal one. Section 4 confronts the translational gap between effective in vitro concentrations and concentrations achievable in human patients. Section 5 proposes a computational docking approach, using existing crystal structures, as a way to generate testable hypotheses about why particular glycosides favor particular branches, while staying explicit about what docking can and cannot demonstrate on its own. Throughout, this review tries to flag where the literature is genuinely thin, contradictory, or extrapolated from a different cancer type or species, rather than smoothing those gaps into one tidy narrative. [Connector/bridge] Figure 1 shows the structures of the four cardiac glycosides this review focuses on, including the lactone-ring size difference discussed below.

Structures of the Four Cardiac Glycosides Examined

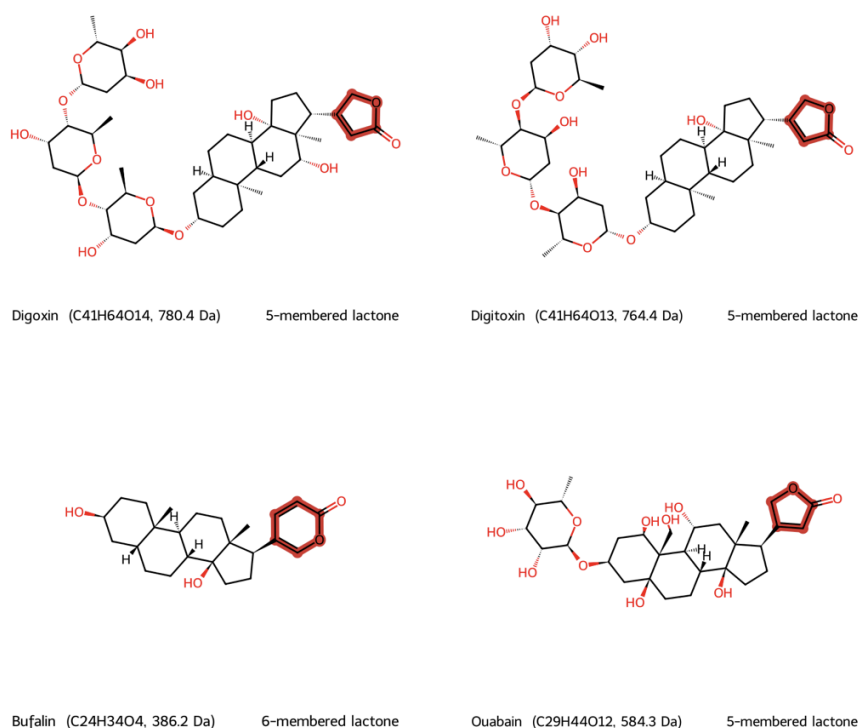


Figure 1. Two-dimensional structures of digoxin, digitoxin, bufalin, and ouabain, generated in RDKit from PDB Chemical Component Dictionary isomeric SMILES (ligand IDs DGX, F9R, BUF, OBN). The steroid D-ring lactone is highlighted in red: five-membered for digoxin, digitoxin, and ouabain, six-membered for bufalin (Section 1.1).

1. The Shared Initiating Event: Na⁺/K⁺-ATPase Inhibition

1.1 Structural Basis of Cardiac Glycoside Binding

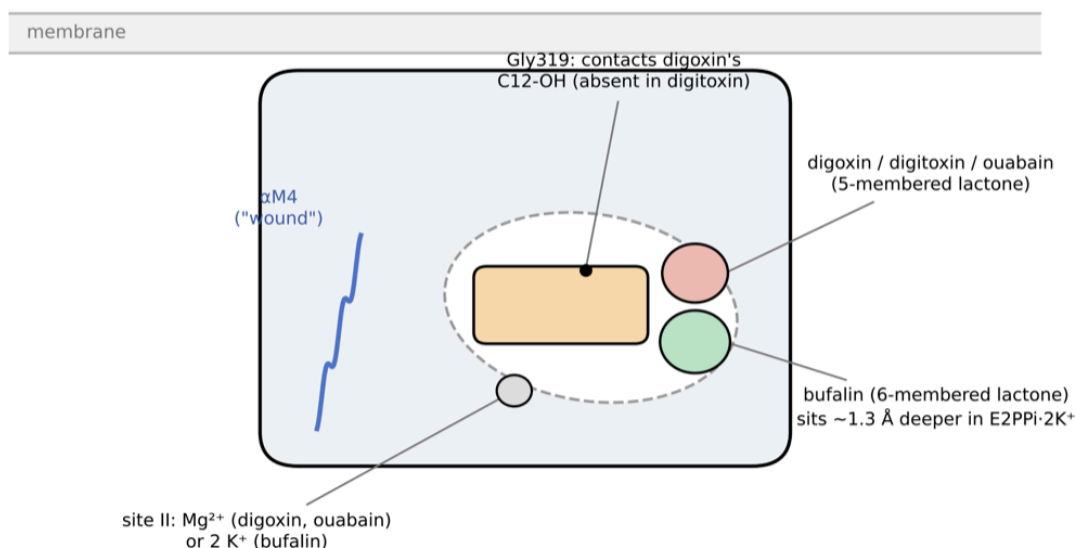
Cardiotonic steroids (CTS) are a family of natural and semisynthetic compounds sharing a steroid core, a lactone ring (five-membered for compounds like digoxin and digitoxin, six-membered for bufalin), and, in some cases, a sugar or trisaccharide substituent. Despite this structural diversity, they act on the same target: the Na^+/K^+ -ATPase (NKA), the ion pump that maintains the transmembrane Na^+ and K^+ gradients essential to nearly every animal cell. CTS bind with highest affinity to the E2P (phosphoenzyme) conformation of the pump (Laursen et al., 2015).

Crystal structures resolved by Kanai et al. (2021) (PDB 7D91–7D94 and 7DDF–7DDL, including 7DDH, the digoxin-bound structure) and by Laursen et al. (2015) (PDB 4RET, digoxin; 4RES, bufalin) show that CTS do not force the pump into a new shape. They instead enter a cavity already present in the $\text{E2PPi}\cdot\text{Mg}^{2+}$ conformation, one that coincides with the pump's normal Na^+/K^+ exchange pathway. This cavity is lined by six negatively charged Asp/Glu residues and narrows sharply where the steroid ring meets the sugar moiety, part of why sugar substituents (as in digoxin) change binding behavior relative to unglycosylated CTS like bufalin (Kanai et al., 2021).

The two crystallography groups identified specific structural correlates of the potency differences among glycosides. Laursen et al. (2015) found that the pump's cation-binding sites are occupied differently depending on which CTS is bound: a single Mg^{2+} occupies site II in the digoxin- and ouabain-bound structures, while both sites are occupied by K^+ in the bufalin-bound structure. In every complex examined, transmembrane helix αM4 adopts a "wound" conformation characteristic of the E2P state and favorable for high-affinity CTS binding; the authors conclude that the identity of the bound cation and the size of the CTS's lactone ring together determine how tightly αM4 winds, and propose this as the trigger for high-affinity binding. Kanai et al. (2021) offer a complementary, more granular explanation for one specific potency difference: the position of K^+ in the $\text{E2PPi}\cdot 2\text{K}^+$ state suits the carbonyl group on bufalin's six-membered lactone ring, but not the five-membered lactones of other CTS, which is offered as the structural reason bufalin's affinity drops more sharply than digoxin's or ouabain's in that particular pump conformation (bufalin's binding site there sits roughly 1.3 Å deeper than the corresponding site for five-membered-lactone compounds). Kanai et al. (2021) further note that the only chemical difference between digitoxin and digoxin is a single hydroxyl group at C12 of digoxin, sitting in van der Waals contact with a glycine residue (Gly319) in the binding pocket: a small structural difference Section 4 returns to as a candidate explanation for why digoxin and digitoxin behave somewhat differently in glioma models.

Figure 2 illustrates these structural relationships.

