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Review

Glutathione Depletion and Stalwart Anticancer Activity of Metallotherapeutics Inducing Programmed Cell Death: Opening a New Window for Cancer Therapy

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Cite This: *ACS Omega* 2024, 9, 20670−20701

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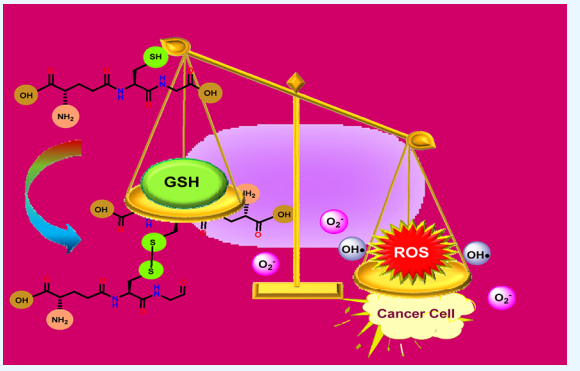
ABSTRACT: The cellular defense system against exogenous substances makes therapeutics inefficient as intracellular glutathione (GSH) exhibits an astounding antioxidant activity in scavenging reactive oxygen species (ROS) or reactive nitrogen species (RNS) or other free radicals produced by the therapeutics. In the cancer cell microenvironment, the intracellular GSH level becomes exceptionally high to fight against oxidative stress created by the production of ROS/RNS or any free radicals, which are the byproducts of intracellular redox reactions or cellular respiration processes. Thus, in order to maintain redox homeostasis for survival of cancer cells and their rapid proliferation, the GSH level starts to escalate. In this circumstance, the administration of anticancer therapeutics is in vain, as the elevated GSH level reduces their potential by reduction or by scavenging the ROS/RNS they produce. Therefore, in order to augment the therapeutic potential of anticancer agents against elevated GSH condition, the GSH level must be depleted by hook or by crook. Hence, this Review aims to compile precisely the role of GSH in cancer cells, the importance of its depletion for cancer therapy and examples of anticancer activity of a few selected metal complexes which are able to trigger cancer cell death by depleting the GSH level.



1. INTRODUCTION

Humans are at high risk of cancer as a result of multiple mutations in genes.[1,2] Cancer morbidity is expected to increase substantially in the imminent decades, as we are still unable to arrest the reckless and rapid proliferation of cancer cells following programmed cell death.[3] To combat this life-snatching disease, scientists are becoming more concerned and ceaseless attention is being paid to find fruitful treatment modalities with the rapid development of appropriate anticancer therapeutics. The distinctive tumor microenvironment (TME), unveiling a more acidic condition (low pH), hypoxia, overexpression of glutathione (GSH), elevation of certain enzymes as a consequence of their uncontrolled proliferation and rapid metabolism,[4] has become a topic of interest to modify the anticancer mechanisms of drugs so that the drugs can compel the cancer cells to undergo programmed cell death (apoptosis). In the TME, GSH (L-γ-glutamyl-L-cysteinyl-glycine), which consists of cysteine, glutamic acid and glycine, is a very significant natural tripeptide, non-protein thiol in that it establishes a cellular antioxidative defense system and plays a pivotal role for tumor initiation, progression and metastasis.[5−11] Actually, an antioxidative system (AOS) is required for cells to preserve reduction−oxidation (redox) homeostasis and carry out normal physiological activities to shield against the introduction of xenobiotics such as pollutants, toxins and drugs.[12,13] It has been seen that increased levels of GSH detoxify the excessive reactive oxygen species (ROS) or reactive nitrogen species (RNS) and other free radicals produced by the anticancer therapeutics.[14] Thereby, GSH deteriorates the chemotherapeutic efficiency of the drugs.[15] Consequently, in order to survive against the treatment with drugs, cancer cells acquire the ability to modulate the redox homeostasis and prevent themselves from undergoing apoptosis if the drugs are incapable of depleting the GSH.[16] A considerable loss of GSH is an indication of regulating the redox signaling events, which trigger the cell death and progression.[17] Plenty of efforts have, therefore, been made over the past few decades to augment the therapeutic efficacy of anticancer drugs by depleting intracellular GSH, as GSH depletion has been validated to recuperate the therapeutic efficacy of ROS-based therapies, viz. photodynamic

Received: November 8, 2023
Revised: March 22, 2024
Accepted: April 5, 2024
Published: April 16, 2024

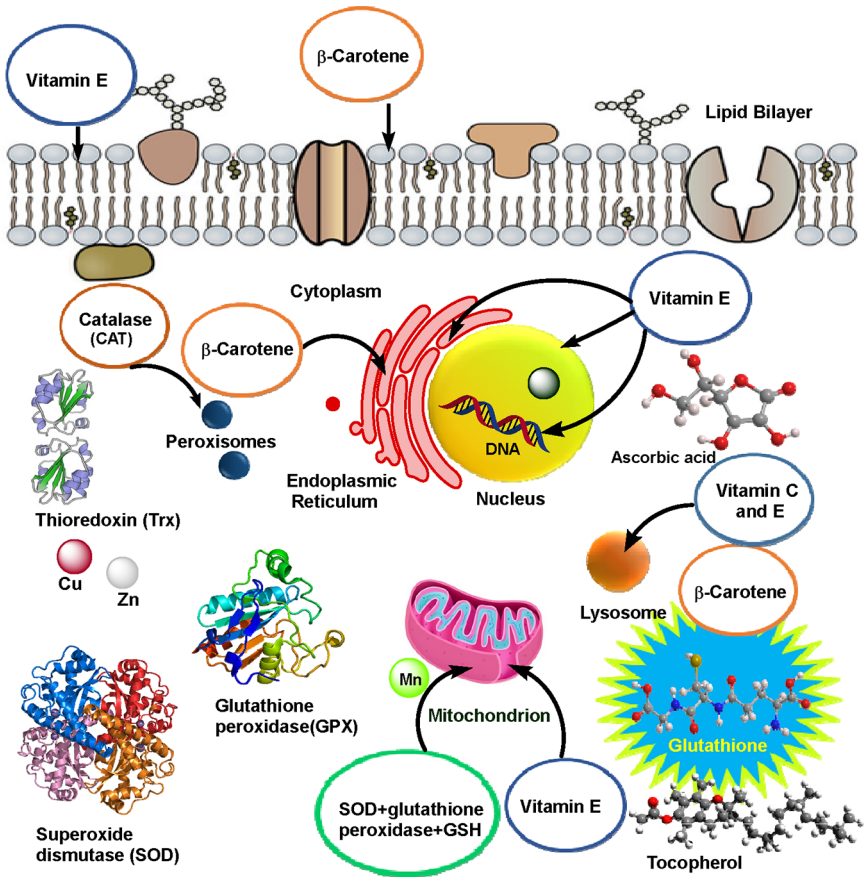


Figure 1. Intracellular enzymatic antioxidative system (AOS).

therapy (PDT), sonodynamic therapy (SDT), chemodynamic therapy (CDT), ferroptosis and chemotherapy.[18−20] Hence, successful cancer therapy encounters great challenges.

The AOS inside the cell comprises enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and thioredoxin (Trx) and non-enzymatic antioxidants including GSH, ascorbic acid, β-carotene and tocopherol. In AOSs, GSH is revealed as the predominant antioxidant among all (Figure 1).[21] ROS are unstable and highly reactive oxygen species with a single electron and an unpaired electron positioned in the outermost orbital. ROS including superoxide anions ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) are produced by endothelial cells, inflammatory cells and the mitochondrial electron transport system. The production of ROS radicals begins with the one-electron reduction conversion of oxygen (O_2) to $O_2^{\bullet-}$, which is converted in turn to H_2O_2 under the influence of the enzyme SOD. These two ROS then form the basis for the generation of further ROS and even more dangerous radicals.[22]

The intracellular redox system remains in a dynamic equilibrium, maintaining a balance between ROS generation and elimination. However, impairment of the GSH level will destroy the redox homeostasis as the ROS elimination will be hindered, which results in copious ROS accumulation in the cells.[23,24] Therefore, an extremely high escalation of the ROS level in cells causes an imbalance between ROS production and antioxidant defenses. In this circumstance, cells suffer from oxidative stress, which triggers a cascade of cellular damage through the destruction of deoxyribonucleic acid (DNA), peroxidation of lipids and modification of proteins, leading to cell death.[25] For that reason, the extent to which the AOS is capable of scavenging ROS is upregulated to prevent oxidative stress-induced cellular damage. The intracellular concentration of GSH is found to be around 2−10 mM, whereas in the extracellular environment it is around 2−10 μM.[26,27] It has been observed that most of the intracellular GSH exists in the reduced form, which is responsible for reducing the oxidizing substances like ROS and itself is oxidized to glutathione disulfide (GSSG). In turn, GSSG can again be reduced back to GSH with the help of nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione reductase (GR), maintaining a redox cycle (GSH ↔ GSSG) (Figure 2).[28]

The high level of oxidative stress makes the cells more vulnerable to GSH shortage, creating a lethal weakness. Therefore, tricks to achieve GSH depletion and thereby increase oxidative stress can be successfully applied in cancer therapy.[29] ROS-based therapy is considered as a therapeutic strategy to annihilate cancer cells by amplifying intracellular ROS levels along with the simultaneous depletion of intracellular GSH.[30] Therefore, exogenous compounds or entities (anticancer drugs) which produce considerable ROS can trigger cell death under light irradiation, ultrasound or chemical reaction after being delivered to cancer tissues.[31] Although ROS-based therapies have been applied in clinical treatments, there are still some barriers that obstruct its therapeutic efficacy. One of the main obstructions is ROS scavenging by cellular antioxidative ROS scavengers, specifically GSH.[32,33] Decreasing the GSH levels in tumor cells has, therefore, made it possible to increase the ROS levels and

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ACS Omega 2024, 9, 20670−20701

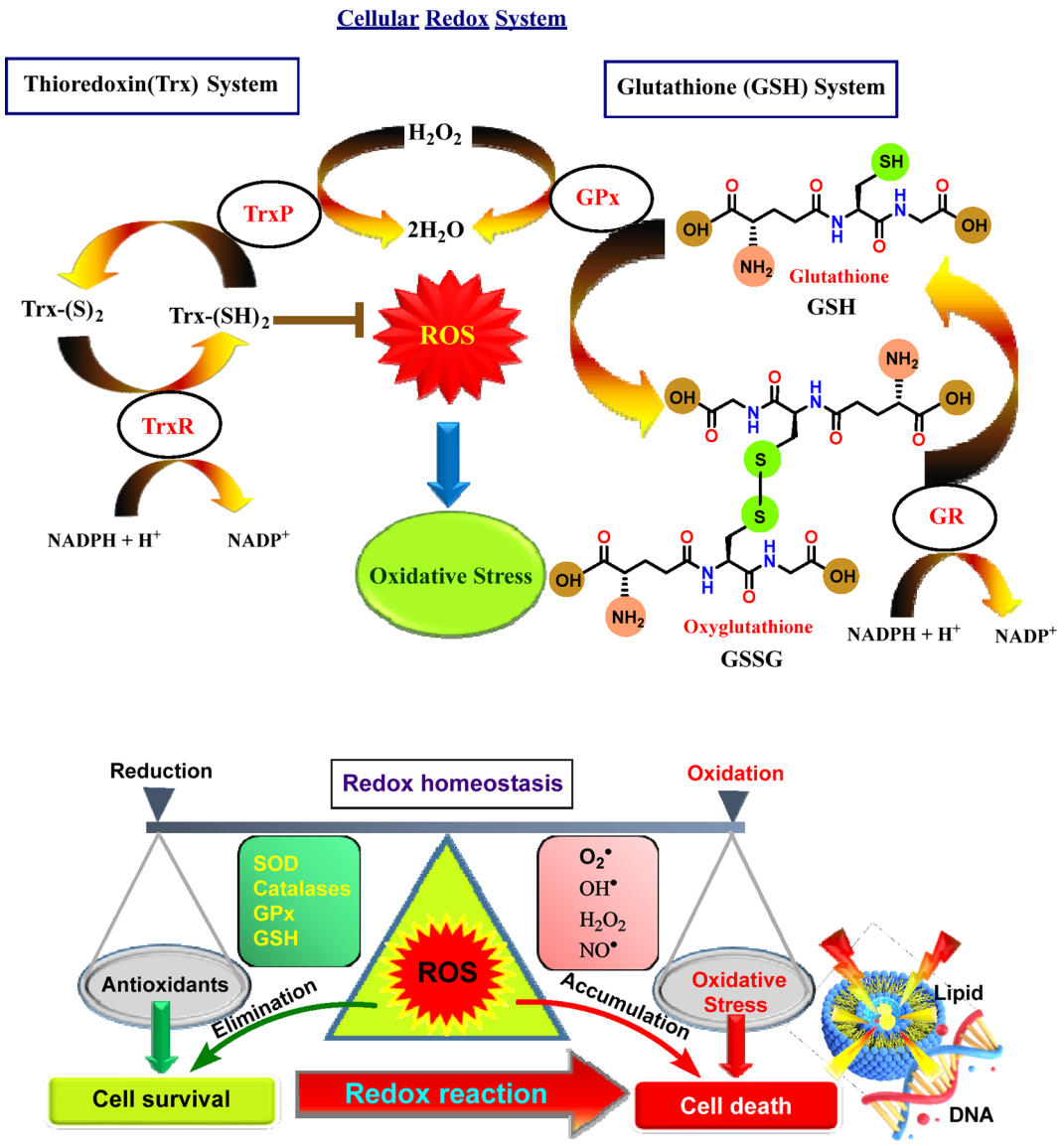

Figure 2. Intracellular redox system.

reinstate the therapeutic efficacy of anticancer scaffolds for ROS-based cancer therapy.[34,35]

In order to elucidate the intimate relationship between GSH and cancers clearly, most of the requirements in clinical treatments centralize on the cancer. Therefore, GSH depletion can be assumed to be the key strategy to amplify the oxidative stress in cancer cells, enhancing the destruction of cancer cells by fruitful cancer therapy. To amplify the therapeutic potential of ROS-based therapy, the concentration of ROS scavenging substances should be diminished. ROS-based therapies include PDT, CDT and SDT. In PDT, photosensitizers are employed to liberate the cytotoxic singlet oxygen under light irradiation after accumulation of photosensitizers in the tumor tissues. Then tumor cell death is initiated through profuse ROS generation.[36] CDT is based on the *in situ* Fenton or Fenton-like reaction, in which H_2O_2 in TME reacts with exogenous catalysts and generates $OH^{\bullet}$.[37] SDT utilizes sonosensitizers to convert O_2 into ROS under ultrasound activation. Due to the high penetrability of ultrasound, SDT is superior in the treatment of deeper tumors compared with light-activated therapy.[38] The thing in common for ROS-based PDT and SDT is the generation of large amounts of ROS in tumor tissues, which initiates oxidative stress and induces cell death. Considering this mechanism, ROS-based therapy usually does not cause tumor resistance as compared with other traditional therapies like photothermal therapy and chemotherapy. However, the efficacy of ROS-based therapy is limited by ROS scavenging, which is mediated by high levels of intracellular GSH.[39] Therefore, this Review aims to delineate the significance of GSH depletion in cancer therapy for enhancing the therapeutic efficacy of anticancer metallodrugs having the capability of both GSH depletion and ROS generation.[40]

2. CANCER CELLS AND THE ROLE OF GSH

Exogenous mutagens like ultraviolet radiation and various chemical entities or endogenous mutagens such as reactive byproducts of enzymatic degradation and cellular metabolism initiate the undaunted mutation of genes, which stimulates the hasty and unabated proliferation of abnormal cells commencing cancer in the body.[41] Therefore, an adequate supply of nutrition, which is essential to fuel up the survival and

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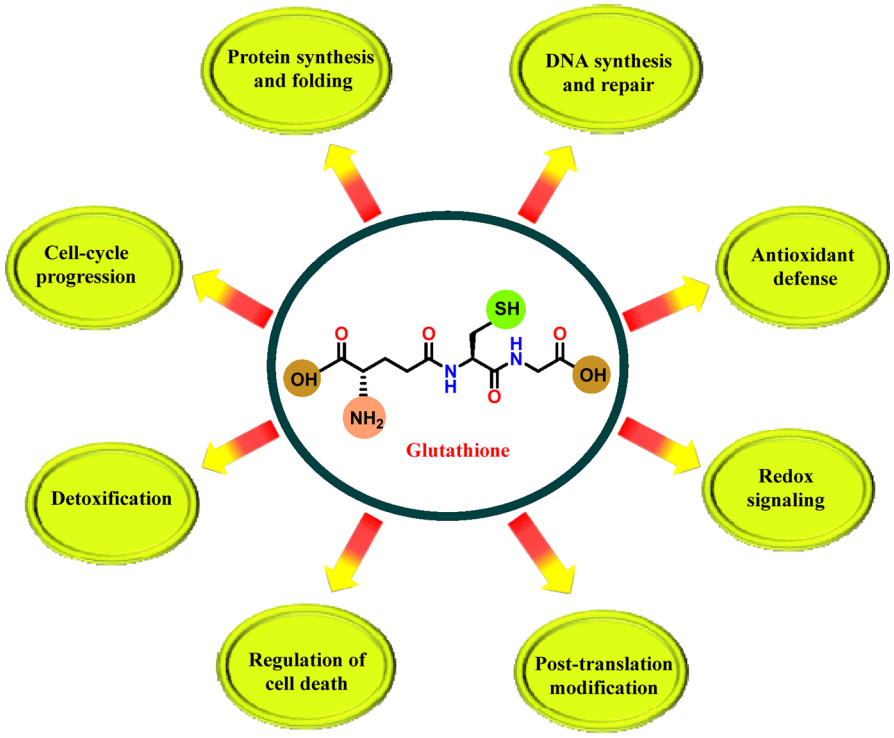

