Molecules and Cells

Minireview

Glyoxalase 1 as a Therapeutic Target in Cancer and Cancer Stem Cells

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Methylglyoxal (MG) is a dicarbonyl compound formed in cells mainly by the spontaneous degradation of the triose phosphate intermediates of glycolysis. MG is a powerful precursor of advanced glycation end products, which lead to strong dicarbonyl and oxidative stress. Although divergent functions of MG have been observed depending on its concentration, MG is considered to be a potential anti-tumor factor due to its cytotoxic effects within the oncologic domain. MG detoxification is carried out by the glyoxalase system. Glyoxalase 1 (Glo1), the ubiquitous glutathione-dependent enzyme responsible for MG degradation, is considered to be a tumor promoting factor due to it catalyzing the removal of cytotoxic MG. Indeed, various cancer types exhibit increased expression and activity of Glo1 that closely correlate with tumor cell growth and metastasis. Furthermore, mounting evidence suggests that Glo1 contributes to cancer stem cell survival. In this review, we discuss the role of Glo1 in the malignant progression of cancer and its possible use as a promising therapeutic target for tumor therapy. We also summarize therapeutic outcomes of Glo1 inhibitors as prospective treatments for the prevention of cancer.

Keywords: cancer, cancer stem cell, glyoxalase 1, methylglyoxal

INTRODUCTION

Cancer metabolism is a process by which cancer cells produce the energy needed to meet the increased demands required for their rapid growth and expansion. This process, known in tumor microenvironments as metabolic reprogramming, maintains the survival and proliferation of cancer cells, and in some cases, drives chemotherapy resistance. This metabolic reprogramming, called the Warburg effect, is a hallmark of cancer cells in which the cells use aerobic glycolysis to convert glucose to lactate, regardless of oxygen availability, to support mitochondrial oxidative phosphorylation (Ferreira, 2010). These changes to cancer cell metabolism in unfavorable environments aberrantly activate growth and survival signals within cells, which accumulate oncogenic alterations to support this disordered and uncontrolled growth.

The glyoxalase system, consisting of glyoxalase 1 (Glo1) and glyoxalase 2 (Glo2), is responsible for the enzymatic metabolism important for homeostatic maintenance through the decomposition of metabolic by-products, such as methylglyoxal (MG). Glo1 is involved mainly in the detoxification of glycolytic MG in a glutathione (GSH)-dependent manner, whereas Glo2 decomposes s-lactoylglutathione to lactate (Sousa Silva et al., 2013). In normal tissue, Glo1 acts as a protective enzyme in anti-glycation defense, since high levels of MG and other endogenous cytotoxic metabolites can induce cellular damage. In the context of cancer progression, cancer

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cells with high Glo1 activity exhibit enhanced growth and proliferation and are associated with multidrug-resistance (MDR) in chemotherapy (Thornalley, 2003; Thornalley and Rabbani, 2011). Conversely, the decrease in Glo1 induces apoptosis due to the high accumulation of MG above the titer. Here, to understand the contribution of Glo1 to tumorigenesis, we review both the role of Glo1 in malignant progression of cancer, as well as its potential to serve as a therapeutic target for cancer cells and cancer stem cells (CSCs). We also summarize the therapeutic outcomes of Glo1 inhibitors for prospective prevention and treatment cancer.

CONTRIBUTION OF GLO1 TO MULTI-DRUG RESISTANCE FOR SURVIVAL OF CANCER CELLS

High expression of Glo1 has been observed in various cancers, including prostate, stomach, breast, ovary, and colon (Cheng et al., 2012; Rulli et al., 2001; Sakamoto et al., 2001). *Glo1* was frequently amplified in 8.4% of human cancers and was more than 3-fold overexpressed in 2.4% of cancers (Santarius et al., 2010). In many cancers, increased expression and activity of Glo1 had a protective effect on cells against the toxicity of anticancer drugs. This protective effect contributed to survival and MDR in more aggressive and invasive cells, and led eventually to chemotherapy failure (Thornalley, 2003). In metastatic prostate cancers, the immunosuppressive microenvironment maintained by overexpression of Glo1 induces the upregulation of 5-hydro-5-methylimidazolone (MG-H1)-mediated immune checkpoint protein, programmed-death ligand 1 (PD-L1), which promotes cancer progression. In addition, the association between Glo1 and PD-L1 was confirmed in the tissues of prostate cancer patients. PD-L1 was highly expressed in most Glo1-positive prostate cancer samples, which closely related to high levels of aggression, invasion, expansion, metastasis, and cancer recurrence (Antognelli et al., 2021).