Na⁺/K⁺-ATPase α-subunit — E2P conformation

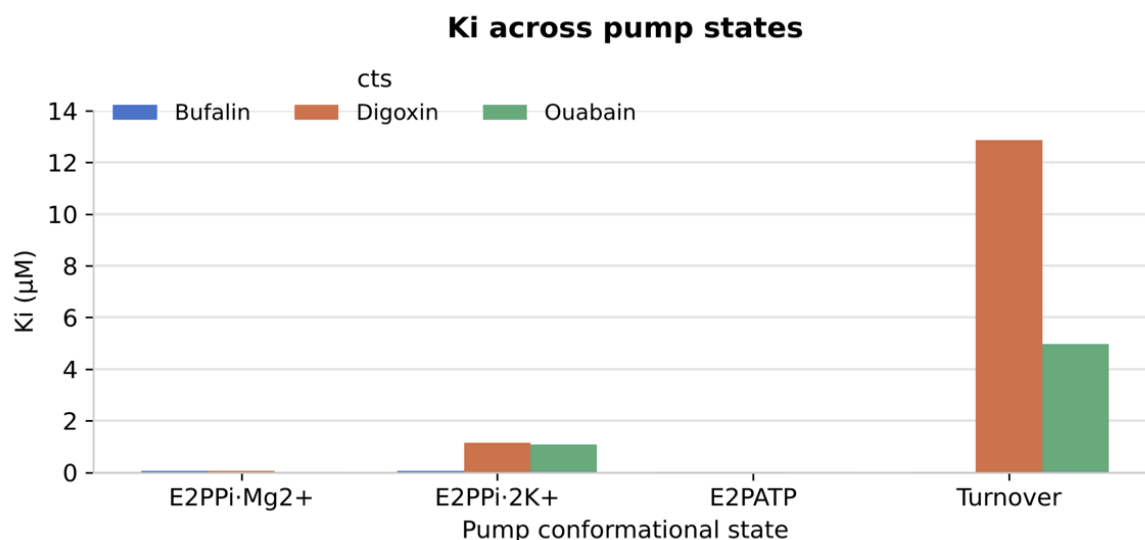


Schematic illustration (not a crystallographic rendering) of the structural relationships described by Kanai et al. (2021, PDB 7D91–7D94, 7DDF–7DDL) and Laursen et al. (2015, PDB 4RET, 4RES).

Figure 2. Schematic illustration (not a crystallographic rendering) of the shared E2P binding cavity and the structural correlates of potency described in the text: cation occupancy at site II, the five- versus six-membered lactone ring, and the Gly319 contact distinguishing digoxin from digitoxin. Based on Kanai et al. (2021, PDB 7D91–7D94, 7DDF–7DDL) and Laursen et al. (2015, PDB 4RET, 4RES).

Kanai et al. (2021) also report apparent inhibitor affinities (K_i) for bufalin, digoxin, and ouabain across several conformational states of the purified pump, shown below. Notice the sharp affinity drop for digoxin and ouabain (but not bufalin) in the Turnover and E2PPI·2K⁺ states, consistent with the lactone-ring-size explanation above.

These values are plotted in Figure 3.



Apparent inhibitor affinity, μM — note the sharp rise for digoxin/ouabain (not bufalin) in E2PPi-2K+ and Turnover. Data: Kanai et al. (2021), Table 1

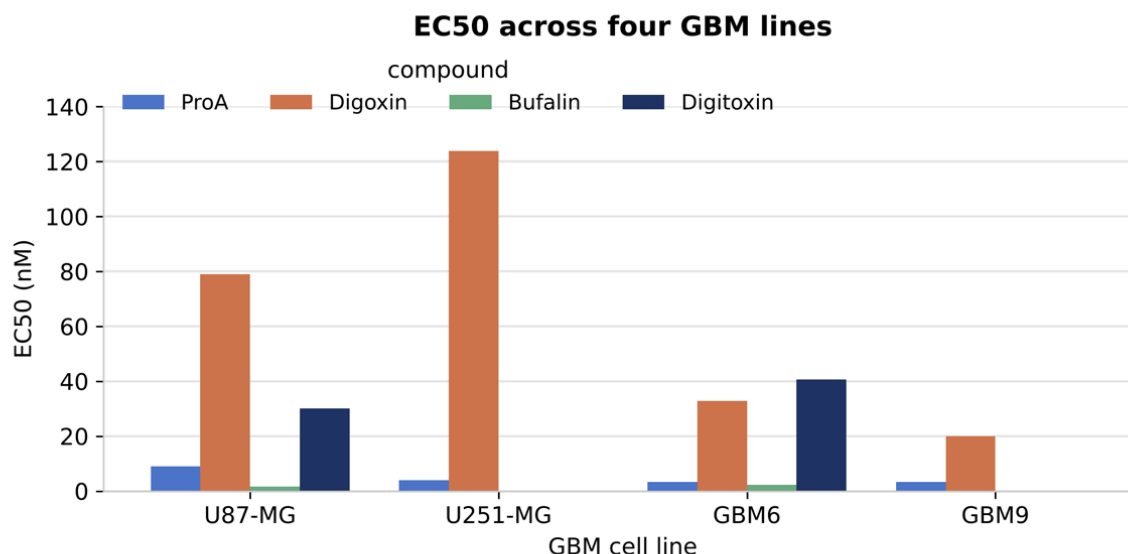
Figure 3. Apparent inhibitor affinity (K_i , μM) for bufalin, digoxin, and ouabain across Na^+/K^+ -ATPase pump conformational states. Data: Kanai et al. (2021), Table 1.

1.2 Relevance to Glioblastoma

NKA inhibition is not incidental to cancer biology. The pump itself is implicated in tumor behavior independent of any drug: it promotes the migration of cancer cells, and its aberrant expression and activity have been associated with several cancers, including glioblastoma (Valluri et al., 2022). When CTS inhibit NKA, they disrupt potassium ion homeostasis, which has been linked to caspase-mediated apoptosis and necrosis in highly proliferating GBM cells (Valluri et al., 2022).

A useful, if partial, quantitative picture of how differently four glycosides (proscillaridin A, or ProA, digoxin, bufalin, and digitoxin) behave against glioma cells comes from Berges et al. (2018), who measured potency across four GBM cell lines (U87-MG, U251-MG, GBM6, GBM9). ProA was consistently the most potent, roughly ten times more potent than digoxin across every line tested; bufalin and digitoxin were tested only in the two lines where comparison data are available (U87-MG and GBM6), not all four.

Figure 4 shows the measured EC_{50} values across all four cell lines.



Bufalin and digitoxin tested only in U87-MG and GBM6. Data: Berges et al. (2018), Table 1

Figure

4. EC50 (nM) for proscillaridin A (ProA), digoxin, bufalin, and digitoxin across four GBM cell lines. Bufalin and digitoxin were tested only in U87-MG and GBM6. Data: Berges et al. (2018), Table 1.

A separate strand of evidence comes from Valluri et al. (2022), who tested digoxin, alone and combined with temozolomide (TMZ), across GBM lines with different intrinsic TMZ sensitivity. In the TMZ-resistant line T98G, temozolomide alone produced no detectable response at any clinically relevant dose, while digoxin alone (10 μ M) produced an 83.9% decrease in viability, and digoxin plus TMZ produced a similar 78.9% decrease. The intermediate-sensitivity line U87MG showed a comparable pattern, and the TMZ-sensitive line BNC-6 showed the largest effect, with digoxin alone producing a 100% viability decrease.

Table 1 summarizes these results across the three cell lines.

Table 1. Digoxin, alone and combined with temozolomide (TMZ), on viability across GBM cell lines of differing intrinsic TMZ sensitivity. Data: Valluri et al. (2022).

Cell line (TMZ sensitivity)	Condition	Viability decrease
T98G (resistant)	Digoxin 10 μ M	83.9%
T98G	Digoxin + TMZ	78.9%
U87MG (intermediate)	Digoxin 10 μ M	79.4%
U87MG	Digoxin + TMZ	81%
BNC-6 (sensitive)	Digoxin 10 μ M	100%

Valluri et al. (2022) also note that rat CNS-1 glioma cells were resistant to digoxin, a useful caution given the broader rodent-versus-human/pig difference in NKA sensitivity to cardiac

glycosides documented outside the cancer literature. Potency numbers from non-human, non-primate systems should not be assumed to transfer directly to human GBM.