Figure 3. Representation of biological roles of GSH.

incessant proliferation of cancer cells under typical physiological circumstances, involves several intracellular redox reactions to liberate energy and reactive chemical species as byproducts.[42] Inevitably, an instantaneous strong antioxidant defense system is established to conserve intracellular redox homeostasis and to prevent cellular demolition by engulfing the reactive chemical species.[43,44] As a consequence, cancer cells are affluent with high antioxidant levels, especially with GSH, whose appearance at an elevated concentration of ∼10 mM (10 times less in normal cells) detoxifies the cancer cells.[45] Therefore, the TME acquires a highly reductive nature due to overexpression of reduced GSH to keep away the DNA damage and disruption of protein homeostasis, rescuing the tumor cells from programmed cell death.[46] Briefly, GSH performs several pleiotropic roles owing to its capability to keep up the intracellular proteins (e.g., antioxidant molecules, metabolic enzymes, and transcription factors) in its reduced state, ensuring cell metabolism and cell survival (Figure 3).[47] GSH can engulf free radicals and ROS directly or enzymatically.[48] In the enzymatic pathway, GSH brings about the reduction of H_2O_2 into water and lipid peroxide into lipid alcohol being employed as a co-substrate of GPX.[49] Moreover, GSH detoxifies the other electron-deficient xenobiotics (X) through direct conjugation with them, forming GS-X adducts upon catalytic action of glutathione S-transferase (GST),[50,51] and then cells thrust them out with the help of the multidrug resistance-associated protein 1 (MRP1) efflux pump.[52] Hence, it is obvious that elevation of GSH level is a common phenomenon for all types of cancer.[53] Moreover, the upregulated GSH plays a crucial role in cancer initiation, progression and metastasis as well as in drug resistance.[54] At the initiation stage of cancer, intracellular GSH shelters the cells from facing ruinous carcinogenesis upon detoxification of carcinogens and thereby impedes ROS-prompted DNA oxidation followed by DNA damage.[55−57] At the stage of progression, profuse production of ROS in cancer cells assists their hurried metabolism and aberrant proliferation.[58] In this stage, the GSH level becomes very high to wipe out the harmful ROS and interrupts the apoptosis of cancer cells by adjusting the anti-apoptotic Bcl-2 family proteins and triggering the caspase activity.[59] In malignant tumors, GSH also plays a pivotal role in the metastasis of cancer cells. Synthesis of GSH in the mitochondrial matrix is facilitated by the action of mitochondrial transporter family members, SLC25A22 and SLC25, stimulating the admission of glutamate to the mitochondrial matrix. However, the tumor necrosis factor α (TNF-α) and matrix metallopeptidase-dependent cancer metastasis show a descending trend in SLC25A22-negative cancer cells, ensuring the obstruction of GSH synthesis.[60] Lastly, the escalation of GSH level triggers the drug resistance property of tumor cells, as GSH is ready to take part in GST-catalyzed cleansing of the toxic heterogeneous substances followed by subsequent removal of the adduct from the cancer cells through MRP efflux pumps.[61] In this way, the affluence of GSH in cancer cells builds a barricade against numerous therapeutic approaches. In fact, successful anticancer drug development should urgently find a route for designing a molecule that is boosted with both GSH-depleting and ROS-generating efficiency.

3. BIOSYNTHESIS, TRANSPORTATION AND METABOLISM OF GSH

In order to gain a profound insight into the intracellular GSH depletion and its importance in cancer therapy, we should have a transparent knowledge of synthesis, transportation and metabolism of GSH in the cellular medium. GSH, which is an essential protagonist for scavenging intracellular ROS and other free radicals to prevent cells from dying, is a ubiquitous linear tripeptide natural molecule. Its skeleton is comprised of three important amino acids, glutamate (Glu), cysteine (Cys)

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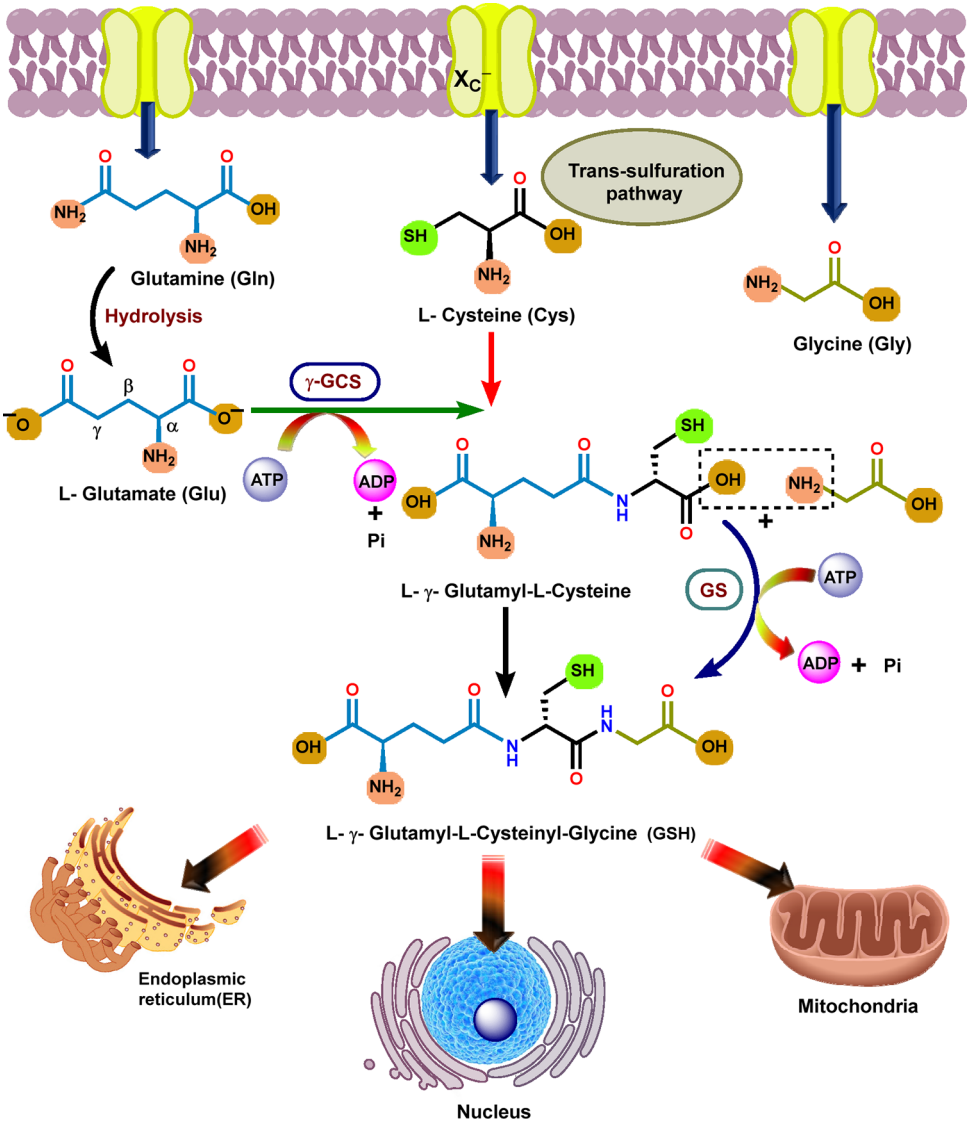

Figure 4. Mechanism of de novo glutathione synthesis and compartmentalization in different organelles.

and glycine (Gly), that undergo enzyme-catalyzed reactions in the cytoplasm at the expense of two adenosine triphosphate (ATP) molecules.[62] After cytosolic synthesis, about 85−90% of the GSH is freely disseminated in the cytosol, and then it is compartmentalized (15%) in various organelles, including the nuclear matrix, mitochondria, endoplasmic reticulum (ER) and peroxisomes.[63−66] Thus, the intracellular GSH concentration remains at 1 mM to 10 mM depending on the cell type. However, its concentration in blood plasma is relatively very low (∼0.01 mM) owing to its rapid catabolism. Both cytosolic and compartmentalized GSH have been shown to take part in discrete biological processes. Above all, the intracellular GSH concentration relies on a dynamic equilibrium between its synthesis, its transport and its rate of metabolism.[67]

The synthesis of GSH begins with the construction of a dipeptide, γ-glutamylcysteine, by the conjunction of Glu and Cys under catalytic action of γ-glutamylcysteine synthetase (γ-GCS), which is also recognized as γ-glutamylcysteine ligase (γ-GCL). In this step, the condensation of the γ-carboxyl group of Glu with the amino group of Cys forms a strong γ-peptidic linkage that protects GSH from hydrolysis, which may occur by the action of intracellular peptidases.[68] At first, glutamine (Gln) is transported into cells with the assistance of numerous transmembrane amino acid (aa) transporters.[69] Then, Gln predominantly undergoes hydrolysis to form Glu in the cytoplasm. On the other hand, the reduction of cysteine dimer (cystine) liberates Cys in the extracellular medium, and then Cys enters into cells by the system X_c^-, which is a vital system for governing the intracellular Cys accumulation.[70] It is very interesting to mention that one Glu molecule is transported out of the cells in exchange for each cystine molecule during the transportation process. Furthermore, Cys can directly cross the cell membrane by dint of an aa transporter.[71] After the initial step, Gly, which is transported to the cytoplasm by glycine transporter (GlyT), is connected to γ-glutamylcysteine by the catalytic activity of an enzyme, glutathione sythetase (GS).

Two enzymatic steps are involved in the consumption of one molecule of ATP for each catalytic cycle, where ATP breaks down into adenosine monophosphate (ADP) and Pi, providing energy for the transportation of amino acids and the synthesis of GSH.[72] The existence of the free C-terminal Gly in the GSH skeleton defends it against the action of intracellular γ-glutamylcyclotransferases (γ-GCTs).[73−75] It can be highlighted

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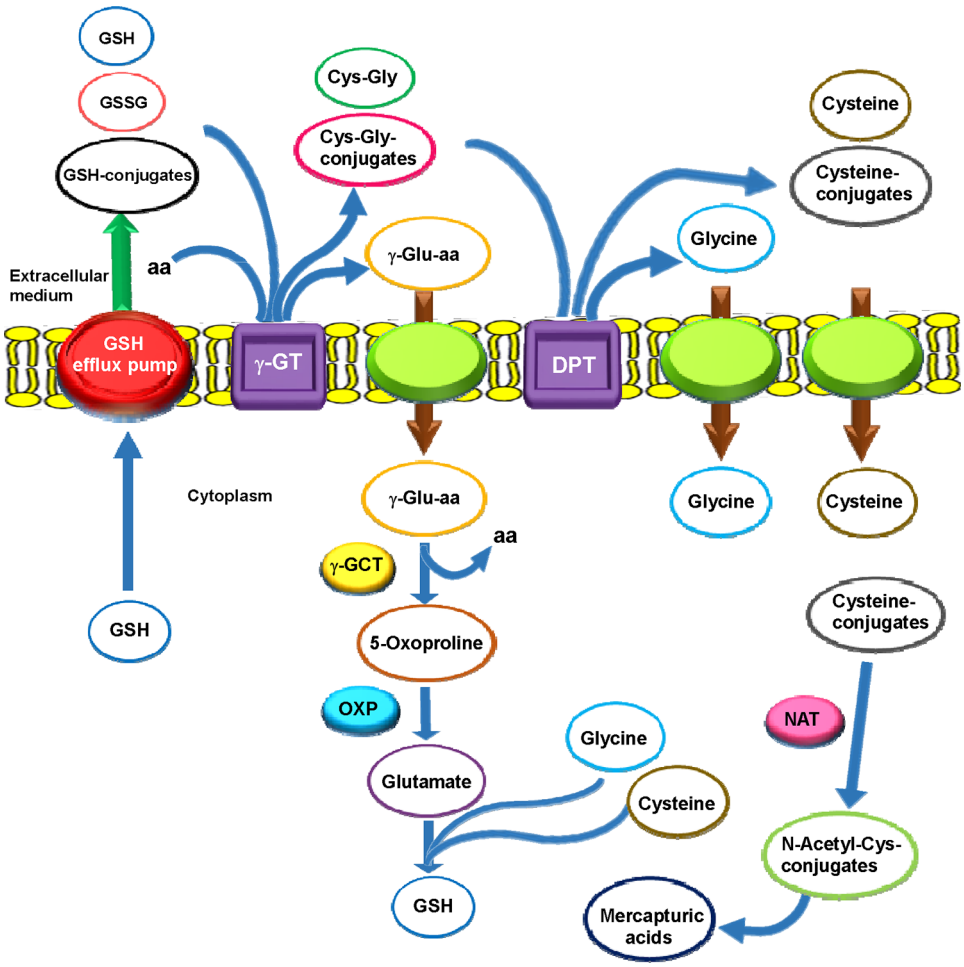

Figure 5. Synthesis of glutathione through the salvage mechanism.

that synthesis of GSH is governed by three factors—raw materials, enzymes and energy—and scarcity of any of these three factors can limit the GSH synthesis. Therefore, it is very important to supply profuse amounts of amino acids in the cytoplasm for the GSH synthesis. Usually, the shortage of Cys in the cellular medium brings about most of the dysfunction of GSH synthesis. In this regard, the role of system X_c^- (Cys transporter) is very crucial in limiting Cys supply and obstructing GSH synthesis. An insufficient supply of Glu or Gly can also restrain GSH synthesis.[76] In line with this, the importance of γ-GCS/γ-GCL enzyme in GSH synthesis is highly vindicated to limit the rate of GSH synthesis, and hence it can be assumed as another important factor for carrying out GSH depletion. In fact, the activity of γ-GCS/γ-GCL mostly depends on its expression level.[77,78] Specifically, the expression level of γ-GCS/γ-GCL is entirely controlled by nuclear factor erythroid 2-related factor 2 (Nrf2), which is a well-known transcription factor for regulating the cellular defense system against oxidative stress.[79] As soon as GSH is formed, it plays a key role in feedback inhibition as well by competitively inhibiting γ-GCS/γ-GCL activity and regulates the formation of its heterodimer.[80] As ATP provides energy throughout the synthesis process, it can also be a factor in controlling the intracellular GSH level (Figure 4).[81,82]

The synthesis of GSH can also happen either through a salvage mechanism involving the catabolism of GSH or through the recycling of GSH after its oxidation to GSSG. In the extracellular medium, GSH is catabolized enzymatically by the action of the γ-glutamyl transpeptidase (γ-GT), which is expressed largely on the apical surface of the cell membrane. Not only GSH but also glutathione S-conjugates as well as glutathione complexes take part in γ-GT-mediated catabolism. Initially, the γ-glutamyl moiety is eliminated from GSH or the GSH-conjugated skeleton by the action of γ-GT. Then, the γ-glutamyl fragment is relocated to other acceptors like other dipeptides or other amino acids, which results in the production of a cysteinylglycine (Cys-Gly) moiety or cysteinylglycine-conjugates (Cys-Gly-conjugates). After that, ectoprotein dipeptidase present at the cell surface further hydrolyzes these products by disrupting the peptide bond between Cys and Gly. Then, specific transporters receive the free Cys moiety as well as the γ-glutamyl-amino acids (γ-Glu-aa) from the extracellular medium and transport them to the intracellular medium. γ-GCT in the intracellular medium acts on γ-glutamyl derivatives to produce 5-oxoproline as well as its analogous aa. Finally, by the action of 5-oxoprolinase (OXP), 5-oxoproline is transformed to Glu, which then conjugates with the Cys and Gly successively to construct the GSH skeleton. At the same time, S-Cys conjugates are converted to mercapturic acids through acylation of the amino group present in the cysteinyl residue by the action of intracellular *N*-acetyl-transferases (NATs) (Figure 5).[83]

Transportation of GSH in the biological medium is important for maintaining its extracellular or intracellular

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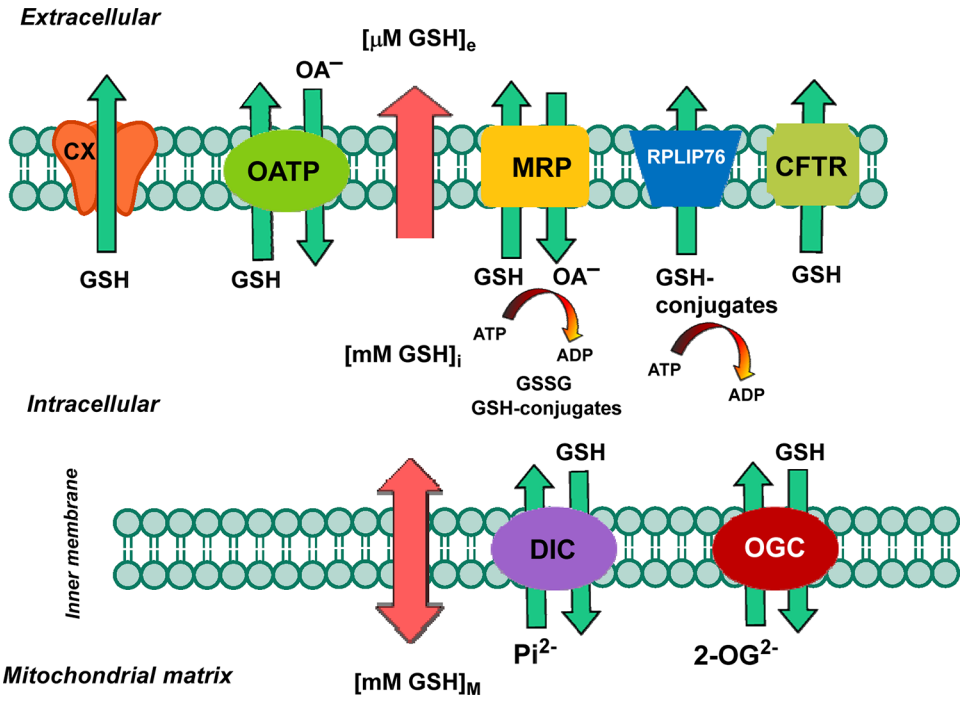