Furthermore, research has characterized the effect of the Glo1-MG pathway on MDR. Proteomic response analysis in Glo1-overexpressed HEK293 cells showed that MG depletion due to overexpression of Glo1 provided a protective shield for the spliceosome involved in tumorigenesis, thereby providing drug resistance to anticancer agents and eventually allowing cancer growth. In HEK293 cells treated with MG, the MG-induced cytotoxicity specifically depleted proteins involved in RNA splicing and mitochondrial respiratory electron transport chains, suggesting spliceosome-targeted damage and activation of mitochondrial apoptosis. Cell lines of different cancer types also showed increased expression of Glo1 and a positive correlation with the spliceosome for MG modification. These findings suggest that inhibition of Glo1 as part of an anticancer mechanism can impact apoptosis through the increase in steady-state cell concentrations of toxic MG levels (Alhujaily et al., 2021). As such, combining anticancer drugs with Glo1 inhibitors in drug resistant cancers may exert synergistic effects with significant clinical implications for therapeutic outcomes.

ROLE OF GLO1 IN CANCER DEVELOPMENT AND PROGRESSION

Determining the expression patterns of specific molecules in the multiple pathways involved in cancer progression may aid cancer diagnoses and tumor progression predictions. The elevated expression of Glo1 observed in many cancers may represent a cellular response to the increased cytotoxic metabolites associated with the glycolytic adaptation necessary for unhampered tumor growth. Studies have confirmed a positive correlation between Glo1 expression levels and the rate of cell proliferation in aggressive prostate cancers during cancer progression (stages 2 and 3). The Glo1 expression pattern in high-grade prostate intraepithelial neoplasia, a precursor of invasive prostate cancer, makes it an attractive candidate for the identification of precancerous lesions (Doherty et al., 2014; Rounds et al., 2021). Furthermore, overexpression of Glo1 showed a positive correlation with PKCλ expression in breast cancer. Co-expression of Glo1 and PKCλ in late-stage breast cancer functions as a cooperator to promote cancer progression and contributes to poor clinical outcomes (Fonseca-Sanchez et al., 2012; Motomura et al., 2021a).

Induced expression and nuclear translocation of Glo1 is a common feature in oropharyngeal squamous cell carcinoma patients. Nuclear Glo1 accumulation in cancer cells correlates significantly with improved cell survival and is a risk factor for unfavorable patient prognosis (Kreycy et al., 2017). Gastric cancer cell lines overexpressing Glo1 exhibit changes in the energy producing pathway dependent on Glo1 function, which results in broad synergistic effects on tumorigenicity and growth-promoting activities. In addition, several cancer-related genes observed in gastric cancer were shown to markedly improve the growth-promoting activity in the presence of *Glo1* (Hosoda et al., 2015). Together, these studies identify Glo1 as a novel metabolic oncogene that promotes the proliferation, survival, and development of cancer cells via the regulation of glucose metabolism used for energy production.

GLO1 IS INVOLVED IN SURVIVAL MECHANISM OF CANCER CELLS

Glo1 plays a pivotal role as a pro-survival factor to protect cancer cells from apoptosis. Mechanistically, Glo1 controls mitochondrial apoptotic mechanisms by maintaining the amounts of MG that are unavoidably generated during cancer development. Thus, Glo1 impacts survival by evading apoptosis, rather than by directly regulating cancer cell growth and proliferation (Chiavarina et al., 2014; Hu et al., 2014). Decreased Glo1 induces apoptosis and inhibits both migration and invasion in the presence of tumor-necrosis factor related apoptosis inducing ligand in human epidermal squamous carcinoma cell. Glo1 silencing also inhibits the growth of squamous cell carcinoma xenografts in a murine model (Zou et al., 2015). Moreover, in human colorectal cancer cells, the inhibition of Glo1 reduces colony formation, migration, and invasion by both upregulating the expression of STAT1, p53, and Bax, and also decreasing the expression of c-Myc and Bcl-2 (Antognelli et al., 2014; Chen et al., 2017).