Whether NKA inhibition kills GBM cells through the same route it uses in cardiac muscle, however, is not settled. In cardiomyocytes, the textbook mechanism is that CTS binding to NKA raises intracellular Na^+ , which the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) then extrudes less efficiently, so Ca^{2+} accumulates inside the cell (Berges et al., 2018). Extending this logic to cancer cells, the argument runs that this calcium rise could activate calmodulin kinases relevant to downstream cell-fate signaling. Berges et al. (2018) are explicit, though, that "the ion pumping function of NKA in the anticancer activity of CG is still under debate," and this review treats that caveat as load-bearing rather than a formality.

2. Where the Pathway Splits: Two Downstream Stress Responses

2.1 The ER Stress / PERK–eIF2 α –CHOP Branch

The most direct evidence that a cardiac glycoside triggers endoplasmic reticulum (ER) stress in glioma cells comes from Shen et al. (2014), who treated U87MG human glioma cells with bufalin and found dose-dependent activation of essentially the full unfolded protein response (UPR): phosphorylation of PERK, IRE1 α , and eIF2 α , and accumulation of ATF6 fragments, all increasing with bufalin concentration, alongside dose-dependent increases in the ER chaperones GRP78 and GRP94 after 24 hours of treatment. Downstream, bufalin upregulated the pro-apoptotic transcription factor CHOP and accelerated cleavage of caspase-4 in U87MG cells. This was shown to be causal rather than incidental: knocking down CHOP with siRNA attenuated bufalin-induced apoptosis, directly implicating the ER-stress response, and specifically the PERK–eIF2 α –CHOP arm of it, in bufalin's cytotoxic effect on glioma cells.

One limitation of Shen et al. (2014) is that it does not explicitly connect this ER-stress cascade back to NKA inhibition; the paper documents that bufalin triggers ER stress in glioma cells, not the mechanistic step connecting NKA inhibition to that ER stress. The clearest single-paper account of that connecting step comes from a different cancer type: Li et al. (2021), studying oleandrin (a related cardiac glycoside) in breast cancer, report that oleandrin, acting as an NKA inhibitor, increases intracellular Na^+ and activates the $\text{Na}^+/\text{Ca}^{2+}$ exchange channel, and that the resulting Ca^{2+} influx and disruption of cellular ion homeostasis activates ER-stress pathways including PERK/eIF2 α /ATF4 and IRE1-XBP1. In that system, phosphorylation of PERK and eIF2 α (Ser52) was enhanced within 6 hours of treatment, followed by ATF4 activation and increased expression of ATF4-dependent targets including CHOP. This paper is not glioma-specific, but it is the clearest available single-paper causal chain from cardiac-glycoside-driven NKA inhibition through to PERK/eIF2 α /CHOP activation, and this review uses it to fill the mechanistic gap that Shen et al. (2014) leaves open for glioma.

2.2 Why ER Stress Is a GBM Vulnerability, and the HIF-1 α Suppression Branch

Independent of cardiac glycosides, there is a separate rationale for why ER stress specifically is a real, exploitable vulnerability in glioblastoma. Liu, Tsung, and Attenello (2020), reviewing cell stress and the ER chaperone GRP78 in glioma, report that GRP78 overexpression is directly correlated with malignant tumor characteristics, including higher tumor grade and increased proliferation and migration; GRP78 expression is also higher in GBM tumors upon recurrence, and knockdown or suppression of GRP78 sensitizes glioma cells to both temozolomide and radiation therapy. In other words, GBM cells appear to lean on chronic, adaptive ER-stress

signaling to survive and resist standard-of-care treatment. That is exactly the kind of dependency a drug that pushes ER stress past a tolerable level could exploit.

A separate downstream branch runs through hypoxia signaling rather than the UPR. The foundational paper here is Zhang et al. (2008), from Gregg Semenza's group, who showed that digoxin and several other cardiac glycosides inhibit synthesis of hypoxia-inducible factor 1- α (HIF-1 α) and block tumor growth. Using pulse-labeling and immunoprecipitation, they found that a 4-hour pretreatment with 100 nM digoxin reduced newly synthesized HIF-1 α to 27% of the vehicle-treated level, a degree of inhibition comparable to cycloheximide, a global protein-synthesis inhibitor used as a positive control:

Table 2. Relative newly-synthesized HIF-1 α after a 4-hour pretreatment, by condition. Digoxin's effect is comparable to cycloheximide, a global protein-synthesis inhibitor used as a positive control. Data: Zhang et al. (2008).

Pretreatment (4 h)	Relative HIF-1 α level
Vehicle	1.00
Rapamycin	0.31
Cycloheximide	0.12
Digoxin (100 nM)	0.27

At 50 nM, ouabain and proscillaridin A inhibited HIF-1 α protein expression under both normoxic (20% O₂) and hypoxic (1% O₂) conditions. More broadly, eleven distinct cardiac glycosides (digoxin, ouabain, proscillaridin A, digitoxin, acetyldigitoxin, convallatoxin, peruvoside, strophanthin K, nerifolin, cymarin, and periplocymarin) produced more than 88% inhibition of HIF-1 α synthesis at a common concentration of 0.4 μ M, and daily intraperitoneal digoxin (0.5 or 2 mg/kg) dose-dependently inhibited the growth of P493-Myc tumor xenografts in mice.

An important limitation of Zhang et al. (2008) is that it was carried out entirely outside glioma: the tumor models used were Hep3B (liver cancer) and P493-Myc (lymphoma), and no GBM cell line was tested. The rationale for extending this finding to glioblastoma comes from a separate literature establishing that hypoxia and HIF-1 α signaling matter enormously in GBM specifically. Bou-Gharios, Noël, and Burckel (2024), reviewing preclinical and clinical strategies to overcome hypoxia in glioblastoma, note that the presence of hypoxia within the tumor bulk activates HIF-mediated signaling, and catalog pharmacological HIF-1 α /HIF-2 α inhibitors tested in GBM (including OKN-007, melatonin, curcumin, EF-24, acriflavine, echinomycin, and KCN1). Cardiac glycosides do not appear on that list. As of this 2024 GBM-hypoxia review, in other words, no single paper has shown that a cardiac glycoside suppresses HIF-1 α in a glioma cell line. The connection this review draws from digoxin to HIF-1 α to GBM currently rests on combining a general-cancer finding (Zhang et al., 2008) with a GBM-specific rationale for why HIF-1 α matters (Bou-Gharios et al., 2024), not on one paper demonstrating both halves of the claim together.

2.3 An Explicit Comparison, and Its Limits

The single best attempt in the literature to place both branches side by side comes from Kumavath et al. (2021), a broad review of cardiac glycosides as anticancer drugs. It states plainly that cardiac glycosides bind the Na^+/K^+ -ATPase pump, inhibiting it and causing intracellular retention of Na^+ and a rise in intracellular Ca^{2+} , and that the resulting lower expression of Na^+/K^+ -ATPase subsequently causes ER stress. It separately diagrams HIF-1 α suppression as a second consequence of the same upstream inhibition. The review also ranks the relative potency and cytotoxicity of five glycosides: proscillaridin A was most potent and cytotoxic, followed by digitoxin, ouabain, digoxin, and lanatoside C.

Kumavath et al. (2021) is, however, a general cancer-pharmacology review, not a glioma paper: it never uses the terms PERK, eIF2 α , CHOP, or GRP78, and it never mentions glioma. It is the best available source for the idea that both branches originate from the same upstream event, but connecting either branch specifically to glioblastoma has to be done by combining it with the more GBM-focused sources discussed above.