Figure 6. Transportation mechanism of glutathione.

concentration, compartmentalization and homeostasis to carry out various biological functions, which may also be a crucial aspect for designing GSH-depleting anticancer drugs, as blocking GSH transportation can deplete the GSH level. Therefore, we should take a closer look at the specific transport mechanism of GSH (Figure 6). Transportation of GSH depends mostly on the expression of various transporter proteins on the surface of the plasma membrane or the membrane of the organelles. The GSH transport influx depends on the intracellular GSH synthesis and GSH efflux through the plasma membrane. For GSH influx, two transporter proteins play key roles. One is OAT1/3 (organic anion transporters 1 and 3), which follows a Na^+-independent mechanism to exchange the GSH for 2-oxoglutarate. The other is SDCT-2 (sodium dicarboxylate cotransporter-2), which follows the Na^+-dependent influx of GSH. On the other hand, degradation (catabolism) of GSH takes place in the extracellular medium. Therefore, the efflux of GSH, GSH-adducts and GSSG to the extracellular medium is a significant step. As per the current investigation, two GSH transporter proteins are known for GSH efflux through the plasma membrane. One of them is the multi-drug-resistant protein (MRP) which is encoded by ABCC genes, and the other is the organic anion transport polypeptide protein (OATP), which is encoded by SLCO genes.[84] The MRP transporters act as cotransporters of GSH and organic anions (OA7). However they play the key role for transportation of GSH-conjugated metabolites and GSH-conjugated xenobiotics, which should be ejected out immediately from the cells to avoid harmful effects. In this way, this efflux system can protect the normal cells from toxic abuse and build up drug resistance in cancer cells. In addition to this, the export of GSSG to the extracellular medium suggests the importance of MRPs in identifying the cellular responses against oxidative stress. It is noteworthy that the hydrolysis of ATP and the presence of GSH are required in any case of transportation of organic anions (drugs and conjugated OA7) with the help of MRP.[85,86] An electrochemical gradient, which is developed through the plasma membrane, is very important to direct the GSH transport by OATPs in an outward direction, and this direction can be reversed with an increase in the extracellular GSH concentration. The concentration of GSH in cytosol is very high (10 mM) compared to its blood plasma concentration (~0.01 mM). At physiological pH, GSH remains negatively charged. Therefore, the intracellular potential becomes highly negative (730 to 760 mV), which expedites the extrusion of GSH from the cell. In this manner, the combined effect of chemical as well as electrical gradients appears to be an influential driving force for the uptake of solutes by OATPs in cells.[87] The transportation of GSH and GSH-conjugates efflux may also be accomplished with the help of Ral-regulated effector protein (RLIP76), cystic fibrosis transmembrane conductance regulator (CFTR) as well as hemichannels (connexins). The CFTR (ABCC7) protein is also the member of the ABCC/MRP family of transporters. The CFTR acts as a chloride channel in association with its involvement in either direct transportation of GSH or modulation of GSH transport by other proteins.[88] On the other hand, RLIP76 (RALBP1) is related to Rho/Rac-GAP, a 76 kDa Ral-binding and Ral effector protein. It has been reported that RLIP76 acts as a novel multispecific transporter of GSH-conjugates as well as xenobiotics and exhibits intrinsic ATPase activity.[89] Lastly, the connexions, which belong to the transmembrane proteins family, regulate the passage of molecules and ions having molecular mass of up to 1 kDa (Figure 6).[90,91]

In association with the transportation, compartmentalization of GSH is also a very important process in various biological events as soon as it is present in the intracellular medium. GSH is generally compartmentalized into three main organelles: (1) mitochondria, (2) nuclear matrix and (3) ER. After compartmentalization of GSH, a significant GSH-pool is observed within mitochondria, nuclear matrix and ER.

3.1. Transportation to the Mitochondria. Oxidative stress exhibited by toxic free radicals is very dangerous as it can

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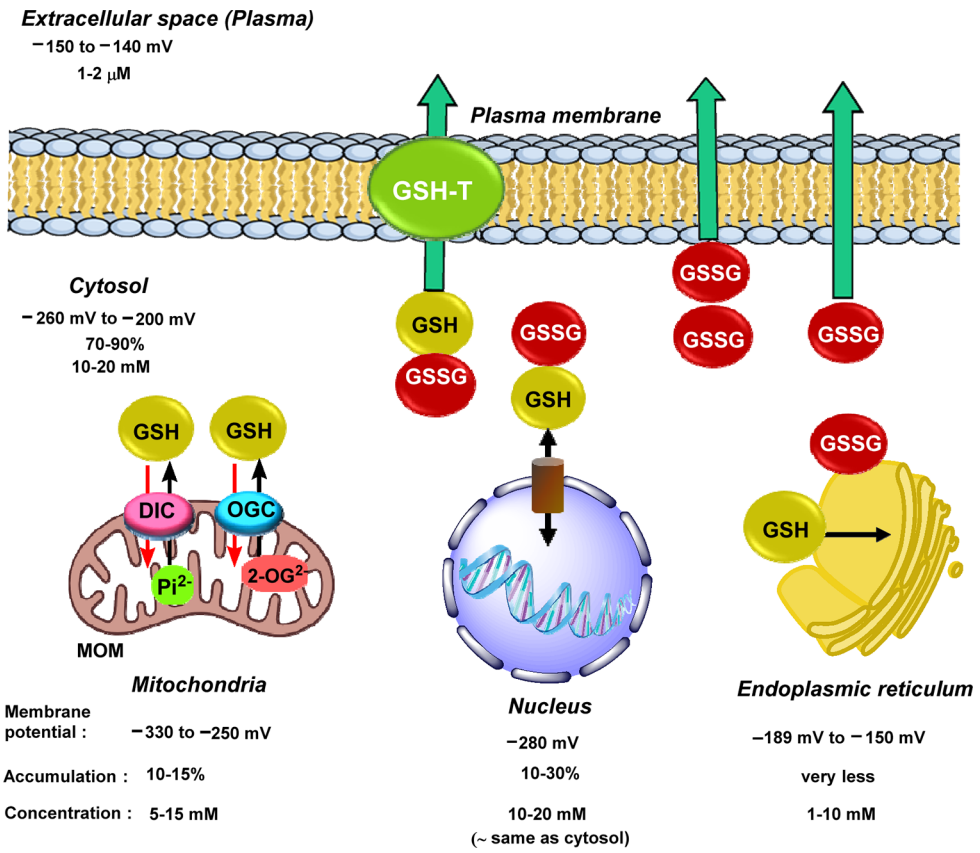

Figure 7. GSH transportation and compartmentalization in mitochondria, nucleus and endoplasmic reticulum.

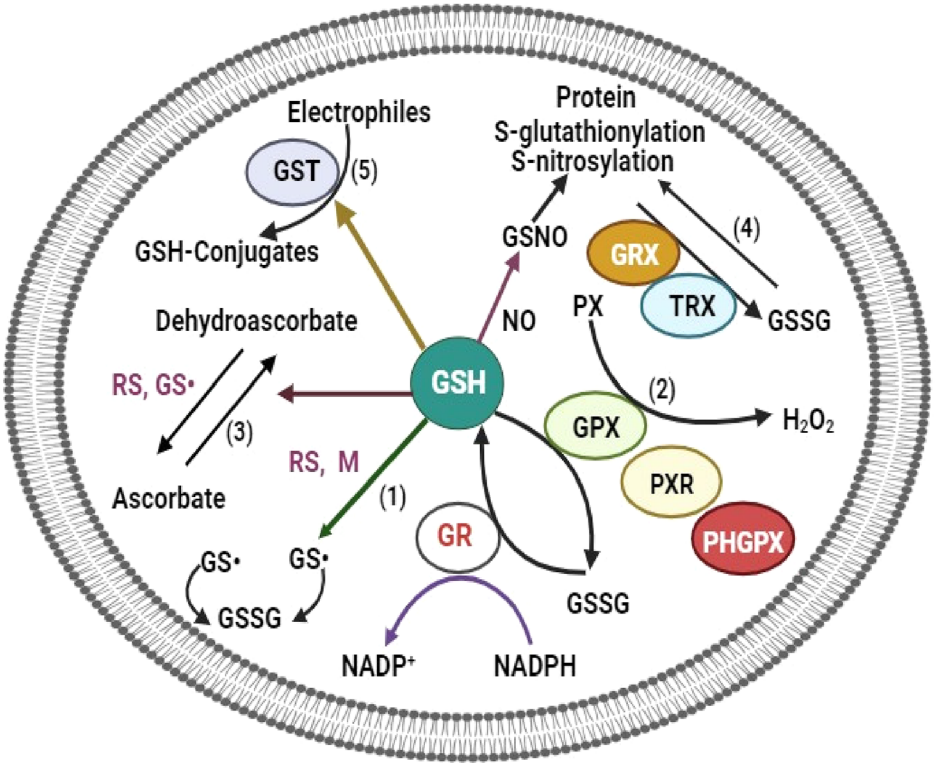

Figure 8. Metabolism process of glutathione.

demolish the mitochondria, causing mitochondrial dysfunction. In order to prevent oxidative damage because of aerobic respiration, mitochondria ardently appeal GSH to be compartmentalized into it. Since the mitochondria are incompetent to synthesize GSH by a de novo mechanism, they mostly rely on the salvage of GSH from GSSG by GR and

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on the acceptance of cytosolic GSH through the outer mitochondrial membrane (OMM). In the intracellular medium, GSH can easily penetrate the OMM through porin channels.

The mitochondrial matrix space bears a negative membrane potential compared with the cytoplasm, and GSH appears as a negatively charged moiety at the physiological pH of cell. Therefore, either GSH must be transported into the mitochondrial matrix aggressively or it must replace another anion. In such cases, 2-oxoglutarate or the monocarboxylate, tricarboxylate, dicarboxylate and glutamate-hydroxide or glutamate-aspartate transporters may be potential candidates for being exchanged with cytosolic GSH in the mitochondrial GSH import process (Figure 7).[92]

3.2. Transportation to the Nuclear Matrix. The matrix space of the nucleus also serves as another important container for reserving GSH. Unfortunately, there is no well-defined transport machinery available for GSH so far for nuclear compartmentalization. In fact, it is assumed that GSH is able to diffuse into the nucleus by penetrating through the nuclear pores. Current reports reveal that the main function of nuclear GSH is to shield the DNA from oxidative alterations and thereby enhance the efficiency of the DNA repair mechanism (Figure 7).[93−95]

3.3. Transportation to the Endoplasmic Reticulum. The lumen of the ER contains a higher concentration of GSSG compared to the cytosol, as in the mitochondria, which triggers the formation of disulfide bonds along with their subsequent isomerization through the function of oxidoreductases and protein disulfide isomerases.

In the ER, the high concentration of GSSG is managed by the influx of reducing equivalents. The reducing equivalent may be protein translocation and/or selective transport of GSH from the cytosol to the ER. The formation of disulfide bonds is catalyzed by the protein disulfide isomerase (PDI), and PDI can be reduced. Then the flavoprotein Ero1 further oxidizes the reduced PDI to PDI. In this process, ROS are generated as byproducts, which are further detoxified by GSH, and the GSSG level becomes high. There are two possibilities where PDI can be either reduced by GSH or oxidized by GSSG. Then the high concentration of luminal GSSG is discharged from the ER.[96]

The reactive thiol group (−SH) present in the cysteinyl moiety of GSH helps GSH to take part in different metabolic processes such as redox reactions as well as nucleophilic substitution or addition-type reactions. The GSH metabolism can occur in three different ways: (i) exhibiting antioxidant properties and maintaining redox balance, (ii) acting as a mediator of thiol/exchange reactions and (iii) inducing nucleophile conjugation. Being a strong reducing agent, GSH interacts with the reactive species (RS) or free radicals and also takes part in a metal redox process leading to the generation of thyil radical ($GS^{\bullet}$). Then, the two reactive $GS^{\bullet}$ species combine to form a GSSG molecule [step (1) in Figure 8]. Moreover, GSH plays a catalytic role in cleansing the cells from harmful peroxides (PX) by the activity of peroxiredoxins (PXRs), GPXs, and phospholipid hydroperoxide glutathione peroxidases (PHGPXs). As soon as GSSG is formed, it is further reduced back to GSH by dint of NADPH through the catalytic action of GR [step (2) in Figure 8]. GSH also displays its antioxidant role in other primary antioxidant systems. For example, the dehydroascorbate reductase (DHR) regenerates the oxidized ascorbate (dehydroascorbate) by the antioxidative role of GSH [step (3) in Figure 8].

As a reversible mechanism for post-translational alterations of proteins, GSH takes part in thiol/exchange reactions (*S*-glutathionylation and *S*-nitrosylation). The *S*-glutathionylation and *S*-nitrosylation reactions include mainly protein-SH/GSSG exchange reactions and the formation of *S*-nitrosoglutathione (GSNO), mediated by NO initiating the *S*-nitrosylation of proteins. By the enzymatic action of glutaredoxin (GRX) or Trx, disulfide linkages are eliminated through dethiolation or deglutathionylation [step (4) in Figure 8]. At last, GSH-conjugates or -adducts are formed by nucleophilic attack of GSH toward electrophiles like endogenous metabolites or xenobiotics, catalyzed by GST [step (5) in Figure 8].[97]

4. GLUTATHIONE AND REDOX HOMEOSTASIS

In the skeleton of a GSH molecule, the strong peptidic γ-linkage between Glu and Cys prevents the hydrolysis of GSH by intracellular peptidases. On the other hand, the cleavage of GSH by intracellular GCTs is resisted due to the presence of the C-terminal Gly. The presence of the −SH group helps GSH to carry out various biological functions such as redox and nucleophilic addition-type reactions. In practice, the overall physiological balance between oxidizing and reducing equivalents within the subcellular compartments preserves the redox homeostasis. The metabolism of xenobiotics as well as thiol/disulfide exchange reactions involving the GSH provide an important source of Cys. As mentioned earlier, the initiation of GSH synthesis occurs through the formation of γ-glutamylcysteine from Glu and Cys under the catalytic action of the γ-GCL and then immediate conjunction of Gly by the action of GS.[98,99] If the ratio of GSH/GSSG is changed, then the intracellular thiol−disulfide balance is disrupted; this phenomenon can be regarded as a major cause of changes in the redox status or redox signaling of the cell.[100,101] GSH can directly detoxify the ROS/RNS, as most of the physiological oxidants involved in the reaction with GSH occur through the −SH present in it. It has been observed that, upon the formation of ROS/RNS, various types of GSH oxidation species can be generated. The chemical profile of these oxidation species depends on the identity as well as the extent of the ROS/RNS generated. GSH can undergo one-electron oxidation of Cys by ROS such as O_2^- through the formation of glutathionyl radical $GS^{\bullet}$ along with the formation of the thiyl peroxyl radical (GSOO). Furthermore, H_2O_2 and peroxynitrite ($ONOO^-$) as ROS/RNS trigger the two-electron oxidation of GSH through the formation of GSSG, glutathione disulfide S-oxide (GS(O)SG), glutathione sulfenic (GSOH), sulfinic (GSO_2H) and sulfonic acids (GSO_3H), glutathione disulfide *S*-dioxide ($GS(O)_2SG$), glutathione *N*-hydroxysulfenamide (GSNHOH), glutathione thiosulfenamide (GSNHSG) and *S*-nitrosoglutathione (GSNO).[102,103] Although there are possibilities of formation of various physiologically relevant oxidized GSH derivatives, except for GSNO and GSSG, detailed studies with them are not possible owing to the lack of handy and selective techniques for their quantification in association with their high instability and reactivity.[104−106] Moreover, the study by Bolanos et al. has revealed that γ-glutamylcysteine can also detoxify ROS by acting as GPX-1 cofactor.[107]

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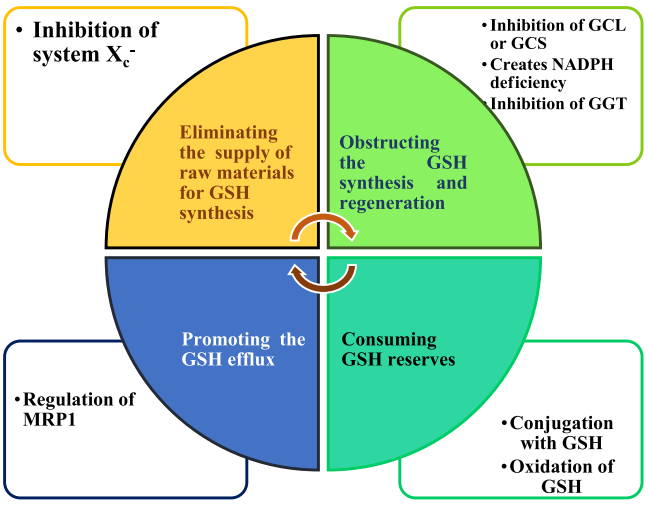

Figure 9. Strategies and targets for GSH depletion.