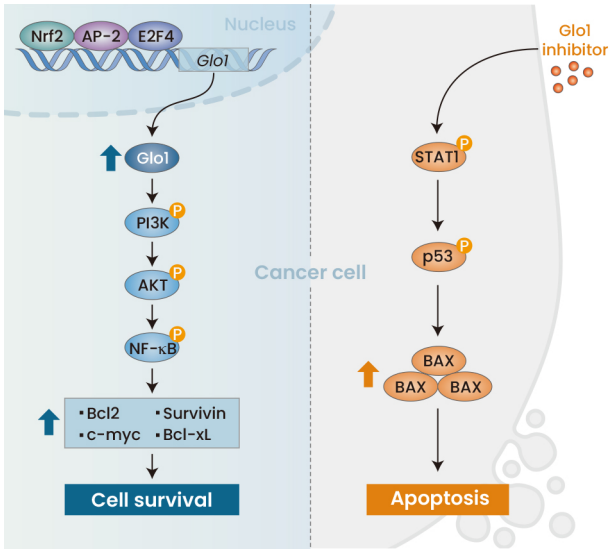

Fig. 1. Schematic representation of survival mechanism of Glo1 in cancer. Increase in Glo1 via activation of *Nrf2*, *AP-2*, and *E2F4* induces phosphorylation of NF-κB, PI3K, and AKT signaling pathways, which therefore increase pro-survival proteins such as Bcl2 and survivin, providing cancer cell survival.

Activation of *AP-2, Nrf2*, and *E2F4* in malignant tumors causes an increase in Glo1 expression, thereby activating NF-κB and PI3K/Akt signaling pathways to improve survival of cancer cells (Xue et al., 2012; Zou et al., 2015). These findings strongly support that Glo1 is an essential factor for the proliferation and survival of cancer cells in various types of malignant tumors that are associated with poor prognosis for patients. Therefore, inhibition of Glo1 activity and its related signaling molecules should be considered an effective therapeutic strategy for the treatment of different types of cancers (Fig. 1).

DUAL ROLE OF MG IN CANCER DEVELOPMENT

MG occurs naturally in the cell as a metabolic byproduct of aerobic glycolysis. MG reacts with arginine and lysine residues to form MG-arginine adducts. Accumulation of these MG-modified proteins relates to aging and various diseases. Although still controversial, MG has been used as a metabolic biomarker of growth for some cancers.

A better understanding of the effects of different MG concentrations on cellular functions is required to make progress toward treatment strategies utilizing MG. Initial studies that focused on the anti-proliferative and cytotoxic effects of MG in cancer showed that the accumulation of toxic MG induced by inhibition of *Glo1* decreases DNA replication, protein synthesis, and mitochondrial respiration (Hosoda et al., 2015; Taniguchi et al., 2012). Low millimolar concentrations of MG induce apoptosis in cancer cells through various pathways. In malignant proliferating PC3 cells, exposure to 3 mM MG induces apoptosis by blocking cell cycle progression and partially degrading poly (ADP-ribose) polymerase (Milanesa et al., 2000). In addition, exposure of MG to colon and lung cancer cells contributes to cellular apoptosis through regulation of the MAPK family (p-ERK, p-JUN, and p-p38) and apoptosis-related genes (Guo et al., 2016; Shimada et al., 2018). Interestingly, in hepatocellular carcinoma, low concentrations (1 μM) of MG caused a decrease in cellular migration, invasion, and adhesion, without affecting viability, in a p53-dependent manner (Loarca et al., 2013). Together, these findings suggest that low concentrations of MG may be a potential apoptosis inducer in malignant proliferating cells.

In addition to anticancer activity, there has also been accumulating evidence that MG promotes cancer progression. In cancer cells with exacerbated glycolytic activity, MG induces post-translational glycation of heat shock proteins (HSPs). These modified HSPs are implicated in cell proliferation, invasion, and metastasis (Ciocca and Calderwood, 2005; Sakamoto et al., 2002). RNA sequencing analysis of the gene expression profile of Glo1-depleted breast cancer cells revealed that the MEK/ERK pathway, through activated SMAD1, is an MG-induced pro-metastatic signature (Nokin et al., 2016; 2019). High endogenous MG induces post-translational glycation of HSP90, which suppresses the expression of large tumor suppressor 1 (LATS1), which negatively regulates Yes-associated protein (YAP). As a result, increased nuclear persistence of YAP upregulates the expression of several YAP target genes (Park et al., 2020), many of which promote cell growth and proliferation of breast cancer cells. In a mouse xenograft model, Glo1-depleted breast cancers also showed increased tumorigenic and metastatic potential, suggesting that increased MG caused by the loss of Glo1 has pro-cancer effects on cancer development (Nokin et al., 2016; Zhang et al., 2018). Similar effects have also been reported in colorectal cancer patients where increased aggressiveness of cancer cells positively correlates with the accumulation of high levels of MG-adducts and inversely correlates with Glo1 activity (Chiavarina et al., 2017). According to the type of cancer, it is suggested that the adaptation of cancer cells to MG stress favorably affects growth and survival, and eventually leads to apoptosis resistance. Therefore, the level of MG-adducts could have the potential serve both as a useful marker for diagnostic applications to monitor cancer progression, but also as an anticancer adjuvant in the future.