Put together, the working model this review proposes (bufalin and its structural relatives tending toward the ER-stress/PERK-eIF2 α -CHOP branch, digoxin and digitoxin tending toward the HIF-1 α suppression branch) is a reasonable synthesis of the available evidence, but it has not been demonstrated head-to-head. No published paper shows a single cardiac glycoside producing both branches within the same glioma cell line, and, more specifically, digoxin has not been shown to trigger PERK/CHOP-mediated ER stress in glioma cells, nor has bufalin been shown to suppress HIF-1 α in glioma cells, in any source reviewed here. The clean two-branch split should be read as this review's organizing hypothesis, not as an established fact.

Two further findings complicate the picture rather than resolve it. First, the direction of the effect is not universal. Tokugawa et al. (2021) found that periplocin and other cardiac glycosides actually suppress IRE1/PERK/ATF6 UPR signaling in myeloma and HEK293 cells, the opposite direction from the ER-stress branch described above. That is a reminder that the relationship between cardiac glycosides and UPR signaling is context-dependent rather than fixed in one direction across all cell types. Second, at least one additional, independent mechanism has been identified for bufalin specifically in glioblastoma: Lan et al. (2019) report that the $\alpha 1$ subunit of the sodium pump (ATP1A1) regulates glioblastoma cells' sensitivity to bufalin through a Src-p53-mitochondrial apoptosis pathway that is independent of both ER stress and HIF-1 α . The two-branch model in this review is therefore best understood as capturing the two most extensively documented downstream consequences of NKA inhibition in cancer cells generally, not as an exhaustive account of every mechanism through which cardiac glycosides kill glioma cells.

3. A Complication: When the Stress Response Protects Instead of Kills

3.1 Autophagy as a Survival Mechanism

Shen et al. (2014), the same study that established bufalin's ER-stress effect in glioma cells, is also the single most directly relevant paper for the claim that this same stress response triggers protective autophagy, because it was carried out in actual glioma lines (U87MG and LN229) rather than a different cancer type. The authors confirmed a dose-dependent increase in LC3-II (the autophagosome-associated form of LC3) in bufalin-treated glioma cell lysates, and found that bufalin treatment significantly increased the number of MDC-stained autophagic vacuoles in both U87MG and LN229 cells.

The key finding for this review's argument is directional: blocking autophagy (with 3-methyladenine or wortmannin) increased expression of ER-stress-associated proteins and increased the proportion of cells undergoing apoptosis, and co-treatment with an autophagy inhibitor augmented bufalin-induced apoptosis relative to bufalin alone. Shen et al. (2014) interpret this as showing that "autophagy played a cytoprotective role": the autophagy triggered by bufalin was, on balance, helping glioma cells survive the ER-stress insult rather than contributing to their death. The authors further show that ER stress preceded autophagy (rather than the reverse) and that the PERK/eIF2 α /CHOP pathway specifically drives this protective response: knocking down eIF2 α or CHOP attenuated bufalin-induced LC3-II conversion, directly tying the survival-promoting autophagy back to the same ER-stress branch described above. The paper's own abstract states the clinical implication directly: "Inhibition of autophagy potentiates the proapoptotic effect of bufalin, suggesting appropriate regulation of autophagy is necessary for sensitizing tumor cells to anticancer therapy."

Škubník et al. (2021), reviewing cardiac glycosides as autophagy modulators, synthesize the same underlying glioma data and add a useful methodological detail: when ER stress in glioma cells was prevented pharmacologically (pretreatment with 500 μ M tauroursodeoxycholate, a chemical chaperone), bufalin-induced expression of LC3-II and other autophagy markers was reduced, confirming that ER stress precedes and drives autophagy in this system rather than the two arising independently. The review frames autophagy's role explicitly as a double-edged sword: "autophagy acts as a tumor suppressor by degrading potentially oncogenic molecules... on the other hand, autophagy strongly contributes to tumor cell survival and progression of tumor growth," and suggests that combining bufalin with autophagy inhibitors could be a productive direction for anticancer regimens.

The clearest verbal articulation of a survival/death threshold governed by stress intensity, however, comes from a non-GBM system and is worth citing on its own terms rather than borrowing its language without attribution: Zhao et al. (2017), working in human gastric cancer cells, found that bufalin-induced autophagy is conducive to cancer cell survival, that "when the ER stress exceeds a certain extent, the activated unfolded protein response (UPR) triggers autophagy," and that inactivating autophagy (via 3-MA or Atg5 knockdown) significantly worsened bufalin-induced apoptosis. Their framing, that "autophagy has a dual nature in the regulation of cell death: mild autophagy protects cells against harmful conditions and promotes cell survival," is the clearest single statement in the literature reviewed here of a threshold governed by stress or autophagy intensity, even though it comes from gastric rather than glioma cells.

3.2 Toward a "Threshold" Model

What molecular machinery actually links CHOP activation to autophagosome formation? Yan et al. (2015), reviewing the interplay between the UPR and autophagy in tumor drug resistance, describe the connecting steps: CHOP increases expression of ATG5 and BH3-only proteins while reducing Bcl-2 levels, which allows the autophagy-initiating protein beclin-1 to be released from its inhibitory complex with Bcl-2; separately, inactivation of mTORC1 (itself a consequence of sustained ER stress) dephosphorylates ATG13 and the ULK1/2 complex, initiating autophagosome formation. Critically for the threshold argument, the same review states that "if the UPR fails to restore ER homeostasis, prolonged and severe ER stress may transform the adaptive UPR response, which protects cells from death in adverse stress, to deadly output by activating the ER-dependent apoptosis signaling pathway." That directly supports the idea that

outcome depends on the duration or severity of stress crossing some threshold, rather than being fixed by which pathway is activated. The review also flags a striking asymmetry worth noting for any future therapeutic strategy: "UPR induced autophagy exerts a pro-survival response in malignant tumor cells but elicits a cell death response in normal cells." If that generalizes, it implies a therapeutic window in which the same drug-induced stress could be more dangerous to tumor cells than to healthy tissue.

Khan et al. (2021), reviewing autophagy's role in glioblastoma treatment resistance specifically (though not in the context of cardiac glycosides or ER stress), provide independent GBM-specific grounding for the threshold argument: autophagy-mediated pro-survival signals drive resistance to anti-angiogenic chemotherapies in glioblastoma, but a continuous or sufficiently intense autophagic signal can itself lead to autophagic cell death through exhaustion of critical organelles and proteins. The authors explicitly flag "what degree of autophagy modulation will facilitate the sensitization of glioma cells" as an open question in the field, which is, in effect, an open invitation for exactly the kind of dose-and-duration-focused work this review's proposed threshold model would need to make precise.

It would be a mistake, however, to treat the survival/death balance as a fixed property of bufalin or of ER-stress-triggered autophagy in general. Miao et al. (2013), studying bufalin in HepG2 human hepatocellular carcinoma cells, found the opposite relationship: blocking autophagy decreased apoptosis (from 35.6% down to 14.2%), meaning that in that cell context bufalin-induced autophagy contributed to cell death rather than protecting against it. This directly contradicts the direction reported in glioma and gastric cancer cells, and is the clearest evidence in this review that the survival-versus-death role of autophagy is cell-context-dependent rather than a fixed rule that can be assumed to generalize across tumor types.

4. Translational Barriers

Any therapeutic argument built on the mechanisms above eventually has to confront a basic pharmacological question: are the concentrations that produce these effects in vitro reachable, safely, in a human patient? The honest answer differs substantially by compound, and even by which specific glycoside is being discussed.

Digoxin has real, if limited, modern human trial data outside its cardiac indication. Kurzeder et al. (2025), in a proof-of-concept trial for reducing circulating tumor cell cluster size in metastatic breast cancer, gave patients a daily maintenance dose of digoxin (0.125 mg or 0.25 mg tablets, taken before 10:00 a.m.) for seven days, targeting a serum concentration of 0.7 ng/mL. Nine patients reached that target serum level; the treatment was well tolerated with no digoxin-related adverse events, and the effect on tumor cell cluster size was statistically significant but described by the authors as mild. This is useful evidence that digoxin can be dosed safely in cancer patients near its established cardiac range. It is also evidence, though, that at that dose the clinical effect was modest, not evidence that this dose reproduces the ER-stress or HIF-1 α effects described in earlier sections.