5. DIFFERENT STRATEGIES FOR DEPLETING INTRACELLULAR GSH

A high level of GSH content in cancer cells is one of the major obstructions for cancer therapy because of its great antioxidant property. As GSH is very influential to inhibit the anticancer potency of therapeutics, significant GSH depletion unveils a new facet for current cancer therapy. Based on the different synthetic and metabolic pathways of GSH, strategies for GSH depletion can be grouped into four major points: (1) eliminating the supply of raw materials for GSH synthesis, (2) obstructing GSH synthesis and regeneration, (3) consuming GSH reserves and (4) promoting GSH efflux (Figure 9).

5.1. Eliminating the Supply of Raw Materials for GSH Synthesis. The first and foremost approach to depleting GSH in the intracellular medium involves the substantial cessation of GSH formation by eliminating the adequate supply of raw materials for GSH synthesis moving from the extracellular medium to the intracellular medium via the plasma membrane, as the usual supply of constituent amino acids can continue the regular biological synthesis of GSH. Therefore, by inhibiting the transportation of extracellular amino acids into cells, GSH synthesis can be restricted, which will significantly diminish the intracellular GSH level and augment the therapeutic efficiency of anticancer agents. As Cys is the leading constituent for GSH synthesis, the starvation of Cys in cells can cause GSH depletion. In this regard, the Cys transporter, system X_c^-, is highly important in controlling the transportation of Cys in cells. Therefore, Cys starvation can be considered as a key approach for lowering the GSH levels in the intracellular medium by inhibiting the function of system X_c^-. As system X_c^- is the transporter of extracellular cystine into cells, the disruption of system X_c^- can bring about substantial Cys starvation, which results in the GSH depletion in cells and thereby enhances the therapeutic efficacies of anticancer drugs.[108]

5.2. Obstructing the GSH Synthesis and Regeneration. *5.2.1. Inhibition of GCL or GCS Activity.* The next point for aiming toward GSH depletion is to obstruct the GSH synthesis and regeneration. As some important enzymes and biomolecules are highly indispensable for regulating the level of intracellular GSH, the pronounced inhibition of these enzymes and biomolecules offers a fruitful approach to GSH depletion. As per the aforementioned synthetic mechanism, γ-GCS or γ-GCL is important to catalyze the synthesis of GSH from Glu and Cys. Therefore, inhibition of the function of GCL can restrict the rate of GSH synthesis. In this context, the role of L-buthionine sulfoximine (BSO) as a GCL inhibitor is highly remarkable. It has often been observed that BSO is very adept in reducing the level of GSH by blocking the GSH synthesis.[109−111] Therefore, in recent scenarios researchers have been fond of attaching the BSO with reactive chemical species-generating agents or with normal chemotherapeutics to diminish ROS hunting by GSH and to defend the drugs from detoxification through considerable GSH depletion. Sinha et al. observed that the total GSH content in MCF-7/ADRR cells was substantially decreased by 80−90% upon treatment with BSO (50 mM, 48 h).[112] The advancement of BSO in cancer treatments has also been considered in clinical trials.[113,114] In brief, the application of BSO or its related inhibitors can increase the therapeutic potential of drugs via (1) depleting the GSH level and escalating the ROS level, inducing apoptosis of cancer cells, and (2) increasing the sensitivity of cancer cells to anticancer agents as well as radiation in radiation therapy.

5.2.2. Creating NADPH Deficiency. Not only the obstruction of GSH synthesis but also inhibition of its regeneration are very important to eradicate the further appearance of GSH in cancer cells. As GSH is easily regenerated from GSSG by the catalytic action of GR through oxidation of NADPH to $NADP^+$, interruption of the reaction of GR and NADPH can abate the reduction process, which will result in the depletion of the intracellular GSH level, inducing increased oxidative stress in cancer cells.[115] To accomplish the inhibition of GSH regeneration, there are mainly two approaches to cutting down the NADPH level: (1) inhibition of the pentose phosphate pathway (PPP) for the production of NADPH or (2) the consumption of NADPH by triggering the reduction process of nitroimidazole (NI) to aminoimidazole (AI) under hypoxia condition in cancer cells. It has been recognized that glucose-6-phosphate dehydrogenase (G6PD) is an important enzyme in carrying out the PPP for NADPH production. Therefore, blocking of G6PD can be a prime target for cutting down NADPH levels in cancer cells.[116]

5.2.3. Inhibiting the Function of γ-Glutamyl Transferase (GGT). After being extruded from the intracellular medium, GSH degrades into its constituent amino acids, which may also

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ACS Omega 2024, 9, 20670−20701

serve as the regenerated source of raw materials for further GSH synthesis. Therefore, inhibition of the degradation process should also be a point of interest for GSH depletion. It has been observed that GGT is overexpressed in a few types of tumors for future survival and proliferation. As GGT is the key enzyme to catalyze this degradation, a disturbance in the function of GGT can lead to GSH depletion. It is noteworthy that acivicin, which is an established irreversible inhibitor of GGT, can demolish the GSH levels in the case of a few cancer cells.[117,118] Moreover, some other inhibitors of GGT were established, including azaserine (*O*-diazoacetyl-L-serine) and L-DON (6-diazo-5-oxo-L-norleucine). However, the toxicities associated with these compounds were a major problem for future utilization,[119] which may be a point of interest for researchers to find fruitful inhibitors of GGT in the near future. Therefore, in-depth investigations are still required to comprehend the intricate roles of GGT in moderating redox homeostasis as well as physiological activity in cancer cells prior to making it a target for cancer therapy.[120]

5.3. Consuming the GSH Reserves. The third strategy for depleting intracellular GSH involves the consumption of GSH reserves. This approach can be devised based on the chemical characteristics of GSH, which can provide ideas for controlling intracellular GSH levels with the introduction of some substances that are capable of reacting with GSH chemically. This objective can be fulfilled by either the conjugation of GSH with some specific electrophilic molecules forming GS-X-type conjugates, which will be subsequently extruded out of the cancer cells, or the oxidation of GSH with some specific oxidizing agents forming GSSG, which will be in turn expelled out of the cancer cells or may be reduced back to GSH again.

5.3.1. Conjugation of Electrophilic Molecules with GSH. For conjugation with GSH, moderately active electrophiles are generally preferred to consume an elevated amount of GSH in cancer cells, as highly active electrophiles are not always selective to bind GSH; rather they bind other macromolecules and thereby create uninvited toxic effects. It has been visualized that numerous exogenous or endogenous electrophilic entities can be directly conjugated with GSH under the proficient catalytic action of GST, thus making possible the detoxification of xenobiotics and endogenous compounds by forming GS-X conjugates.[121,122] In turn, these GS-X conjugates are extruded out of the cells by the role of MRP1 as soon as they are formed in cytosolic medium. Therefore, this process can also be regarded as an important strategy for the depletion of intracellular GSH levels. Furthermore, α,β-unsaturated aldehydes and ketones are very prolific to react specifically with the thiol group of GSH by Michael addition, exhibiting avidity toward GSH conjugation and hence depletion.[123] Isothiocyanates (ITC), naturally occurring compounds from cruciferous vegetables, are very familiar for GSH depletion by undergoing conjugation with GSH between the −SH of Cys residues and the highly electrophilic central carbons of ITC.[124,125] Along with the induction of rapid depletion of GSH, ITC is also found to inhibit mitochondrial respiration. Among several reported ITCs, a few, such as β-phenylethyl isothiocyanate (PEITC) and sulforaphane (SFN), were identified to deplete GSH effectively and reached phase II clinical trials for the treatment of oral and lung cancers as well.[126] In addition to this, some reported α,β-unsaturated compounds, such as diethyl maleate (DEM) and phorone (PHO), have also shown a remarkable propensity toward GSH conjugation.[127]

5.3.2. Oxidation of GSH. In practice, GSH undergoes oxidation by reacting with oxidizing substances to form GSSG to conserve the redox homeostasis in cells. By considering the benefit of this antioxidant property of GSH, an ample amount of oxidizing compounds can be delivered into cells to swallow the intracellular GSH in a continuous manner. Metals and disulfides have frequently been used to accomplish this strategy. Therefore, the conversion of GSH to GSSG with the application of oxidizing agents is now being considered to consume intracellular GSH substantially. Actually, GSH is oxidized through the donation of a lone pair of electrons on the sulfur atom in the thiol group to electron-deficient endogenous receptors and then leaves the proton by cleaving the S−H bond. In this way, the formation of free radicals can be suppressed by donating hydrogen atoms to free radicals upon cleaving the S−H bond in GSH.[128] Although *tert*-butyl hydroperoxide, nitric oxide, diamide or some ROS can be considered as powerful oxidizing agents for the oxidation of GSH to GSSG, the consumption process in such cases is temporary, as the action of GR and NADPH reduces GSSG back to GSH again. However, applications of transition metal ions have been studied to see if they are the right choice for the permanent consumption of GSH. For example, transition metal ions like iron(III) and copper(II) have been seen to consume GSH permanently through oxidation of GSH and reduction of metal ions. Besides, iron(II) and copper(I) in their reduced states can catalytically reduce molecular oxygen to $O_2^{\bullet-}$ and hydrogen peroxide to $OH^{\bullet}$, which in turn can induce cellular apoptosis and necrosis. Therefore, the oxidation of GSH with transition metal ions can be thought of as the "killing two birds with one stone" strategy in cancer therapy.[129,130]

5.4. Promoting GSH Efflux. MRP1, which belongs to the ATP-binding cassette (ABC) transporter family, plays a key role in thrusting GSH out of cells, creating a substantial intracellular GSH depletion.[131,132] Vinca alkaloids, anthracyclines and camptothecins are some reported anticancer agents which take part in conjugation with GSH and are wiped out from the cancer cells in the form of GS-X conjugates by MRP1-dependent transportation. Therefore, MRP1 creates chemoresistance for those agents. Aside from this, MRP1 is also engaged in solo transport of GSH without the help of any co-substrate; therefore, the overexpression of MRP1 in cancer cells can be regarded as an indication of its hyperactive empathy to GSH modulation.[133,134] Thus, suitable inflection of MRP1 can be implemented to endorse GSH efflux for considerable GSH depletion in tumor cells.[135] A few compounds like verapamil, chrysin, apigenin, PAK-104P and staurosporine were reported to escalate the function of MRP1 for promoting GSH efflux. Verapamil displayed outstanding MRP1-moderating activity and triggered the apoptosis of cancer cells with overexpressed MRP1, accelerating GSH efflux and creating GSH depletion.[136−138]

6. CONSEQUENCES OF GSH DEPLETION—"CELL DEATH"

Glutathione depletion is an important phenomenon in biological systems, and its consequences lead to cell death. Cancer cell death is essential to annihilate cancer from the body by arresting its rapid proliferation, growth, and metastasis. But, the eradication of cancer cells is difficult due

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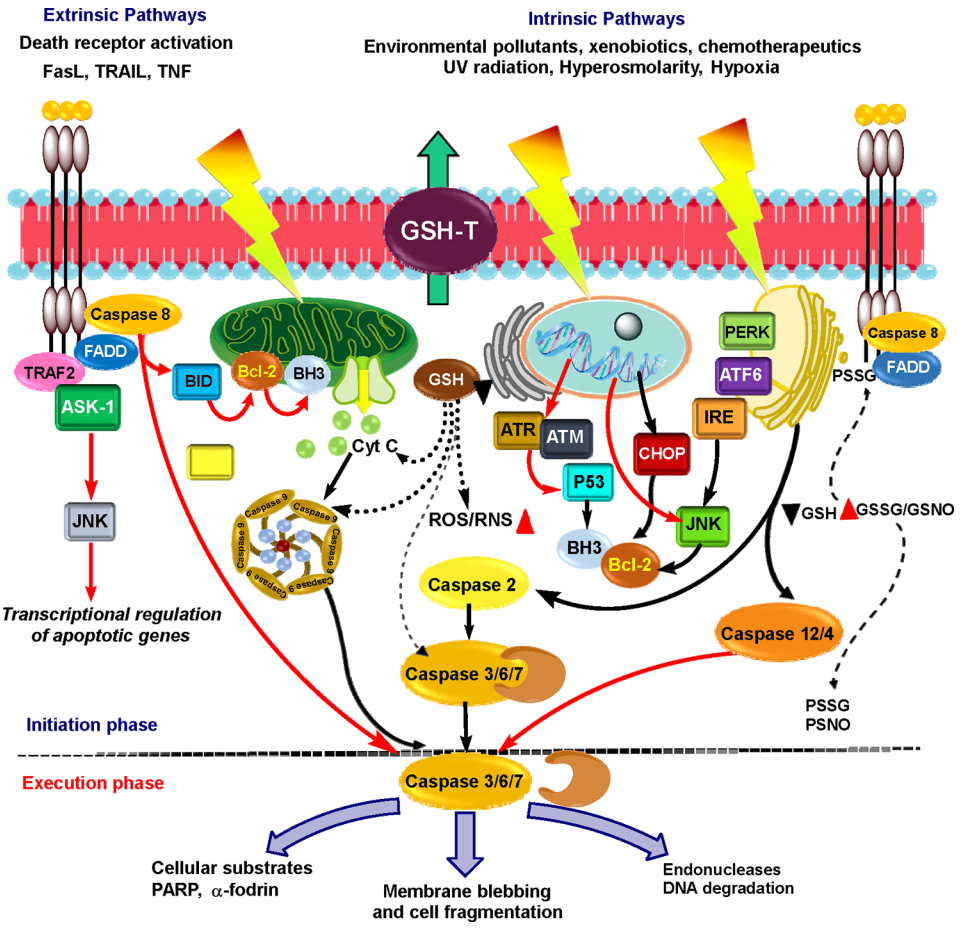

Figure 10. Molecular mechanisms involved in the regulation of apoptosis by GSH.

to the presence of many obstructions, the most important of which is the escalation of intracellular GSH levels in cancer cells to defend them from dying. Therefore, strategies for GSH depletion offer the best mean of annihilation of cancer cells upon application of anticancer therapeutics. Cell death may occur in four different ways: (1) apoptosis, (2) necrosis, (3) ferroptosis and (4) autophagy, based on the various biochemical as well as morphological criteria of cell death.[139]

6.1. GSH Depletion and Apoptotic Cell Death. Apoptosis or programmed cell death is an essential homeostatic process which is involved in numerous biological systems. It is very important not only in the substantial turnover of the cells but also in the normal growth and senescence of any organism under a physiological environment. Its deregulation is seen to be a cause of different pathologies.[140] Early stages of apoptosis are characterized by formation of RS, alterations in intracellular homeostasis, loss of plasma membrane lipid asymmetry, cell shrinkage, chromatin condensation and activation of initiator caspases. The subsequent stage is known as the execution phase of apoptosis, which is regarded as the activation of executioner caspases as well as endonucleases through the formation of apoptotic bodies along with cell fragmentation.[141,142] It can be mentioned that GSH depletion is regarded as an early hallmark for the initiation of cell death in response to a number of apoptotic stimuli in various types of cells.[143−145] Therefore, the intracellular GSH content is a leading factor in progression toward cell death. Many studies have revealed that a high concentration of intracellular GSH is an indication of apoptotic-resistant phenotypes, while GSH depletion leads to apoptosis. The depletion of intracellular GSH prior to the cell death progression has been attributed to its oxidation due to the formation of ROS/RNS. The signaling steps which control the progression of apoptosis have been studied carefully and categorized into two pathways: (1) extrinsic and (2) intrinsic pathways for the initiation of apoptosis (Figure 10). The introduction of apoptosis via extrinsic pathways is elicited by death receptors, which are activated by Fas ligand or FasL (CD95/Apo-1, Fas), by TRAIL (DR4, DR5) or TNF-related apoptosis-tempting ligand and by TNF-α (TNFR1). It is well studied that the triggering of CD95, DR4 and DR5 compels the formation of the death-inducing signaling complex (DISC) in connection with Fas-associated death domain (FADD) and caspase 8 (or in some cases caspase-9) along with the cellular FLICE-inhibitory protein (FLIP). The progression of apoptosis commences with the intensification of the loop prompted by caspase 8-responsive cleavage of the Bcl-2 family proteins, Bid, and then their translocation to the mitochondria, which triggers the release of cytochrome *c* (Cyt c) subsequently. On the other hand, TNFR1 signaling produces two signaling complexes: (a) Complex I is formed by the induction of TNF and mediates the employment of receptor-interacting protein, TRAF-1/2 (TNFR-associated factor) and TRADD (TNFR-associated death domain protein). Complex I lacks FADD and procaspase 8. (b) Complex II or traddosome is formed by the FADD, caspase 8/10 and FLIP in the cytosol. In fact, complex II has been observed to facilitate the other pro-apoptotic signaling cascades which are involved in the

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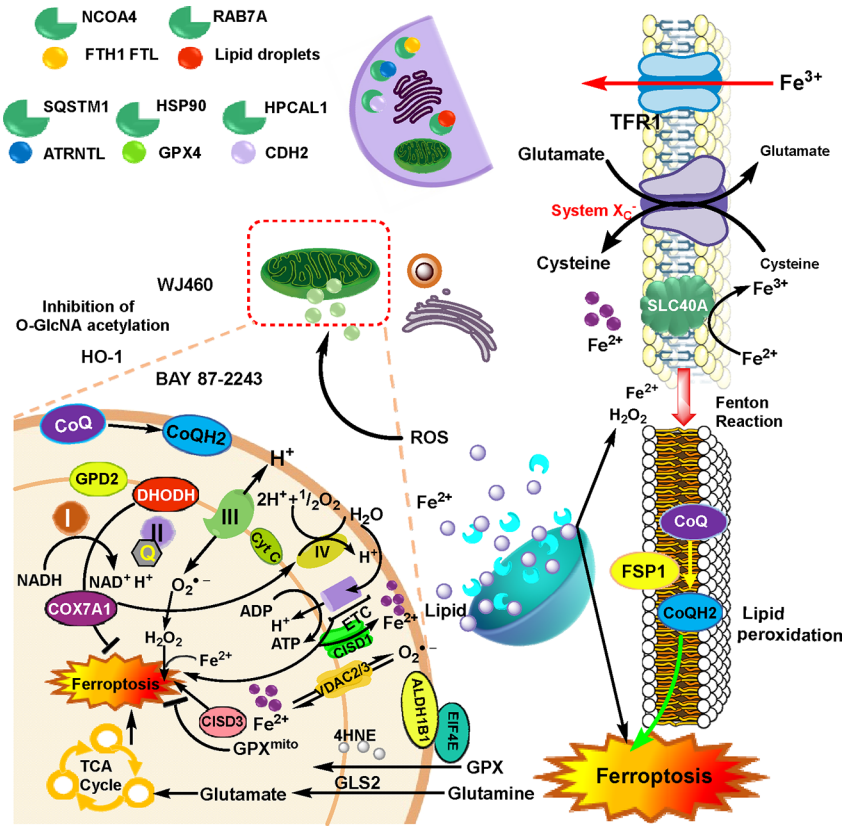

Figure 11. Mechanism of ferroptosis.

activation of the apoptosis signal-regulating kinase 1 as well as the stress-activated kinase (SAPK), JNK. This phenomenon results in the transcriptional or post-transcriptional regulation of apoptotic genes and thereby causes cell death.