THE ROLE OF GLO1 IN CSCs

CSCs are a quiescent subpopulation of cancer cells that have tumorigenic potential (Koh et al., 2020; Reya et al., 2001; Visvader and Lindeman, 2012). They share many characteristics with normal stem and progenitor cells in terms of capacity for self-renewal, multipotency and cell surface markers (Walcher et al., 2020). During differentiation and development of stem cells, not only growth factors and cytokines but also metabolic state is important factor. Thus, metabolic change in CSCs are also expected to consistently affect tumorigenesis. The disruption of Glo1 and an increased MG in human induced pluripotent stem cells exacerbates vulnerability to oxidative stress, which leads to reduction of neuronal expansion and movement via mitochondrial dysfunction and MG-advanced glycation end product (AGE) accumulation in neurons (Hara

et al., 2021; Yang et al., 2016). Therefore, Glo1 regulates neural development through metabolic state, suggesting that continuous changes due to obstruction of Glo1-MG pathway may cause neurologic disorder. Upregulated Glo1 in bone marrow cells reduces oxidative stress and increases the expression of angiogenic factors such as VEGF and HIF1-α, to protect damaged endothelial cells and improve angiogenesis (Vulesevic et al., 2014; Zhang et al., 2021). In addition, the persistent oxidative stress instigates differentiation of CSCs into tumor endothelial cells (Movahed et al., 2021). These findings indicate that alteration of Glo1-MG pathway in CSCs may affect angiogenesis for cancer cell metastasis.

CSCs play an essential role in cancer progression from the onset, and are known to influence MDR and relapse. Since CSCs were first identified in leukemia, numerous studies have focused on CSCs as therapeutic targets of cancer-specific treatments (Bonnet and Dick, 1997; Lapidot et al., 1994). As described above, Glo1 contributes to homeostasis and cell survival through the detoxification of MG. Although the interactions and molecular mechanisms between CSCs and Glo1 are poorly defined, studies have reported that Glo1 is essential for the growth and survival of CSCs. In leukemia, CSCs are identified by expression of Bcr-Abl and have strong resistance to hypoxic conditions (Takeuchi et al., 2010). Bcr-Abl-positive leukemia cancer cells exhibit higher expression levels of Glo1 and other stem cell markers in hypoxic conditions. The viability of CSCs was significantly reduced by a Glo1 inhibitor and the levels of apoptosis increased. In breast cancer, CSCs are identified by expression of $CD44^{hi}/CD24^{-/low}$, but especially by aldehyde dehydrogenase 1 (ALDH1) (Ricardo et al., 2011). Basal-like breast cancers, especially grade 3 tumors, exhibit significantly higher Glo1 expression levels compared with other breast cancer subtypes. Grade 3 tumors in Basal-like breast cancer are characterized as undifferentiated and aggressive, and highly express both ALDH1 and Glo1 (Elston and Ellis, 1991). Interestingly, there are no differences in Glo1 expression levels between $ALDH1^{high}$ and $ALDH1^{low}$ Basal-like cancer cells, but $ALDH1^{high}$ cells have higher Glo1 activity than $ALDH1^{low}$ cells. In $ALDH1^{high}$ cells, Glo1 inhibition using TLSC702 reduced viability, induced apoptosis of cancer cells and decreased tumor-sphere formation. In addition, Glo1 knockdown using siRNA significantly suppressed viability and tumor-sphere formation (Motomura et al., 2021b; Tamori et al., 2018). Together, these studies suggest that Glo1 is important to the survival of CSCs and is a potential therapeutic target for cancer therapy. The specific interaction between CSCs and Glo1 remains unclear and further investigation in various cancer types is needed.