That concentration gap is stated explicitly, and quantitatively, in the foundational HIF-1 α paper itself. Zhang et al. (2008) write that "therapeutic plasma concentrations of digoxin in cardiac patients ($\approx$ 10–30 nM) are somewhat lower than the concentration required for maximal inhibition of HIF-1 α expression in hypoxic cancer cells after 24 h of drug treatment." Their own effective concentrations were 100 nM digoxin (giving 73% inhibition) and 50 nM for ouabain and proscillaridin A. The authors offer a partial hedge, suggesting effective inhibition at lower

concentrations might be observed after more prolonged exposure, but this remains, by their own admission, unconfirmed. This is arguably the single clearest, most citable statement of a quantitative gap between in vitro effective dose and achievable human plasma concentration anywhere in the literature reviewed for this paper.

The most directly GBM-relevant source on this question, however, complicates a simple "digoxin doesn't reach effective concentrations" story by drawing a sharp distinction between digoxin and its close relative digitoxin. Lee et al. (2017), working in human glioma stem cells (GSCs), found that digitoxin (DT) at clinically achievable concentrations (10–25 nM) suppressed HIF-1 α accumulation under hypoxic conditions, with no significant effect on cell viability at that same range, a favorable therapeutic window. By contrast, they report that "digoxin required higher concentration than used for cardiac therapy (2–3 nM)" to achieve a comparable anti-HIF-1 α effect. (This 2–3 nM figure for digoxin's cardiac range, as stated by Lee et al., differs numerically from the $\approx$ 10–30 nM figure Zhang et al. (2008) give for the same clinical range, a discrepancy this review flags rather than resolves, since the two papers may be using different measures, such as free versus protein-bound plasma drug levels.) The practical upshot, in Lee et al.'s own framing, is that digitoxin, not digoxin, is the pharmacokinetically better-supported candidate for the HIF-1 α branch in glioma specifically: it is lipid-soluble and likely able to cross the blood–brain barrier, and the authors argue it has the potential to address a real unmet need in an incurable cancer with limited treatment options. This is an important correction to keep in mind: much of the general cardiac-glycoside-and-cancer literature (including Zhang et al., 2008, and much of Section 2 above) is built around digoxin, but the GBM-specific pharmacokinetic case is actually stronger for digitoxin.

Table 3 lists computed physicochemical properties for all four compounds relevant to this question.

Table 3. Computed physicochemical properties relevant to CNS penetration, calculated in RDKit (Crippen LogP, topological polar surface area) from PDB Chemical Component Dictionary isomeric SMILES. All four compounds exceed the 500 Da molecular-weight cutoff conventionally associated with passive blood–brain barrier permeability; bufalin is the only one whose TPSA falls under the commonly cited $\sim$ 90 Å² threshold, consistent with digoxin's known poor, P-glycoprotein-limited brain penetration. These are computed estimates, not measured brain-penetration data.

Compound	MW (Da)	cLogP	TPSA (Å ²)	H-bond donors	H-bond acceptors	Rotatable bonds
Digoxin	780.9	2.22	203.1	6	14	7
Digitoxin	765.0	3.25	182.8	5	13	7
Bufalin	386.5	4.24	70.7	2	4	1
Ouabain	584.7	−1.51	206.6	8	12	4

Figure 5 plots these same properties against the thresholds conventionally used to judge blood–brain barrier permeability.

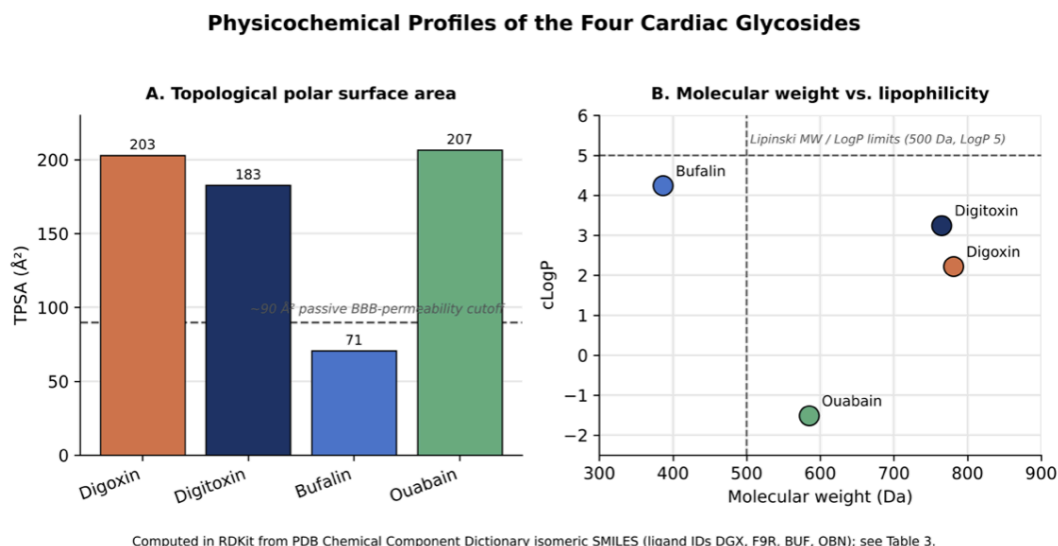


Figure 5. (A) Computed topological polar surface area (TPSA) for each compound against the $\sim 90 \text{ \AA}^2$ cutoff conventionally associated with passive blood–brain barrier permeability. (B) Molecular weight vs. computed LogP against the Lipinski Rule-of-Five boundaries (500 Da, LogP 5). Bufalin is the only compound inside both boundaries; the other three are well outside, consistent with digoxin's documented poor, P-glycoprotein-limited brain penetration. Computed in RDKit from PDB Chemical Component Dictionary isomeric SMILES; see Table 3 for exact values.

Bufalin's translational picture is considerably weaker, because essentially no human pharmacokinetic data exist for it at all. Ye et al. (2023), reviewing a decade of bufalin research in refractory and drug-resistant cancers, describe bufalin plainly as "a toxic agent," and note that it was tested in a single Phase II clinical trial in China for pancreatic cancer (NCT00837239, initiated 2009), for which no results were ever posted, with no other clinical trials following; bufalin has, by their account, been tested almost exclusively in mainland China. Reported in vitro potency (IC_{50}) figures for bufalin across cancer types range roughly from 10 nM (multiple myeloma) to 500 nM (triple-negative breast cancer), with in vivo animal doses around 0.1–2 mg/kg reported without accompanying human-translatable pharmacokinetic parameters. Set against this, LingHu, Luo, and Gang (2020), working specifically in glioma cell lines (U87 and U-373), used bufalin concentrations from roughly 15.6 nM up to 1,000 nM in dose-response experiments, with 1,000 nM as the primary working concentration in their mechanistic experiments, a concentration roughly 30 to 100 times higher than digoxin's ~ 10 –30 nM clinical range (there being no established human range for bufalin itself against which to compare). Any claim about bufalin's achievable or safe human dose should therefore be treated as unknown rather than estimated from these in vitro figures.

Two further sources point toward where translational strategy might go next, though both should be read cautiously: their full text could not be verified in the course of this review. Teng et al. (2014) report, at the abstract level only, combining a cardiac glycoside (lanatoside C) with systemic anticancer neural stem cells for glioblastoma therapy, with lanatoside C sensitizing glioma cells to TRAIL-induced apoptosis, which would be directly relevant to a combination-therapy strategy if the full study can be obtained and verified. Mijatovic et al.

(2013), a broader "trial watch" on cardiac glycosides and cancer therapy, likely contains a wider survey of the clinical trial landscape but was similarly inaccessible for direct verification here. Taken together, the translational path forward for either branch most plausibly runs through combination strategies (pairing a glycoside with an autophagy inhibitor, as suggested in Section 3, or with existing chemoradiation) and through further work specifically on digitoxin's blood–brain-barrier penetration and dosing in glioma patients, rather than through digoxin or bufalin alone at concentrations shown to be safe today.