The intrinsic pathways of apoptosis emerge in the mitochondria and ER following DNA-damaging pathways. These pathways are initiated by the action of a number of different provocations like the application of chemotherapeutic or cytotoxic agents (xenobiotics, environmental pollutants, drugs) or stress (radiation, hypoxia, hyperglycemia, oxidative and osmotic stress) as well as the withdrawal of cytokine. Therefore, these different types of factors activate the mitochondrial intrinsic pathway through the release of Cyt c in cytosol. The release of Cyt c helps in opening the mitochondrial permeability transition pore (MPTP) and thereby induces the loss of the mitochondrial membrane potential (MMP).

GSH depletion in the course of apoptosis can be accomplished via discrete pathways: (a) induction of GSH loss through the activation of death receptors as soon as GSH is extruded across the plasma membrane with the help of GSH transporters or pumps (GSH-T), (b) loss of GSH due to its rapid oxidation and formation of GSSG by ROS as well as RNS generated from impairment of mitochondrial function, (c) apoptotic signals arising from pro-oxidant stimuli which mediate GSH depletion by its direct oxidation and/or conjugation, (d) further reduction or extrusion of GSSG and GSH adducts to avoid their deleterious effects inside the cell. Other mechanisms such as impairment of GSH synthesis and recycling might also contribute to preventing GSH replenishment during apoptosis, initially proposed to be formed by the voltage-dependent anion channel, the adenine nucleotide translocator and the cyclophilin D. Recent studies have suggested that mitochondrial apoptosis is independent of these complexes, which in contrast might be involved in cell death by necrosis. The intrinsic mitochondrial pathway is regulated by Bcl-2 family proteins. The BH3-only proteins (members of the Bcl-2 family) Bad, Bid, Bim, NOXA and PUMA regulate the anti-apoptotic Bcl-2 proteins (Bcl-2 and Bcl-xl) to promote apoptosis. Bcl-2 and Bcl-xl inhibit Bax and Bak. Induction and/or activation of BH3-only proteins de-repress Bax and Bak by direct binding and inhibition of the Bcl-2 anti-apoptotic family members. Bax and Bak are crucial for inducing the permeabilization of the OMM and the release of Cyt c. This leads to the recruitment of Apaf1 into the apoptosome and activates caspase 9 to further regulate execution caspases.[146]

6.2. GSH Depletion and Necrosis. Necrosis is another effective way to achieve cell death, and it is termed as the morphologically distinct procedure of destruction of cells. It is initiated in cells through tissue destruction in an irreversible way because of the bioenergetic failure along with a high level of oxidative damage. It is fundamentally different from apoptosis, as it occurs due to energy depletion as well as failures in the ion pump/channel, resulting in quick loss of cellular membrane potential and leading to swelling, rupture and ultimately cytolysis. Therefore, necrotic cell death can be defined by the increase in cell volume, which leads to the significant swelling of organelles, rupture of plasma membrane and thus loss of intracellular contents subsequently. Necrotic cell death is accomplished with the initiation of different signal transduction pathways as well as catabolic processes.[147] It is noteworthy that the huge amount of GSH depletion in association with oxidative stress switch the apoptotic cell death

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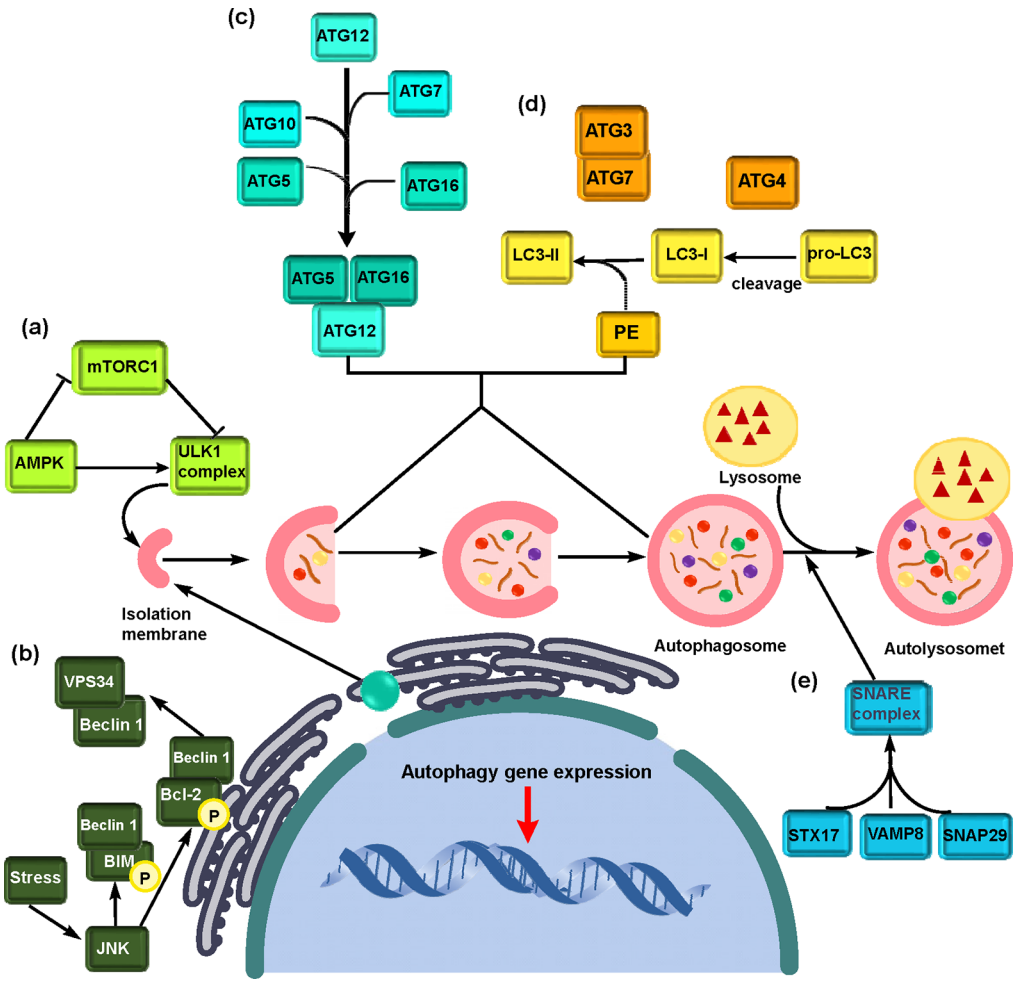

Figure 12. Mechanism of autophagy.

to necrotic cell death.[148] As discussed in an earlier section, GSH-depleting agents like DEM deplete the mitochondrial GSH content at high doses and thereby initiate the necrosis. However, decent doses of GSH-depleting agents can only cause GSH depletion in the cytoplasm, and this induces minimal change in cell viability, leading to TNF-α-induced apoptosis.[149]

6.3. GSH Depletion and Ferroptosis. Ferroptosis is characterized by non-apoptotic iron-dependent regulatory cell death (RCD),[150] which happens due to the down-regulation of a glutathione peroxidase (GPX4) and thereby creation of disorder in the metabolic system of intracellular lipid oxides and excessive accumulation of ROS/RNS.[151] Many pathways have been recognized for ferroptosis in cancer cells, which include system X_c^-/GSH/GPX4, ATG5/ATG7/NCOA4 and FSP1/CoQ10/NAD(P)H pathways,[152] and these pathways are regarded as prospective therapeutic targets for the healing of cancers. Therefore, GPX4 is regarded as the key metabolic enzyme which is highly responsible for degradation of lipid peroxides in the body.[153] GSH is known to be the essential co-factor for the function of GPX4, and the depletion in GSH level causes the down-regulation of GPX4 in cells. Therefore, controlling GSH and GPX4 is a fruitful way to disrupt the cellular redox balance, which induces the assembly of lipid peroxides and finally results in the initiation of ferroptosis.[154] This distinct cell death mechanism was discovered and recognized by Dixon in 2012.[151] In ferroptotic induced cells, the mitochondria become smaller and the mitochondrial membrane density is increased progressively. As a result of this, mitochondrial cristae vanishes or is reduced.[155] The term "ferroptosis" was coined due to the fact that iron chelation reduced the intracellular iron levels and thereby restricted the formation of reactive radical species, making a shield against cell death.[151] Moreover, ferroptosis is considered a type of RCD, which has distinct biochemical, morphological and genetic hallmarks.[156] For ferroptotic cell death, GPX4 and system X_c^- are assumed to be the principal signaling pathways. System X_c^- belongs to the family of heterodimeric aa transporters. SLC7A11, an aa transporter, functions to exchange L-cystine and L-glutamate.[157] Additionally, iron metabolism and lipid peroxidation are two essential events in ferroptosis. Inactivation of GPX4 leads to the accumulation of lipid peroxides, which further leads to an increase in ROS.[158] Circulating iron, ferric ion (Fe^{3+}), is imported into cells by the transferrin receptor (TFR) and subsequently converted to Fe^{2+} in the endosome.[159] Excessive amounts of Fe^{2+} lead to the accumulation of lipid ROS through the Fenton reaction, which in turn causes ferroptosis (Figure 11).

6.4. GSH Depletion and Autophagy. Autophagy is also a crucial pathway for cell death which involves the degradation of cells as well as the recycling of proteins and organelles to keep up the intracellular redox homeostasis. It has been reported that autophagy performs a dual role in numerous diseases. In the initial stage of tumorigenesis, the role of

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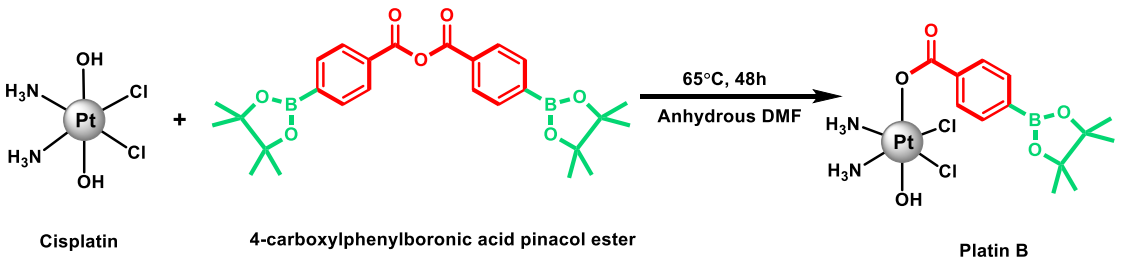

Figure 13. Synthetic route of TDBA-based anticancer Pt(IV) prodrug PlatinB.

autophagy is tumor suppression in order to maintain genomic integrity and prevent tissue damage and tissue swelling by involving quality control systems along with the regulation of oxidative stress responses.[160,161] In the final stage of tumor growth, autophagy supplies nutrients to cancer cells and thereby stimulates their immune escape process through dilapidation of MHC-I on the cancer cell surface.[162]

The cell death mechanism of autophagy is divided mainly into two categories: (1) autophagy-dependent cell death (ADCD) or simply autophagic cell death (ACD), which relies on the autophagy mechanism, and (2) autophagy-mediated cell death (AMCD), which triggers the initiation of other approaches of cell death such as apoptosis, necrosis and ferroptosis. Therefore, AMCD fuels the different ways to cell death as the root of the initiation of the mechanism of other cell death processes.[163]

In the 1990s Ohsumi discovered autophagy-related genes (ATGs) in brewer's yeast.[164] Later, the scholars of Ohsumi isolated more than 40 ATGs in yeast by the process of genetic screening, and approximately half of those genes were perfectly matched with the ATGs in mammals. It is known that mTOR signaling as well as AMPK signaling can bring about autophagy by functioning through cellular stress responses such as oxidation or starvation (Figure 12). In this process, protein phosphatase 1D magnesium-dependent delta isoform (PPM1D)[165] and protein phosphatase 2A (PP2A)[166] dephosphorylate the ULK1 Ser757 as soon as mTOR is dissociated from ULK1. On the other hand, AMPK phosphorylates the ULK1 Ser 317 as well as ULK1 Ser 777 to stimulate the creation of the ULK1 complex.[167] After the completion of acetylation of ULK1 Ser757 by O-GlcNAc, OGT co-mediates the phosphorylation of ATG14 Ser29 with GABARAPs.[168,169] Upon activation of the ULK1 complex, PIKfyve Ser1548 also undergoes phosphorylation to trigger the transformation of PI to PI(5)P. After the transformation, PI(5)P binds with the WIPI2 to detach the isolation membrane from the ER membrane.[170,171] Then, the PI3KC3 complex combines with the ATG5-ATG12 complex as well as the ATG8/LC3 system with the help of WIPI2 in order to carry out the extension of the isolation membrane. Therefore, ubiquitination of the ATG8/LC3 system and ATG5-ATG12 complex is promoted via the formation of a positive feedback loop until the isolation membrane is closed, which leads to the formation of an autophagosome.[172,173] As soon as the autophagosomes are formed, they exploit the fusion proteins of lysosomes at the time of successive removal of ATGs on the outer membrane of autophagosomes. Then, deacetylation of STX17 takes place. The C-terminal hairpin-like structures are implanted into undamaged autophagosomes, where they interact with HOPS and SNAP29 to accelerate the binding of the autophagosome with the lysosome to form the autolysosomet.[174,175] Then, the cellular cargo is finally dissociated into small molecules by means of lysosomes followed by recycling (Figure 12).

7. METALLOTHERAPEUTICS TOWARD GLUTATHIONE DEPLETION AND CANCER THERAPY

The highly antioxidant competency of GSH in cancer cells, as elucidated elaborately in earlier sections, is the major obstruction for anticancer therapeutics. In practice, GSH builds up a powerful defense system either by directly interacting with the chemotherapeutic drugs, forming GSH adducts, or by scavenging the reactive chemical species produced by these therapeutic agents in dynamic cancer therapy (PDT, SDT or CDT), to protect the cells from attack by exogenous substances maintaining redox homeostasis. For this very reason, it is one of the prime causes of cancer therapeutics failing to exert their ultimate potency against cancer. Therefore, strategies toward the depletion of GSH levels in cancer cells will be the boon of anticancer agents for effective annihilation of cancer, augmenting their anticancer potential. A multitude of anticancer agents including metal complexes have been developed so far, but their capability of depleting GSH levels was unexplored. The first metallodrug, which underwent clinical trials in the late 1970s, was cisplatin to treat various types of cancer. The mechanism of action (MoA) of cisplatin and its other platinum-based analogues in cancer cells was the proficient binding to DNA N-heterocyclic bases, specifically to guanine, resulting in cross-linking of the DNA strand and thus preventing the replication of cells by mitosis. As platinum-based metallodrugs were associated with lots of side effects, numerous other metal ions, viz. Fe, Ru, Ir, Zn, Au, Cu etc., have been suggested during the past few decades as alternatives. There are a few anticancer compounds whose GSH-depleting competency in destroying cancer cells has been reported, although their efficacy may be interrupted by some other factors. Therefore, the interference of GSH with metallodrugs can be restricted by following several strategies, such as depleting the concentration of GSH in cells and exploiting GSH as an activator as well as shielding metallodrugs from GSH binding.[176] The main objective of this Review is to highlight the importance of GSH depletion in cancer therapy. Hence, in this section we are going to represent a few examples of metal complexes which have the competency to deplete the intracellular GSH level along with a profound insight into their different GSH-depleting strategies, viz. oxidizing GSH to GSSG, blocking the source of GSH production by forming an adduct with the GSH precursor and being activated by GSH from their inactivated forms, which will be beneficial for destruction of cancer cells by triggering various cell death mechanisms.