APPLICATION OF GLO1 INHIBITORS FOR CANCER CHEMOTHERAPY

The two main therapeutic applications of Glo1 inhibitors are adjuvant therapy for cancers with high Glo1 expression, and cancer chemotherapy against Glo1-related MDR (Table 1). Prototype S-p-bromobenzylglutathione cyclopentyl diester (BBGC), a Glo1 inhibitor with high cell permeability, was first proposed by Vince and Wadd in 1969. Studies have showed that inhibition of Glo1 in human leukemia 60 cells inhibited

Table 1. Effects of BBGC (Glo1 inhibitor) in different types of cancer

Cancer type	Concentration	Effect	Mechanisms	Reference
Myeloid leukemia cell line (HL-60)	GC_{50} 10 μM	Induction of apoptosis	Increase the frequency of DNA strand breaks	Thornalley et al., 1996; 2010
NCI-H460, NCI-H226, A549, NCI-H23, DMS273, DMS114, NCI-H522	GC_{50} 4.4-29.7 μM	Induction of apoptosis	Activation of caspase and stress-activated protein kinase (JNK, p38)	Sakamoto et al., 2001
Human leukemia cell lines (UK711, UK110)	12.5 μmol/L	Induction of apoptosis	Activation of caspase 3	Sakamoto et al., 2000
Human hepatocellular carcinoma cell lines (HepG2, Huh7)	1-10 μM	Reduction of proliferation and migration	Decrease of PDGFR-β, VEGFR2, VEGF, pERK/ERK, *NF-κB* Increase of *Nrf2*	Michel et al., 2019
Glioblastoma multiforma	Human glioblastoma multiforma tumor (T98): GC_{50} 100.6 μM; malignant glioma (U87): 9.9 μM	Induction of apoptosis	Increase of CEdG and RAGE	Jandial et al., 2018
MG53, NCI-H522, YAPC, LB771, A549, NCI-H460, CCF-STTG-1, SW1710	GC_{50}: MG53, 3.8 μM; NCI-H522, 7 μM; YAPC, 10 μM; LB771, 9.5 μM; A549, 23.5 μM; NCI-H460, 19.8 μM; CCF-STTG-1, 17.5 μM; SW1710, 15 μM	Reduction of proliferation and increase apoptosis	Not studied	Santarius et al., 2010
Oropharyngeal squamous cell carcinoma (FaDu and Cal27)	GC_{50} 1-5 μM	Increase of Glo1 nuclear localization Decrease in cell survival	Not studied	Kreycy et al., 2017

CEdG, DNA-AGE N^2-1-(carboxyethyl)-2′-deoxyguanosine.

growth by cellular accumulation of MG. In addition, BBGC has an anticancer effect that inhibits cancer growth by inducing apoptosis through p38 and JNK pathway in a mouse model with xenografts of DMS114 lung cancer and DU145 prostate cancer (Sakamoto et al., 2000; Thornalley et al., 1996) (Fig. 2).

Several studies of drug-resistant cancers have investigated if a combination treatment of a Glo1 inhibitor with an anticancer agent can improve the sensitivity of cancer cells to a drug, compared to treatment with a Glo1 inhibitor alone. In human hepatocellular carcinoma with high Glo1 expression, the combination of ethyl pyruvate (EP), a Glo1 inhibitor, with sorafenib reduced cancer cell proliferation to a higher degree than EP alone. Importantly, Glo1 inhibition caused the MG levels to reach antiproliferative and cytotoxic levels that induced apoptosis via PDGFR, VEGFR2, and pERK pathways (Michel et al., 2019). In this context, inhibition of Glo1 could be used as a targeted therapy in cancer that upregulates glycolysis, and can explain how Glo1 inhibition induces apoptosis by increasing intracellular MG. A related study on human glioblastoma multiforme (GBM), one of the most aggressive types of cancer that begins in the brain, and the AGE pathway, an immunoglobulin-like receptor for AGEs, found that Glo1 is mainly expressed in the intracellular cytoplasm in tissues of GBM patients, whereas receptor for AGE (RAGE) accumulates in the cytoplasm and nucleus/perinuclear region. The inhibition of Glo1 using BBGC induced apoptosis by increasing the expression of both DNA-AGE N2-1-(carboxyethyl-2'-deoxyguanosine) and RAGE in cells (Jandial et al., 2018) (Fig. 2).