5. Where Computation Could Resolve the Divergence

Kanai et al. (2021) (PDB 7DDH) and Laursen et al. (2015) (PDB 4RET, digoxin; PDB 4RES, bufalin) together provide crystallographic ground truth for exactly the comparison this review has been building toward: high-resolution structures of the same pump bound to different cardiac glycosides. The subtle structural distinction Kanai et al. (2021) identify between digoxin and digitoxin (a single hydroxyl group at C12 of digoxin in van der Waals contact with Gly319) is a specific, testable candidate explanation for why these two closely related compounds might differ in their downstream cellular effects (Section 4), but the crystallography papers themselves are purely structural: they say nothing about ER stress or HIF-1 α , and neither paper attempts to connect binding-pocket geometry to which downstream branch a glycoside activates. That inferential connection, between subtle differences in how a glycoside sits in the NKA binding pocket and which stress pathway it preferentially triggers, is not established anywhere in the literature reviewed for this paper. It is proposed here as this review's own hypothesis, to be tested computationally and, ultimately, experimentally.

Molecular docking of digoxin-scaffold compounds against Na,K-ATPase is not a novel approach: Pessôa et al. (2018) synthesized a series of γ -benzylidene digoxin derivatives with substitutions on the lactone ring and used molecular docking to show that specific derivatives favored NKA-isoform-specific interactions correlating with the compounds' observed biological activity. This establishes docking against NKA as an accepted, precedented computational method, and suggests a concrete next step: docking digoxin, digitoxin, bufalin, and their derivatives against the 7DDH/4RET/4RES structures to look for pocket-level features, such as contacts near the C12 position Kanai et al. (2021) identified, that might correlate with which glycosides have been reported to favor the ER-stress branch versus the HIF-1 α branch.

A second, more speculative target is the kinase domain of PERK itself, since PERK sits at the center of the ER-stress branch described in Section 2. A crystal structure of the PERK kinase domain is available (Cui et al., 2011; PDB 3QD2, 2.8 Å resolution), and the paper describing it is explicit about the domain's construct boundaries: "the coordinates of mPERK KD have been deposited in the PDB under accession code 3qd2," from a construct spanning residues Arg584–Asn1114 with an internal segment deletion (Δ Lys702–Thr866), amplified from full-length mouse PERK cDNA. This construct boundary is itself the important fact for any proposed use of this structure: 3QD2 captures only the cytoplasmic kinase domain, not the ER-luminal sensor domain or the transmembrane region that oligomerizes and triggers PERK's trans-autophosphorylation (including at Thr980) in response to ER stress (Cui et al., 2011). Any docking work against 3QD2, in other words, would be probing a secondary, downstream node in the ER-stress pathway (the kinase's catalytic site, relevant to small-molecule PERK modulators) rather than the primary ER-stress sensing event this review is actually interested in. That is a

real limitation, not a minor caveat, and one worth stating up front in any docking proposal built on this structure.

Table 4 lists the crystal structures referenced above.

Table 4. Crystal structures proposed as computational targets in Section 5. Resolutions are listed only where stated in the source cited; — marks a value not given in the reviewed text.

PDB ID	Structure	Resolution	Source	Proposed use in this review
7DDH	Na ⁺ /K ⁺ -ATPase, E2P state, digoxin-bound	—	Kanai et al. (2021)	Primary docking target for digoxin/digitoxin/bufalin scaffold comparison
4RET	Na ⁺ /K ⁺ -ATPase, digoxin-bound	—	Laursen et al. (2015)	Cross-validation structure for the digoxin pose
4RES	Na ⁺ /K ⁺ -ATPase, bufalin-bound	—	Laursen et al. (2015)	Cross-validation structure for the bufalin pose
3QD2	PERK kinase domain (res. 584–1114, Δ702–866)	2.8 Å	Cui et al. (2011)	Secondary, exploratory target; excludes the ER-luminal sensor

Docking studies of this kind can generate hypotheses; they cannot, on their own, confirm a mechanism. Pantsar and Poso (2018), assessing whether docking reliably predicts binding affinity, conclude bluntly that "the short answer is no, and this has been concluded in several comprehensive analyses"; despite substantial effort to improve scoring functions, "the accuracy of binding affinity predictions is only as good as it was 10–20 years ago," and a docking program can predict the correct binding pose while still getting the predicted binding affinity wrong. Their closing observation is worth keeping in view for any student project built around docking: "even after implementing all of these user-based interventions, we always use the most sophisticated scoring function, the eye. If you trust your docking pose, you might be right." Any proposed docking work in this project should therefore be framed explicitly as hypothesis-generating: a way to narrow down which structural features and residue contacts are worth testing experimentally (through binding assays, co-crystallography, or cell-based CHOP and HIF-1α reporter assays), rather than a way to confirm, on its own, why different cardiac glycosides activate different downstream branches.

Discussion

Pulling the sections above together, the model this review proposes is: cardiac glycosides bind a single, structurally conserved cavity on the Na⁺/K⁺-ATPase in its E2P conformation (Section 1); this inhibition, through a still-debated calcium-dependent intermediate step, triggers two distinguishable downstream stress responses: an ER-stress/PERK-eIF2α-CHOP response most strongly documented for bufalin, and a HIF-1α suppression response most strongly documented for digoxin and digitoxin (Section 2); the ER-stress branch specifically also triggers protective

autophagy that can delay or prevent cell death until some not-yet-quantified threshold of stress is exceeded (Section 3); and translating either branch into a usable GBM therapy is presently limited by a real, and in places explicitly stated, gap between effective in vitro concentrations and concentrations reachable safely in human patients, with digitoxin the most promising exception (Section 4).

Figure 6 diagrams this proposed model.

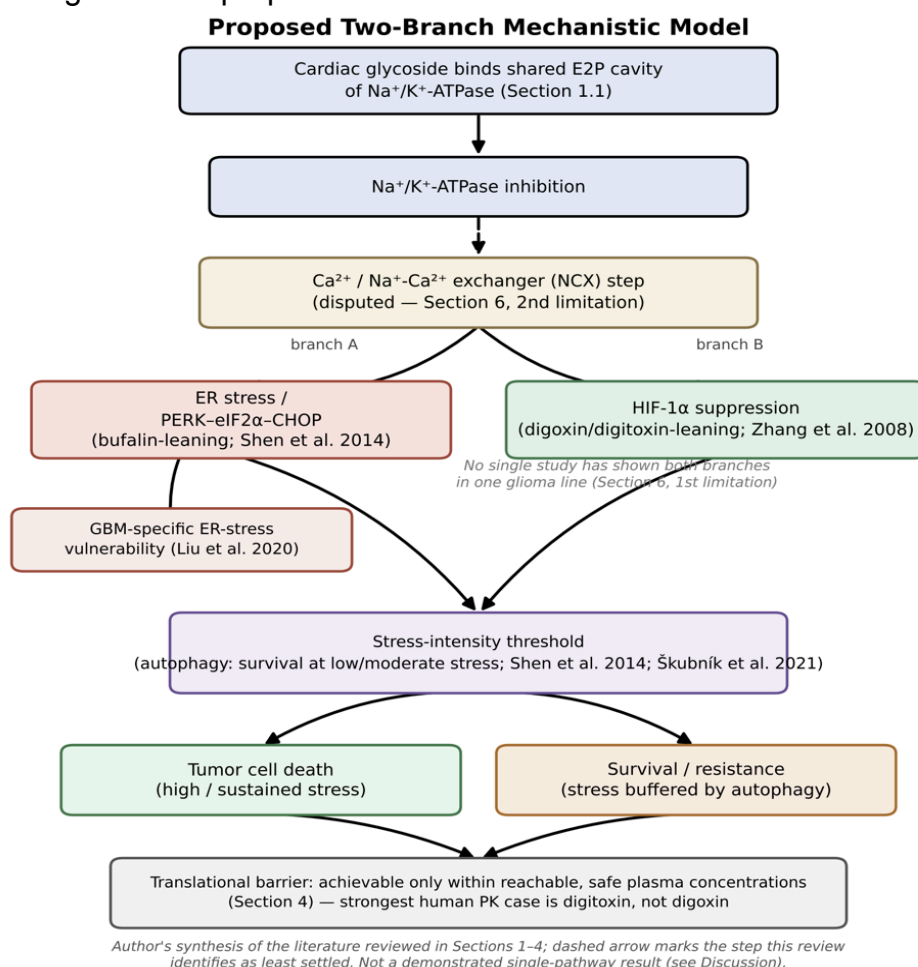


Figure 6. Proposed two-branch mechanistic model synthesizing Sections 1–4. The $\text{Ca}^{2+}/\text{NCX}$ step (dashed arrow) is this review's least-settled link (see Discussion, limitation 2); no single study has shown both downstream branches in one glioma line (limitation 1). This is the author's synthesis across the literature reviewed, not a demonstrated single pathway.