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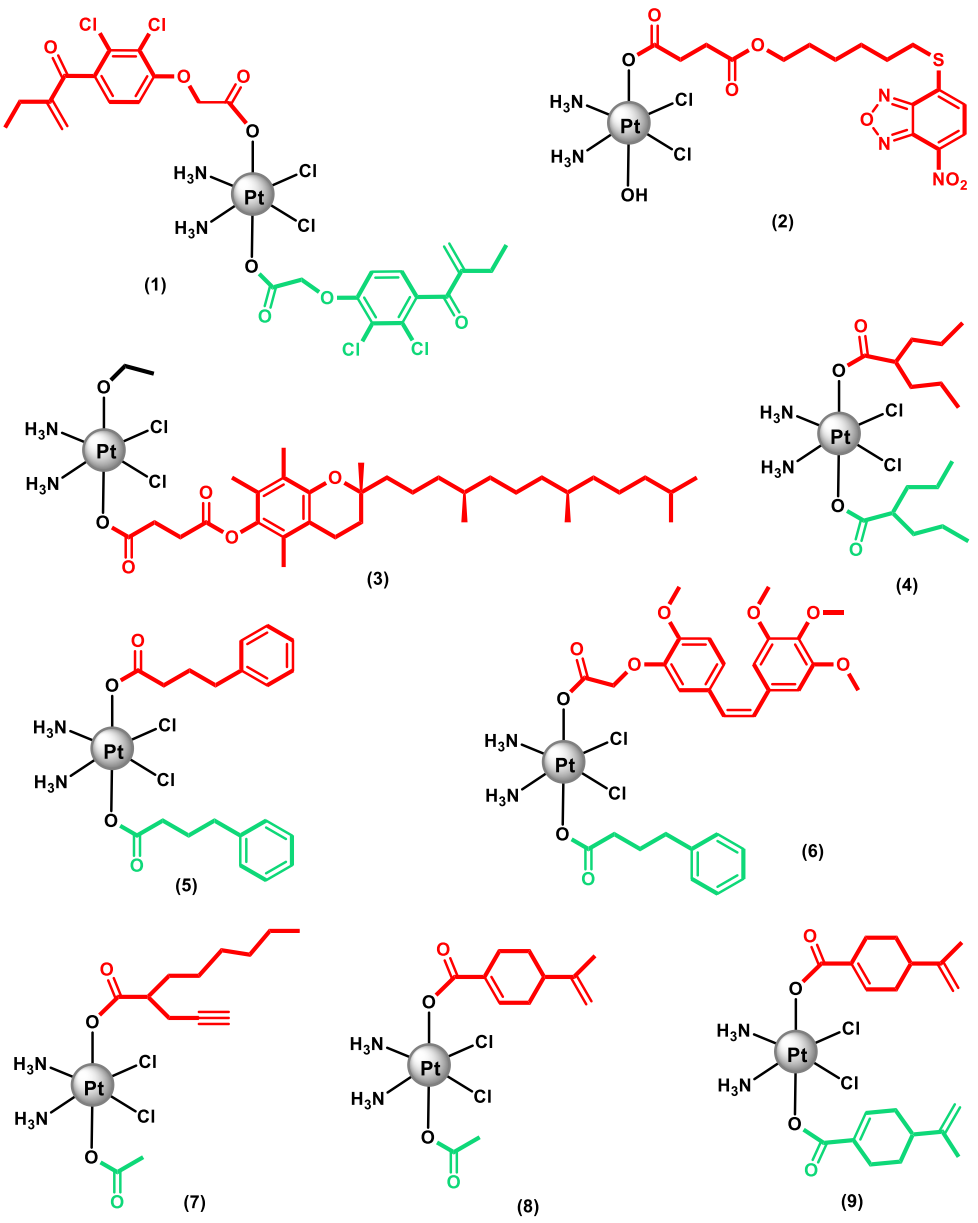

Figure 14. Structures of GSH-activable Pt(IV) prodrugs which liberate bioactive molecules and active Pt(II) drugs upon reductive cleavage of metal−ligand bonds.

7.1. GSH-Activable Metallo-prodrugs and GSH Depletion. *7.1.1. GSH-Depleting Pt(IV) Prodrug.* Cisplatin was the first FDA-approved anticancer metallo-drug, which was used for the treatment of various types of solid tumors, having a wide range of antitumor efficacy. However, this classical Pt(II) drug suffers from severe side effects in association with decreased sensitivity due to the high GSH content in tumor cells, and this consequence restricted its future clinical applications. In this case, the approach toward the synthesis of the prodrug revealed a good omen to resist against high levels of GSH. Consequently, the rapid development of tetravalent platinum(IV) complexes displayed significant antitumor activities for their distinctive chemical structures as well as unique physicochemical and pharmacodynamic properties. The concept of Pt(IV) prodrugs implies that the reduction of prodrugs by GSH will release the cisplatin to act as an anticancer agent concomitant with the depletion of the GSH level and enhancement of drug efficacy. Therefore, Zhang et al. used cisplatin and 4- carboxyphenylboronic acid pinacol ester (TDBA) to synthesize a Pt(IV)-based prodrug, Platin B (Figure 13), which successfully depleted the GSH level along with the release of cisplatin in cancer cells and was used for destruction of breast cancer through the ferroptosis pathway.[177] It was deliberated that as soon as Platin B entered into cancer cells, it was initially reduced by the GSH to liberate cisplatin and derivatives of phenylboronic acid. After that, cisplatin takes part in hydration and crosses the nuclear membrane to come into the cell nucleus. Consequently, DNA replication as well as transcription are inhibited, leading to cell apoptosis. Besides, the cisplatin hydrate can cause mitochondrial damage by enhancing the production of ROS, which reduces the MMP and ultimately leads to apoptosis. Concurrently, the unconfined phenylboronic acid derivative is involved in formation of methylquinone upon interaction with H_2O_2, which again depletes the intracellular GSH. Thus,

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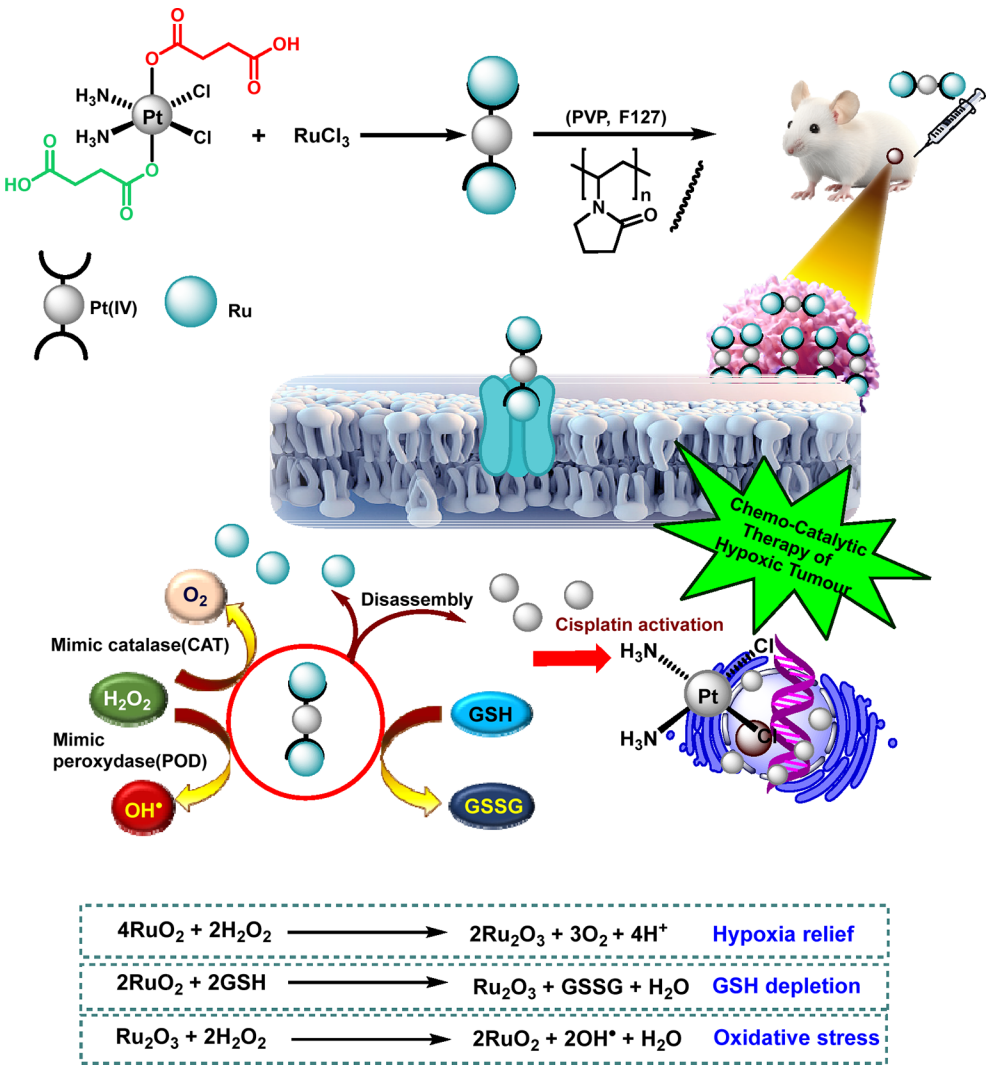

Figure 15. Schematic representation of the anticancer activity of a self-assembled GSH-depleted Pt-Ru hybrid prodrug having multienzyme activities (CAT, GPX and POD) for disturbing redox homeostasis and synergistic chemo-catalytic therapy for hypoxic tumors.

the consumption of intracellular GSH augmented the cisplatin activation and efficiently induced apoptosis/ferroptosis.

In connection with the concept of GSH-activable Pt(IV) prodrug synthesis, many other researchers tried to attach bioactive ligands to the axial position of cisplatin for development of various types of Pt(IV) prodrugs in order to heal lung cancer.[178] GST is overexpressed in cancer cells and causes cisplatin resistance.[179] Etanercept (EA) is a well-known compound possessing a wide range of GST inhibition activity and can reverse the GST-intervened cisplatin resistance.[180] Therefore, Ang et al. used EA in the preparation of a Pt(IV) prodrug and they developed the EA-Pt (IV) complex (**1**) (Figure 14), which substantially subdued the GST activity upon treatment in lung cancer A549 cells.[181] The anticancer activity of this complex was stronger in comparison to those of cisplatin and EA alone. However, the long-term efficiency of EA was restricted due to fluid imbalance and diuresis, and EA is transported out of the cells through particular pumps.[182] As a consequence, researchers were looking for a stronger GST inhibitor than EA which would not be transported out of the cells. Chen et al. developed Pt(IV) prodrug (**2**) upon conjugation of oxoplatin with succinic anhydride.[183] This complex showed significant tumor suppression capability along with low biotoxicity compared to cisplatin. In the same way, Suntharalingam et al. developed a novel dual-targeted Pt(IV) prodrug (**3**) connecting the α-tocopheryl succinate (α-TOS), a vitamin E analogue, at the axial position. As α-TOS is very efficient to disrupt the Bcl-xL and Bax interactions, it can induce mitochondria-mediated apoptosis.[184] They observed that complex (**3**) revealed an aggressive ability to destroy lung cancer cells compared to cisplatin alone, along with 15−20 times higher cellular uptake capacity than that of cisplatin. On the other hand, the conjugation of histone deacetylase inhibitors (HDACi) with Pt-based anticancer drugs captivated the minds of researchers. Therefore, Yang et al. combined valproic acid (VA) with cisplatin and thereby developed VA-Pt(IV) prodrug (**4**), which displayed remarkable anticancer activity both *in vitro* and *in vivo*, having lesser nephrotoxicity.[185] The success of histone deacetylase inhibition by the complex (**4**) encouraged other researchers to further explore the numerous superior HDACi complexes. As a result, Raveendran and co-workers synthesized complex (**5**) by attaching two HDAC-inhibiting phenylbutyrate (PhB) groups, and this complex exhibited almost 50-fold higher cytotoxicity against A549 cells than cisplatin.[186] Schmidt et al. developed a combretastatin A-4 (CA4)-conjugated Pt(IV) prodrug (**6**) to

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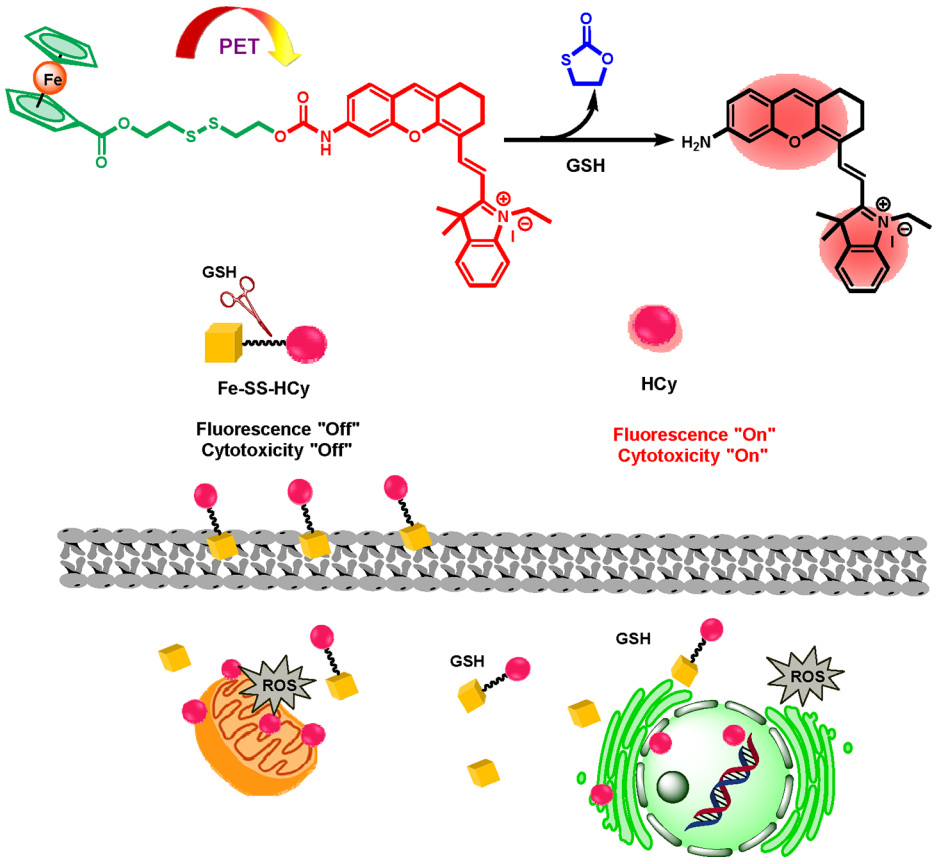

Figure 16. Representation of an organoiron-based prodrug having GSH-activable fluorescence emission and cytotoxicity.

inhibit tubulin polymerization that showed extremely high potency against lung cancer.[187] Complex (**7**), which contained a racemic mixture of 2-(2-propynyl)octanoic acid (POA) and an inert acetate as axial ligands, was established by Gabano et al. and exhibited better *in vivo* anticancer activity upon treatment against Lewis lung carcinoma (LLC).[188] Perillic acid (PA), which is an active metabolite of limonite, conveys a significant anti-metastatic property. Therefore, Ravera et al. developed highly active Pt(IV) prodrugs (**8, 9**) in combination with PA, and both complexes were much stronger than cisplatin, exhibiting significant toxicity against A549 cells in a multicellular spheroid model.[189]

7.1.2. GSH-Depleting Ruthenium–Platinum(IV) Hybrid Prodrug. Hypoxia is the situation of low oxygen content in the TME, which plays important roles in angiogenesis, metastasis and tumor recurrence. Therefore, the regulation of hypoxic condition and redox homeostasis can be regarded as a promising approach for cancer therapy. In this circumstance, the idea of "nanocatalytic medicine" unveils an efficient strategy, which adeptly alters the competency and selectivity of the prodrug into an effective therapeutic drug with reduced side effects. As a high concentration of GSH (10 mM) in cancer cell reduces the efficiency of cisplatin, Mao and co-workers contrived a plan to develop a platinum(IV)–ruthenium hybrid prodrug (Pt-Ru) for chemo-catalytic synergistic treatment of hypoxic tumors by decreasing the GSH concentration (Figure 15). It was observed that hybridization of ruthenium bestowed the Pt(IV) prodrug with multienzyme catalytic functions like mimicking the role of CAT to produce O_2 *in situ*, mimicking the activity of peroxidase (POD) to liberate the ROS and mimicking the role of GPX to deplete the increased level of GSH. Here, ruthenium was present in mixed-valence states, i.e., Ru^{3+} and Ru^{4+}, and played the role of a glue to affix the Pt(IV) complexes to it. Therefore, the administration of Pt-Ru hybrid prodrug effectively overcame the tumor hypoxic condition and revealed a remarkable antitumor proficiency compared to cisplatin both *in vitro* and *in vivo*. This study suggested that the developed Pt-Ru prodrug accelerated the efficiency of cisplatin being boosted with multienzyme activities to control tumor hypoxia and redox homeostasis. Hence, this approach will be a unique strategy to develop GSH-depleted, hypoxia-active platinum drugs.[190]

7.1.3. GSH-Activable Organoiron Theranostic Prodrug. A high GSH content in cancer cells plays the pivotal role in making the GSH-activable prodrug strategy encouraging. In practice, the monitoring of drug release can be made possible by designing theranostic prodrugs by tagging a fluorophore to the therapeutic drugs. Sun et al. developed a GSH-activable mitochondrial organoiron-based theranostic prodrug (**Fe-SS-HCy**), which was activated selectively by the elevated amounts of GSH in cancer cells and displayed enhanced efficacy and decreased systemic toxicity (Figure 16). It has been seen that the fluorescence property of theranostic prodrugs remains in the turned-on state. Therefore, it is important to design the prodrugs having a fluorescence "off–on" property, which can be utilized as a switch to track the drug release by turning on the fluorescence. This theranostic prodrug, **Fe-SS-HCy**, was made of a ferrocene moiety, GSH-activable disulfide linkage and fluorescent hemicyanine (HCy) for diagnosis and therapeutic effects. Ferrocene is renowned as a powerful quencher owing to its electron-donating ability, which can