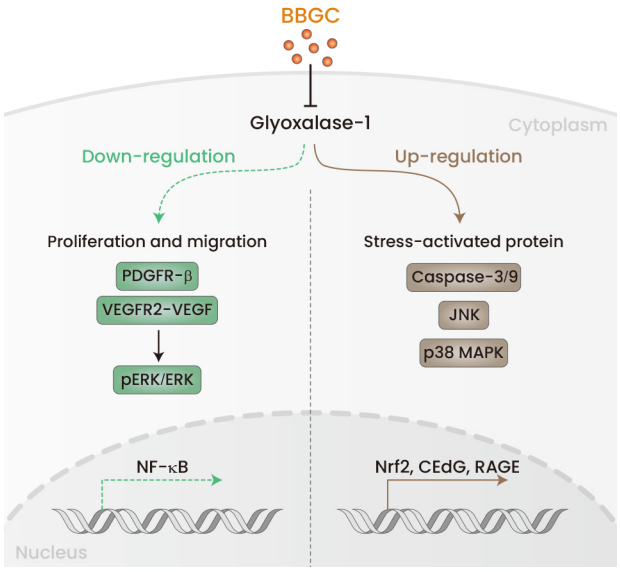

Fig. 2. Schematic representation of mechanism by which Glo1 inhibitor (BBGC) induces apoptosis in human cancer cell lines. In cancer cells, Glo1 inhibitor suppresses the expression of major molecules pathways and transcription factors involved in proliferation and migration, while increasing the expression of stress-activated proteins, eventually inducing apoptosis. The blue and red arrows indicate down-regulation and up-regulation, respectively.

In addition to BBGC, several other drugs were used as Glo1 inhibitors, such as carmucine (BCNU), curcumin, shikonin, and TLSC702 (Table 2). These putative Glo1 inhibitors regulate the proliferation and growth of cancer cells by inducing accumulation of MG and arresting the cell cycle (Finkelstein et al., 2002; Santel et al., 2008; Takasawa et al., 2016; Zhang et al., 2019). Curcumin is found in the plants of the *Curcuma longa* species and inhibits cancer development through accumulation of MG in various cancer types, including breast and prostate cancers. TLSC702 leads to accumulation of argpyrimidine, which is another adduct produced in glycolysis that promotes cancer cell apoptosis. Shikonin and BCNU have different molecular mechanisms used in anticancer therapy. Shikonin induces ROS (reactive oxygen species) production and cell cycle arrest, whereas BCNU enhances the cytotoxicity of cancer cells. Although they have various strategies to suppress the progression of cancer cells, they all induce apoptosis.

CONCLUSION

The importance of the Glo1 system for cancer treatment strategies and CSC inhibition has been demonstrated through early studies on cancer metabolism and the cytotoxic activity of MG. Cancers with high fluxes of MG formation and Glo1 expression are very sensitive to Glo1 inhibitor therapy. Despite the effective anticancer activity of BBGC, the most potent Glo1 inhibitor, on cancers with high Glo1 expression and resistance to anticancer drugs, it has not been used in clinical practice. Although it provides high permeability, it is rapidly

Table 2. Effects of other types of Glo1 inhibitors in different types of cancers

Compound	Cancer type	Concentration	Effect	Mechanisms	Reference
Carmustine	Prostate cancer cells (PC3)	0-300 μM	Enhances cytotoxicity	Not studied	Finkelstein et al., 2002
Curcumin	PC-3, JIM-1, MDA-MD 231, 1321N1	GC_{50} 7.9 μM	Inhibition of proliferation and growth	Induces of accumulation of MGO Decrease of cellular ATP	Santel et al., 2008
Shikonin	Myelogenous leukaemia cell line (K562), breast cancer cell line (MCF-7)	5-25 μM	Induces ROS production	Hyper-phosphorylation of CDKS Cell cycle arrest	Zhang et al., 2019
TLSC702	HL60, NCI-H522, NCI-H460	GC_{50} 0-1,000 μM	Induction of apoptosis	Leads to accumulation of Argpyrimidine adducts	Takasawa et al., 2016

cleared from cells and requires high doses to achieve a strong therapeutic effect. If the barriers surrounding Glo1 inhibitors can be overcome, they could be an effective treatment for refractory cancers with high Glo1 expression.

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AUTHOR CONTRIBUTIONS

S.S.H. and S.H.H. supervised the overall process and wrote the manuscript. J.Y.K., J.H.J., and S.J.L. designed the figure and tables. All authors discussed the related studies, drafted and contributed to the final manuscript.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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