This model is, though, a synthesis across many separate papers studying different cancer types, different species, and different assay systems, not a single demonstrated pathway. Several honest qualifications matter enough to restate directly rather than let them fade into the background.

First, no single published study has shown one cardiac glycoside producing both the ER-stress branch and the HIF-1 α -suppression branch within the same glioma cell line. The two-branch

split is this review's organizing hypothesis, built by combining separate literatures, not an established dichotomy.

Second, the calcium/NCX step connecting NKA inhibition to downstream stress signaling is itself disputed, even within the cardiac-glycoside-and-cancer literature. Berges et al. (2018) call the ion-pumping function's role in anticancer activity "still under debate," and the one glioma-specific study of NCX behavior found here (Chovancova et al., 2020) argues against the straightforward reverse-mode-NCX calcium-overload version of the mechanism in glioma cells specifically.

Third, the pharmacokinetically strongest case for the HIF-1 α branch in glioma belongs to digitoxin, not digoxin. That is an important correction, since digoxin is the compound most cardiac-glycoside-and-cancer literature (including the foundational Zhang et al., 2008, paper) is built around.

Fourth, bufalin has essentially no human pharmacokinetic data, so any claim about a clinically achievable bufalin dose is, at present, aspirational rather than evidence-based.

Fifth, the underlying biochemistry and the cancer biology come from different systems that should not be assumed interchangeable: the crystal structures defining the shared binding mechanism (Kanai et al., 2021; Laursen et al., 2015) were solved using purified pig NKA, while cell-based cancer studies use human glioma lines, and at least one rodent glioma model (rat CNS-1 cells) was reported to be resistant to digoxin entirely (Valluri et al., 2022). That is a reminder that species differences in NKA sensitivity to cardiac glycosides are well documented and should temper any direct transfer of potency numbers across systems.

Sixth, the two-branch model is a simplification, not an exhaustive account: cardiac glycosides suppress rather than activate UPR signaling in at least one other cell type (myeloma; Tokugawa et al., 2021), and at least one additional, ER-stress- and HIF-1 α -independent mechanism (a p53-dependent apoptotic pathway) has been identified for bufalin in glioblastoma specifically (Lan et al., 2019).

Table 5 pulls together the evidence and translational-readiness picture for each compound discussed above.

Table 5. Summary of the evidence and translational readiness discussed in Sections 1–4 for each compound. This restates, rather than adds to, claims already made in the text above.

Compound	ER-stress branch (PERK-eIF2 α -CHOP) in GBM	HIF-1 α branch in GBM	Human PK data	Key caveat
Digoxin	Not reported	Indirect (general-cancer evidence + GBM-hypoxia rationale, not a direct GBM study)	Yes (cardiac dosing; Kurzeder et al. 2025 trial)	Achievable concentrations likely below the HIF-1 α -suppressing dose (Section 4)
Digitoxin	Not reported	Yes — direct, GBM-specific (glioma stem cells; Lee et al. 2017)	Cardiac dosing exists; no GBM/oncology dosing data	Pharmacokinetically the strongest GBM candidate here, but untested in any cancer trial
Bufalin	Yes — direct, GBM-specific (U87MG; Shen et al. 2014)	Not reported in glioma	None at all	Most mechanistically direct GBM evidence, but zero human PK data (Section 4)
Ouabain	Not reported in glioma	General-cancer evidence only (Zhang et al. 2008); not GBM-specific	Not covered in this review	Least GBM-specific characterization of the four compounds

Limitations and Open Questions

- No unified potency comparison (K_i , IC_{50} , or EC_{50} on one common assay) exists across digoxin, digitoxin, and bufalin in glioma lines; the closest available data (Kanai et al., 2021) is purified-pump biochemistry, not glioma cell viability data.
- GBM-specific evidence for the initiating calcium/NCX step is thinner than the evidence for either downstream branch, and the one glioma-specific NCX study found argues against the simplest version of that step.
- No single cell-line experiment has demonstrated both downstream branches (ER stress and HIF-1 α suppression) side by side for one glycoside.
- Bufalin has no human clinical pharmacokinetic data of any kind.
- The PDB structure available for PERK (3QD2) is a kinase-domain-only construct and excludes the ER-luminal stress-sensing domain, limiting what any PERK-focused docking study could actually demonstrate.
- Docking, even when successful, generates hypotheses about binding and structural preference; it does not confirm cellular mechanism, and any proposed computational work here would need wet-lab follow-up (binding assays, reporter assays, co-crystallography) to mean anything.
- Two potentially important sources (Teng et al., 2014; Mijatovic et al., 2013) could not be fully verified in this review and are cited at the abstract level only; their claims should be re-checked against full text before being relied upon.

Conclusion

Cardiac glycosides converge on a single, well-characterized structural target, the Na⁺/K⁺-ATPase in its E2P conformation, and the crystallographic record of how digoxin, digitoxin, bufalin, and ouabain each occupy that pocket is unusually detailed for a natural-product drug class. What happens after that shared binding event, in glioblastoma specifically, is far less settled: the evidence points toward two distinguishable stress responses, an ER-stress/PERK-eIF2 α -CHOP branch and a HIF-1 α suppression branch, each with real but incomplete GBM-specific support, complicated by a protective autophagy response that may make outcome dependent on a stress threshold rather than on which branch is activated, and constrained by real gaps between effective laboratory concentrations and concentrations achievable in patients.

The single most concrete, testable structural hypothesis to emerge from this review is that the small chemical difference between digoxin and digitoxin (a single hydroxyl group at C12, resolved crystallographically by Kanai et al., 2021) may be mechanistically connected to their apparently different downstream behavior in glioma, with digitoxin the pharmacokinetically better-supported candidate for the HIF-1 α branch (Lee et al., 2017). Molecular docking of these compounds and their derivatives against the existing NKA structures (7DDH, 4RET, 4RES), following the precedent set by Pessôa et al. (2018), offers a concrete next step for probing that hypothesis computationally. Any such work should be undertaken with the methodological humility the docking literature itself recommends (Pantsar and Poso, 2018): a way to generate specific, testable predictions about binding-pocket contacts and structural preference, not a substitute for the wet-lab validation, meaning binding assays, reporter assays for CHOP and HIF-1 α , and ultimately cell-based and in vivo testing, that would actually be needed to confirm any of it.