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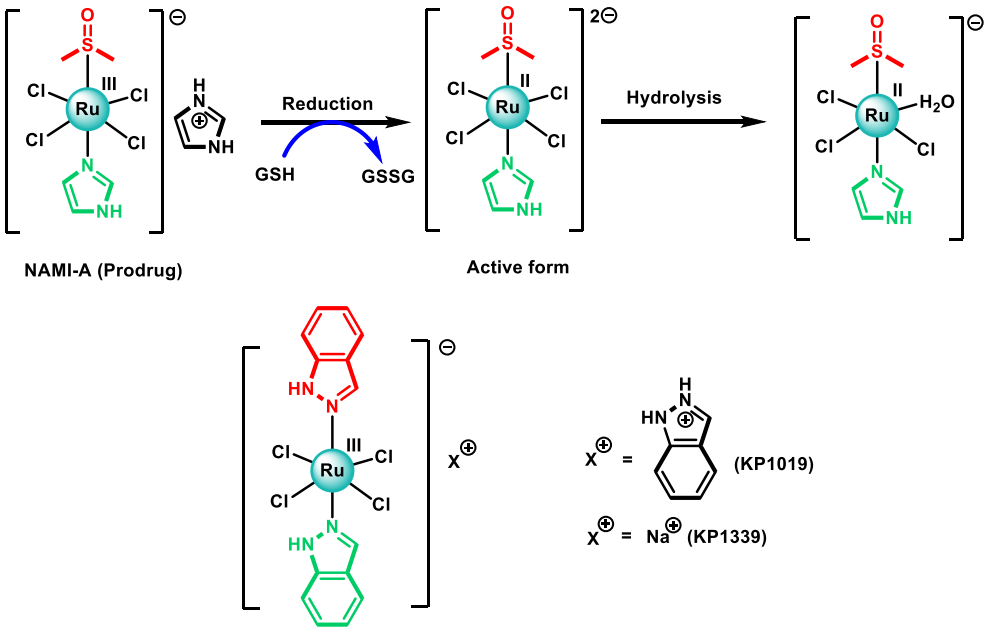

Figure 17. GSH-responsive Ru(III) prodrugs that entered clinical trials.

reduce the fluorescence intensity of organic fluorophores via a photoinduced electron transfer (PET) process. On the other hand, HCy has a profound therapeutic impact along with its strong red fluorescence property. Hence, the use of HCy is very promising for developing an ideal theranostic anticancer drug. Initially, the fluorescence of **Fe-SS-HCy** remains in the "off" mode because of the PET process by ferrocene. Although the cytotoxicity of **Fe-SS-HCy** prodrug is low, the function of this complex was initiated by overexpressed intracellular GSH, which cleaved the disulfide linkage in **Fe-SS-HCy** and helped to release HCy in cytosol as soon as **Fe-SS-HCy** was delivered to cancer cells. Then, the released HCy specifically accumulated in the mitochondria, where HCy generated ROS to destroy cancer cells, and immediately fluoroscence was "on" as HCy was freed from the ferrocene. This strong red fluorescence was utilized for monitoring the release of the drug in real-time. It is important to mention that the activation of **Fe-SS-HCy** by GSH is only possible in cancer cells, which further advances the selectivity for cancer tissues.[191]

7.1.4. GSH-Activable Organoruthenium Prodrug. Ruthenium complexes possess distorted octahedral geometry, having the oxidation states II to IV.[192,193] In 1952, Dwyer et al. observed that ruthenium complexes were highly biologically active,[194] and in the 1980s, Clark et al. reported prospective target-oriented anticancer Ru(III) complexes.[195] Ru(III) complexes have been observed to be activated in tumor tissue under low pH, high GSH and hypoxic conditions. It is noteworthy that innumerable ruthenium complexes have been developed in recent decades which are affluent in significant anticancer activities against lung cancer, and a few of them have revealed diminished side effects compared to cisplatin. Similar to the concept of Pt(IV) prodrugs, the first ruthenium-based anticancer prodrug that entered into clinical trials was NAMI-A (Figure 17).[196−198] NAMI-A was a Ru(III) complex bearing a heterocyclic N-donor ligand which showed low potency against primary tumors and exhibited high toxicity against lung metastases *in vivo*, and it underwent a phase I/II clinical trial for selective administration against lung metastasis.[199] Moreover, Keppler et al. established two analogues of NAMI-A, viz. KP1019 and KP1339, which also entered clinical investigations.[200,201] The mechanistic approach of these complexes revealed that complexes were reduced by GSH or ascorbic acid in cancer cells to form the active Ru(II) under physiological conditions and thereby exhibited anti-metastatic activity.

7.1.5. GSH-Activable Ruthenium(II) Two-Photon Azophotosensitizer. The concept of GSH depletion and creation of enhanced toxicity in cancer cell through unabated production of ROS led Chao et al. to construct a GSH-activable ruthenium(II) azophotosensitizer for two-photon (TP) photodynamic therapy.[202] PDT is very promising and well-established selective strategy for treatment of cancer, where photoactivated chemotherapeutics are activated upon irradiation with light. It has been visualized that photosensitizers trigger the production of cytotoxic singlet oxygen ($^1O_2$) after being excited with light of a particular wavelength. In most cases, a one-photon laser beam having high energy (e.g., UV, green or blue light) is employed for PDT, but that may cause unexpected light toxicity, whereas irradiation with NIR light is very popular, causing less photodamage and having the power of deep tissue penetration. Therefore, implementation of NIR light irradiation during PDT is more appropriate in biological systems. For this very reason, scientists are more prone to develop photosensitizers having the capability of being activated by TP-NIR light irradiation for PDT. For TP-PDT, less cytotoxic photosensitizers are generally used, which can absorb two photons less energy from the light in the infrared spectral range and then get excited to the higher energy electronic state. Then the excited molecules react with triplet oxygen to generate singlet oxygen to annihilate cancer cells.

As the intracellular GSH concentration is markedly higher than the extracellular levels, the aim of Chao et al. was to construct a Ru(II)-based photosensitizer associated with a GSH-activable azo bridging ligand to improve the targeting ability and tumor selectivity as well as drug potency. Therefore, this approach provides a more promising route for selective intracellular activation of the drug molecule after entering into cancer cells. Chao et al. reported a dinuclear Ru(II)-azo

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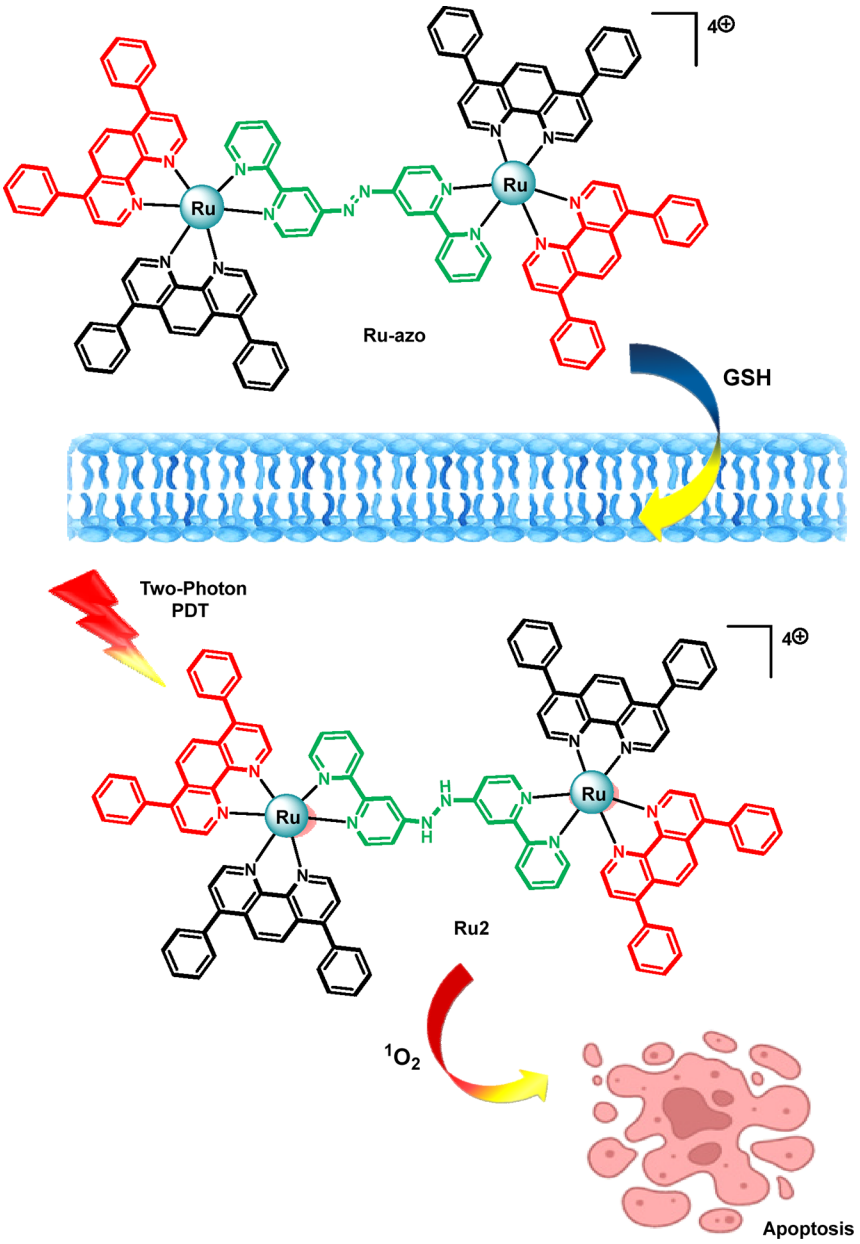

Figure 18. Representation of GSH activation of Ru-azo complex for two-photon phototoxicity.

complex using the ligand 4,4″-azobis(2,2′-bipyridine) (azobpy) for accomplishing TP-PDT (Figure 18). The azobipyridine complex has high electron-withdrawing capacity and hence acts as a lock to turn on or turn off the luminescence property of the Ru(II) complex, whereas GSH reduces the azo ligand and acts as the key for retrieving the optical properties of the photosensitizer. Moreover, the presence of four DIP co-ligands attributed the enhanced lipophilicity to the complex improving the cellular uptake.

7.1.6. GSH-Activable Organoiridium Prodrug. As cancer cells possess high contents of GSH compared to normal cells, Lo et al. applied the concept of prodrug release, constructed a few photoresponsive and GSH-activable Ir(III) complexes and studied their anticancer activities against HeLa cells. They reported two novel cyclometalated iridium(III) polypyridyl polyethylene glycol (PEG) complexes with a disulfide (S−S) linkage as thiol-responsive metallo-therapeutics (Figure 19). The attractive concept of a disulfide linkage was utilized as the disulfide remains stable in the bloodstream and, on the other hand, can be cleaved by intracellular thiols like GSH. Therefore, this concept facilitates the consumption of excess intracellular GSH, which simultaneously increases the drug potency as well. Moreover, the incorporation of a PEG pendant with phosphorescent iridium(III) polypyridyl complexes through a S−S bond can also bring about stimuli-responsive cleavage of prodrugs, triggering the release of the cytotoxic drugs. It was observed that the disulfide linkage was broken down and iridium(III) complexes $[Ir(N^C)_2(bpy\text{-}SH)]^+$ (N^C = pqe, pq) were released upon interaction with GSH, which was examined by ESI-MS. The study revealed that PEG-attached complex **1a**, having a disulfide linker, displayed higher cytotoxicity toward HeLa cells than that of its disulfide-free analogue **1c**, as the complex **1c** was deprived of attachment of PEG pendant through disulfide linkage, which would cause GSH-mediated release of $Ir(pqe)_2(bpy\text{-}SH)]^+$ via the breaking of the S−S bond. However, the bimetallic

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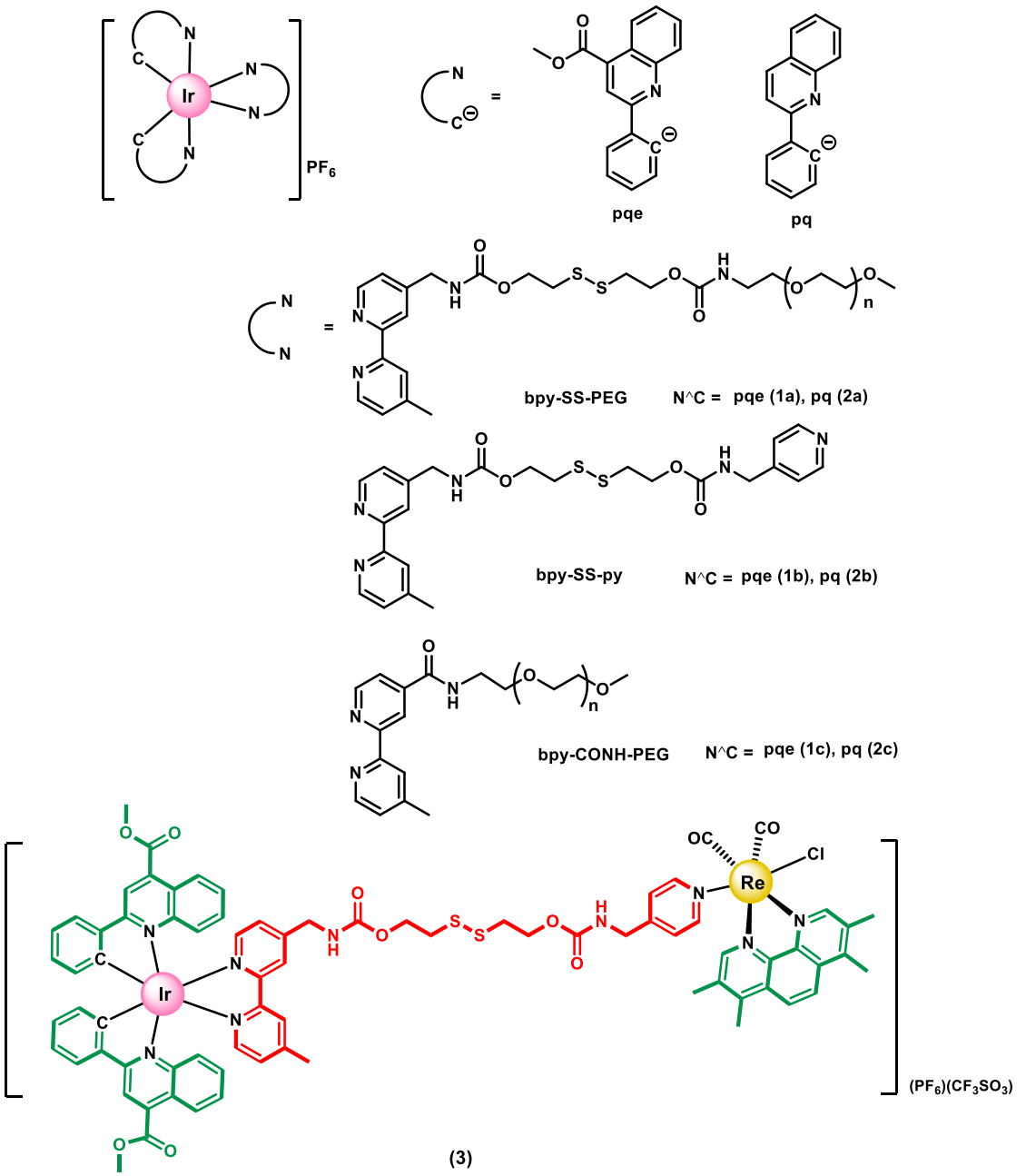

Figure 19. Structures of GSH-activable iridium(III) prodrugs.

complex **3** was poorly taken up in live HeLa cells, which might be attributed due to its bipositive charge. In addition to this, thiol-sensing complex **3** was constructed by the combination of a green-emitting rhenium(I) complex with Ir(III)-polypyridyl complex to establish a fluorescence resonance energy transfer (FRET) couple. All the complexes were eligible to display long-lived and intense green to red emission upon photoexcitation. However, under ambient conditions the heteronuclear bimetallic complex **3** unveiled red emission because of prominent FRET from the rhenium(I) to the iridium(III) component.[203]

7.2. GSH-Activable Ion Transporter. *7.2.1. Cyclometalated "Off–On" Switchable Anion Transporter.* Transmembrane transport of ions is assumed to be the essential biological process involved in various physiological functions like neuroexcitation, cell proliferation and migration, muscle contraction, in maintaining membrane potential, in maintaining cellular pH and cellular secretions. Recently, anion transporters have displayed prospective applications as promising anticancer agents, conveying cytotoxicity by disrupting the redox homeostasis and thereby prompting the cell death. As the GSH concentration is higher in tumors than in healthy tissues, the approach of using GSH-activable ion transporters offers a route to target the tumors. Therefore, Gale and co-workers developed cyclometalated gold complexes as switchable anion transporters that were "switched on" *in situ* by dint of GSH reducing through the decomplexation of gold (Figure 20).

They synthesized novel switchable cycloaurated anion transporters based on 1,3-bis(benzimidazol-2-yl)pyrimidine (BisBzImPy). Gold N-heterocyclic carbene or gold chloride was used as blocking agent to make the switchable complexes.