References

1. Berges R, Denicolai E, Tchoghandjian A, Baeza-Kallee N, Honore S, Figarella-Branger D, Braguer D. (2018). Proscillaridin A exerts anti-tumor effects through GSK3 β activation and alteration of microtubule dynamics in glioblastoma. *Cell Death & Disease*, 9, 984. [10.1038/s41419-018-1018-7](https://doi.org/10.1038/s41419-018-1018-7)
2. Bou-Gharios J, Noël G, Burckel H. (2024). Preclinical and clinical advances to overcome hypoxia in glioblastoma multiforme. *Cell Death & Disease*, 15, 503. [10.1038/s41419-024-06904-2](https://doi.org/10.1038/s41419-024-06904-2)
3. Chovancova B, Liskova V, Babula P, Krizanova O. (2020). Role of Sodium/Calcium Exchangers in Tumors. *Biomolecules*, 10(9), 1257. [10.3390/biom10091257](https://doi.org/10.3390/biom10091257)
4. Cui W, Li J, Ron D, Sha B. (2011). The structure of the PERK kinase domain suggests the mechanism for its activation. *Acta Crystallographica Section D*, 67, 423–428. [10.1107/S0907444911006445](https://doi.org/10.1107/S0907444911006445)
5. Kanai R, Cornelius F, Ogawa H, Motoyama K, Vilsen B, Toyoshima C. (2021). Binding of cardiotonic steroids to Na⁺,K⁺-ATPase in the E2P state. *PNAS*, 118(1), e2020438118. [10.1073/pnas.2020438118](https://doi.org/10.1073/pnas.2020438118)
6. Khan I, Baig MH, Mahfooz S, et al. (2021). Deciphering the Role of Autophagy in Treatment of Resistance Mechanisms in Glioblastoma. *International Journal of Molecular Sciences*, 22(3), 1318. [10.3390/ijms22031318](https://doi.org/10.3390/ijms22031318)

7. Kumavath R, Paul S, Pavithran H, et al. (2021). Emergence of Cardiac Glycosides as Potential Drugs: Current and Future Scope for Cancer Therapeutics. *Biomolecules*, 11(9), 1275. [10.3390/biom11091275](https://doi.org/10.3390/biom11091275)
8. Kurzeder C, Nguyen-Sträuli BD, Krol I, et al. (2025). Digoxin for reduction of circulating tumor cell cluster size in metastatic breast cancer: a proof-of-concept trial. *Nature Medicine*, 31(4), 1120–1124. [10.1038/s41591-024-03486-6](https://doi.org/10.1038/s41591-024-03486-6)
9. Lan YL, et al. (2019). The sodium pump $\alpha 1$ subunit regulates bufalin sensitivity of human glioblastoma cells through the p53 signaling pathway. *Cell Biology and Toxicology*, 35, 521–539. [10.1007/s10565-019-09462-y](https://doi.org/10.1007/s10565-019-09462-y)
10. Laursen M, Gregersen JL, Yatime L, Nissen P, Fedosova NU. (2015). Structures and characterization of digoxin- and bufalin-bound Na⁺,K⁺-ATPase compared with the ouabain-bound complex. *PNAS*, 112(6), 1755–1760. [10.1073/pnas.1422997112](https://doi.org/10.1073/pnas.1422997112)
11. Lee DH, Oh SC, Giles AJ, Jung J, Gilbert MR, Park DM. (2017). Cardiac glycosides suppress the maintenance of stemness and malignancy via inhibiting HIF-1 α in human glioma stem cells. *Oncotarget*, 8(25), 40233–40245. [10.18632/oncotarget.16714](https://doi.org/10.18632/oncotarget.16714)
12. Li X, Zheng J, Chen S, et al. (2021). Oleandrin, a cardiac glycoside, induces immunogenic cell death via the PERK/eIF2 α /ATF4/CHOP pathway in breast cancer. *Cell Death & Disease*, 12(4), 314. [10.1038/s41419-021-03605-y](https://doi.org/10.1038/s41419-021-03605-y)
13. LingHu HR, Luo H, Gang L. (2020). Bufalin Induces Glioma Cell Death by Apoptosis or Necroptosis. *OncoTargets and Therapy*, 13, 4767–4778. [10.2147/OTT.S242567](https://doi.org/10.2147/OTT.S242567)
14. Liu K, Tsung K, Attenello FJ. (2020). Characterizing Cell Stress and GRP78 in Glioma to Enhance Tumor Treatment. *Frontiers in Oncology*, 10, 608911. [10.3389/fonc.2020.608911](https://doi.org/10.3389/fonc.2020.608911)
15. Miao Q, et al. (2013). Anticancer Effects of Bufalin on Human Hepatocellular Carcinoma HepG2 Cells. *International Journal of Molecular Sciences*, 14(1), 1370–1382. [10.3390/ijms14011370](https://doi.org/10.3390/ijms14011370)
16. Mijatovic T, et al. (2013). Trial watch: Cardiac glycosides and cancer therapy. *Oncolimmunology*. PMID 23525565. (Abstract-level source; full text not verified for this review.)
17. Pantsar T, Poso A. (2018). Binding Affinity via Docking: Fact and Fiction. *Molecules*, 23(8), 1899. [10.3390/molecules23081899](https://doi.org/10.3390/molecules23081899)
18. Pessôa MTC, Alves SLG, Taranto AG, Villar JAFP, Blanco G, Barbosa LA. (2018). Selectivity analyses of γ -benzylidene digoxin derivatives to different Na,K-ATPase α isoforms: a molecular docking approach. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 33(1), 85–97. [10.1080/14756366.2017.1380637](https://doi.org/10.1080/14756366.2017.1380637)
19. Scranton K, John S, Angelini M, et al. (2025). The mechanism of action of digoxin requires the sodium-dependent inactivation of the sodium-calcium exchanger. *Science Advances*, 11(51). [10.1126/sciadv.ady9596](https://doi.org/10.1126/sciadv.ady9596)
20. Shen S, Zhang Y, Wang Z, Zhang R, Gong X. (2014). Bufalin Induces the Interplay between Apoptosis and Autophagy in Glioma Cells through Endoplasmic Reticulum Stress. *International Journal of Biological Sciences*, 10(2), 212–224. [10.7150/ijbs.8056](https://doi.org/10.7150/ijbs.8056)
21. Škubník J, Svobodová Pavlíčková V, Psotová J, Rimpelová S. (2021). Cardiac Glycosides as Autophagy Modulators. *Cells*, 10(12), 3341. [10.3390/cells10123341](https://doi.org/10.3390/cells10123341)
22. Teng L, Hejazi S, et al. (2014). Systemic anticancer neural stem cells in combination with a cardiac glycoside for glioblastoma therapy. *Stem Cells*, 32(8), 2021–2032. (Abstract-level source; full text not verified for this review.)

23. Tokugawa M, et al. (2021). Periplocin and cardiac glycosides suppress the unfolded protein response. *Scientific Reports*, 11, 9528. [10.1038/s41598-021-89074-x](https://doi.org/10.1038/s41598-021-89074-x)
24. Valluri A, Lawrence L, Denning KL, Cuda J, Zhu GZ. (2022). Cardiac Glycosides Increase Temozolomide Anticancer Activity in Therapy Resistant Glioblastoma Cells. *International Journal of Translational Medicine*, 2(2), 148–155. [10.3390/ijtm2020012](https://doi.org/10.3390/ijtm2020012)
25. Yan M-M, Ni J-D, Song D, Ding M, Huang J. (2015). Interplay between unfolded protein response and autophagy promotes tumor drug resistance. *Oncology Letters*, 10(4), 1959–1969. [10.3892/ol.2015.3508](https://doi.org/10.3892/ol.2015.3508)
26. Ye Q, Zhou X, Ren H, et al. (2023). An overview of the past decade of bufalin in the treatment of refractory and drug-resistant cancers. *Frontiers in Pharmacology*, 14, 1274336. [10.3389/fphar.2023.1274336](https://doi.org/10.3389/fphar.2023.1274336)
27. Zhang H, Qian DZ, Tan YS, et al. (Semenza GL, senior author). (2008). Digoxin and other cardiac glycosides inhibit HIF-1 α synthesis and block tumor growth. *PNAS*, 105(50), 19579–19586. [10.1073/pnas.0809763105](https://doi.org/10.1073/pnas.0809763105)
28. Zhao H, Li Q, Pang J, Jin H, Li H, Yang X. (2017). Blocking autophagy enhances the pro-apoptotic effect of bufalin on human gastric cancer cells through endoplasmic reticulum stress. *Biology Open*, 6(10), 1416–1422. [10.1242/bio.026344](https://doi.org/10.1242/bio.026344)