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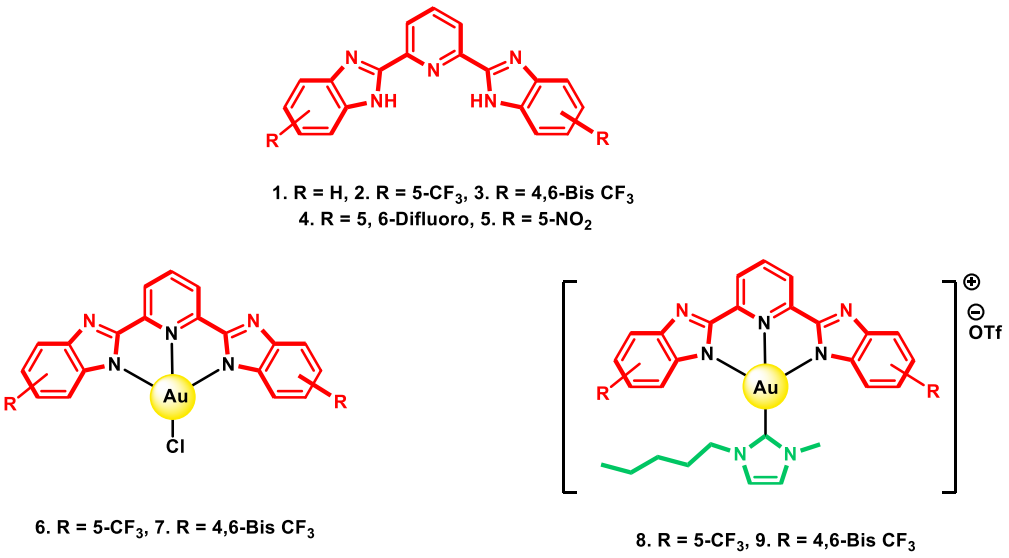

Figure 20. Structures of GSH-active gold complexes as ion transporters.

These complexes were designed in such a way that they will be reduced by the stimulating effect of GSH to liberate the anion transporters in the cytoplasm as soon as the complexes enter into the cells. Consequently, the liberated gold can bind chloride and then expedite the transmembrane transport of ions.[204]

7.3. Metal Complexes, Glutathione Depletion and Anticancer Activity. *7.3.1. Titanium Complexes.* Budotitane and titanocene dichloride were the first nonplatinum titanium-based anticancer metallodrugs to reach clinical trials. But they were not recognized as promising therapeutics because of their insufficient stability in aqueous medium and because they decomposed in the biological environment. This shortcoming of budotitane and titanocene dichloride led researchers to think of developing better titanium-based anticancer agents having good aqueous stability. As a consequence, Tshuva et al. established phenolato-Ti(IV)-based anticancer chemotherapeutics having improved hydrolytic stability (Figure 21). They

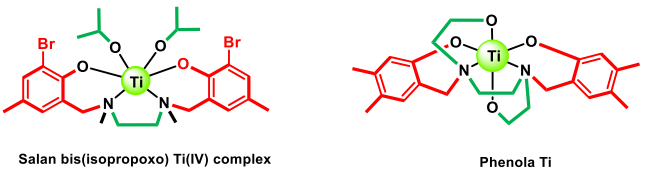

Figure 21. Structures of heteroleptic Ti(IV) complexes.

observed that the complexes exhibited extreme cytotoxicity *in vitro* and *in vivo* against A2780 ovarian carcinoma and HeLa cervical adenocarcinoma cells with enriched hydrolytic stability. Although the MoA of these complexes was not precisely detected initially, they were capable of exhibiting p53-dependent apoptosis upon significant accumulation in both the mitochondria and nucleus. It was monitored that PhenolaTi was stable in aqueous medium as well as in other biological media for weeks. Also, these complexes were highly selective to cancerous cells compared to noncancerous cells. It was thought that, like cisplatin, these complexes might interact with DNA upon displacement of labile ligands. Glutathione is the primary antioxidant present in human cells, and NADPH is capable of restoring the GSH from GSSG. Hence, Tshuva et al. investigated the influence of the mitochondrial ROS on cytotoxicity in two ways: (1) by monitoring the elevation or reduction of the mitochondrial GSH level upon addition of the complex and thus observing the affect of oxidative stress on the cells or (2) by scavenging the mitochondrial ROS. They also studied the interaction of PhenolaTi with the GSH biosynthesis regulator *N*-acetylcysteine (NAC) with respect to the well-known GSH biosynthesis inhibitor BSO. The result demonstrated that PhenolaTi was capable of depleting the GSH level by arresting the biosynthesis of GSH upon interaction with NAC. Therefore, the cells experienced oxidative stress with elevated amounts of ROS, and thus the cells were unable to resolve the oxidative stress, which ultimately led to apoptosis. Above all, it was identified that the main cellular MoA of PhenolaTi was the creation of ER stress under hypoxia, and generation of ROS in the mitochondria and ER was pointed out as the possible target organelle for the cell death mechanism of PhenolaTi.[205]

7.3.2. Copper Complexes. Copper is a very important group 11 transition metal, which plays imperative roles in the human body in keeping the immune system and nervous system healthy and helping the formation of red blood cells and collagen; it also has a key role in bone and connective tissues. Moreover, it exhibits antioxidant properties along with the power of damaging DNA. Therefore, the study of anticancer activities of copper complexes started long ago. Recently, Seco et al. developed Cu(II) complexes coordinating with tetradentate 2-{[(3-chloro-2-hydroxypropyl)pyridin-2-yl-methylamino]methyl}phenol ligand in view of mimicking the antioxidant metalloenzymes CAT and SOD along with the depletion of the GSH level (Figure 22). These complexes were

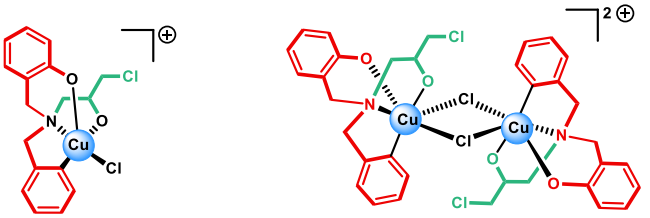

Figure 22. Structures of glutathione-depleting Cu(II) complexes.

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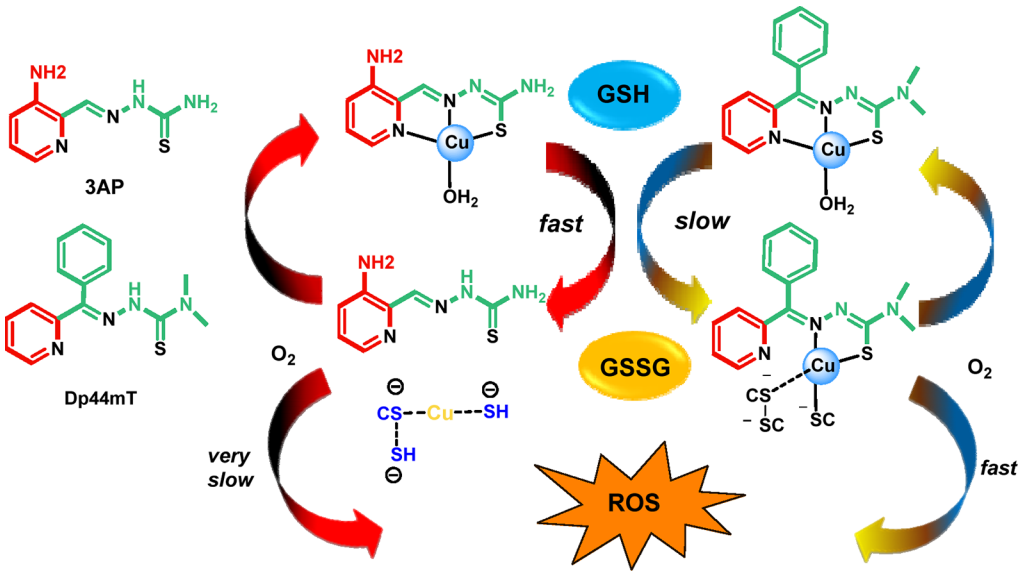

Figure 23. Structures of glutathione-depleting 3AP- and Dp44 mT-based Cu(II) complexes.

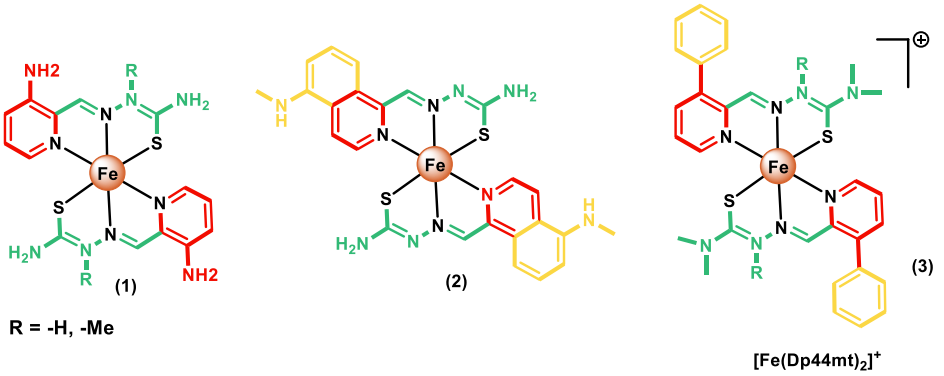

Figure 24. Structures of Fe(II) and Fe(III) complexes.

capable of interfering in the intracellular ROS as well as interaction with some signaling biomolecules related to initiation of the epithelial−mesenchymal transition (EMT) in cancer cells to resist migration and to diminish the aggressiveness of cancer cells. The researchers visualized that, although the complexes showed moderate toxicity (IC_{50} = 82 ± 1 μM), they were very efficient to rectify the other aspects of cancer development. These complexes acted as mimetics of SOD and CAT in H4 glioma cells compared to control along with the obstruction in relocation of monolayer-grown H4 cells by decreasing the expression of EMT markers. They were also competent to impart GSH metabolism and they evidently dropped the intracellular GSH/GSSG ratio by depleting the GSH level.[206]

However, these results are highly anticipative, as modulation of GSH levels leading to the tumor cells being sensitized toward treatment modalities with anticancer therapeutics is the subject of this Review. Therefore, these types of copper complexes can be assumed to be good candidates to fulfill the goal with their remarkable impact on the invasive ability of cancer cells.[206]

Triapine (3AP) and di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44 mT) (Figure 23), which are α-pyridyl thiosemicarbazone (TSC)-type molecules, are well-known for their astounding anticancer activities. Therefore, Sicilia et al. prepared 3AP- and Dp44 mT-based ROS-generating Cu(II) complexes in order to investigate their anticancer potential by withstanding elevated GSH levels in cancer cells. Although 3AP underwent more than 30 clinical trials, it showed undesirable effects, and thus Dp44 mT was developed subsequently. A Cu(II) complex with the Dp44 mT ligand, which is presently in clinical trials (NCT02688101), exhibited the highest anticancer competency compared to the Cu(II) complex with 3AP through balancing between moderate depletion of GSH level and ROS generation. The researchers determined that the mode of anticancer activity of these complexes was through iron chelation along with the hindrance of the activities of the Fe-dependent enzyme such as ribonucleotide reductase and thereby resisted the cell proliferation ceasing the DNA synthesis.[207]

7.3.3. Iron Complexes, Glutathione Depletion and Anticancer Activity. Iron is very essential metal in the human body. It is the key element for constructing hemoglobin, indispensable for carrying oxygen from the lungs to all parts of the body. As the semicarbazole moiety is very important in cancer therapy, as discussed already, Plamthottam et al. reported three complexes of iron(II) and iron(III) with different semicarbazole ligands (Figure 24). Activation of an Fe(III)-triapine complex upon reduction with thioredoxin reductase-1 (TrxR1) and GR led to the formation

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ACS Omega 2024, 9, 20670−20701

of RS. Especially, TrxR1 exhibited high activity with Fe(III)−thiosemicarbazone derivatives. The specific characteristics of the Fe(III) complexes and the redox centers of TrxR were also demonstrated.[208] Among the complexes, the Fe(II)(triapine)$_2$ fragment was redox-active and triggered the human ribonucleotide reductase (RNR) inhibition. Iron complexes with triapine analogues (Figure 24) showed remarkable *in vitro* toxicity and demonstrated that redox events were important for RNR inhibition. They were also capable of inhibiting the proliferation of cells at similar or lower concentrations (250 nM to 0.7 μM) compared to triapine alone.[209]

Iron(III) complex **3** (Figure 24) with a thiosemicarbazone derivative was reduced with the application of ascorbate ions to iron(II), which facilitated the lipid peroxidation. Upon addition of ascorbate to the iron(III) complex, the ascorbyl radical anion (Asc^-) was formed, resulting in the production of profuse ROS.[210] Moreover, complex **3** exhibited promising anti-proliferative activity against the human melanoma cell line (SK-MEL-28), having IC_{50} = 3.125−25 μM, in the presence of 1000 μM of ascorbate.[211] The Fe(II) complex in Figure 25

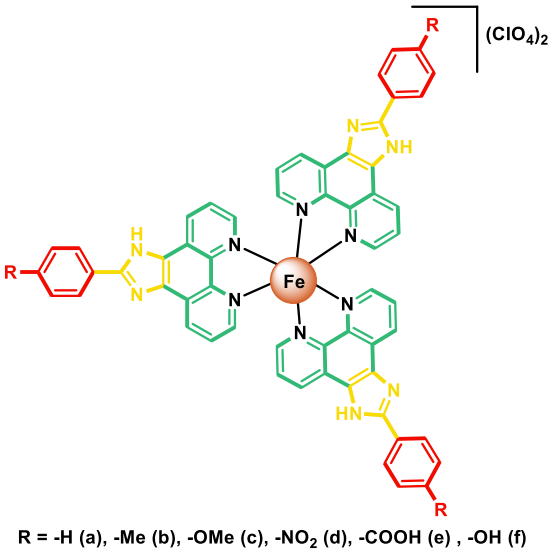

Figure 25. Structure of imidazophenanthroline-based glutathione-depleting Fe(II) complex.

showed appreciable cytotoxicity. Among all the complexes, the complex with methoxy group substitution on the ligand exhibited outstanding cytotoxicity against CaSki cancer cells. The IC_{50} values for the methoxy-substituted complex were 0.75, 6.73, 7.32 and 23.71 μM for Caski, SiHa, HeLa and LO2 cells, respectively. It was observed that complex (**c**) inhibited the cancer cell growth following an apoptotic pathway. In addition to this, it exhibited the TrxR activity.[212]

7.3.4. Ruthenium Complexes, Glutathione Depletion and Anticancer Activity. For many decades, ruthenium complexes have been proved as promising and selective anticancer agents, and a few of them are in clinical trials (Figure 26). Although researchers have been developing many ruthenium complexes and studying their anticancer mode of action, the investigation of their GSH-depleting potency is still under the mask. Therefore, the GSH-depleting capability of previously developed ruthenium complexes should be investigated to open a new facet for enhancing their anticancer potency, as GSH is a chief reducing agent, liable for cellular detoxification of transition metals and ROS/RNS. However, very few

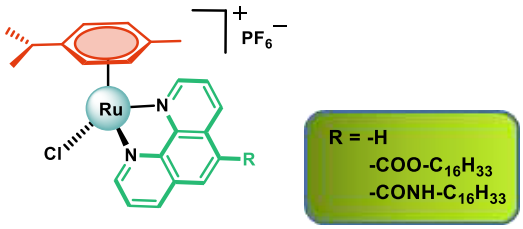

Figure 26. Structure of phenanthroline-based glutathione-depleting Ru(II) complexes with an appended C16 fatty acid chain.

scientists have emphasized the GSH-depleting character of some ruthenium complexes. For example, Berger and co-workers studied the MoA with the prepared ruthenium(II)−*p*-cymene complexes, and they concluded that these complexes triggered cancer cell death through apoptosis with the ability for GSH depletion. As observed before, NAC is the key entity to restore GSH level, scavenge ROS and diminish the anti-proliferative activity of metal complexes. Therefore, they screened the cytotoxicity of the complexes against Hs683 and A549 cancer cell lines in the presence of a Cys precursor, NAC, as Cys is the main precursor for GSH synthesis in the intracellular medium. They visualized a decent but significant increase in the IC_{50} value in case of A549 cell line but it was totally different for Hs683, suggesting that GSH depletion occurred prominently in the case of Hs683 by either a direct or an indirect mechanism.[213]

In connection with this, Chao et al. prepared a set of three ruthenium(II) complexes (Figure 27) in a quest to investigate their anti-metastatic and anti-proliferative functions toward a series of cancer cell lines using MDA-MB-231 (highly aggressive triple-negative breast cancer), MCF-7 (breast cancer), SW620 (colon cancer) and SW620AD300 (drug-resistant colon cancer) cell lines. The percent of selectivity toward cancer cells was justified by screening their activity against normal healthy human cells like MCF-10A (normal breast cells) and LO2 (normal liver cells). The anticancer activities of the complexes were well-justified by their mitochondrial accumulation, ROS generation, disruption of physiological processes, which includes the redox balance, GSH depletion, elevation of iron content and creation of disturbance in energy generation in cancer cells. The three Ru(II) complexes progressively decreased the intracellular GSH level in the respective cancer cells and pushed the cancer cells toward oxidative damage by ROS. Moreover, they successfully depleted the ATP and thereby cut down the energy sources for surviving. The outcomes of the transwell invasion assay, the wound-healing assay and the tube formation assay proved their anti-angiogenesis and anti-migration properties. Upon elevation of the intracellular iron content, these complexes were capable of converting intracellular H_2O_2 to the extremely toxic $OH^\bullet$ radical and depleting the intracellular GSH level, leading the cancer cells to failure in combating against excessive production of ROS. These complexes were also competent to downregulate the expression level of VEGF and thus exhibited significant anti-metastatic properties. However, they brought about cancer cell death through the apoptotic pathway.[214]

Sadler et al. developed new approaches for designing catalytic metallodrugs. Their main objective was the construction of redox-modulating drugs capable of depleting the GSH level through thiol oxidation and subsequent hydrogenation reactions. This concept is actually very beneficial to

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ACS Omega 2024, 9, 20670−20701

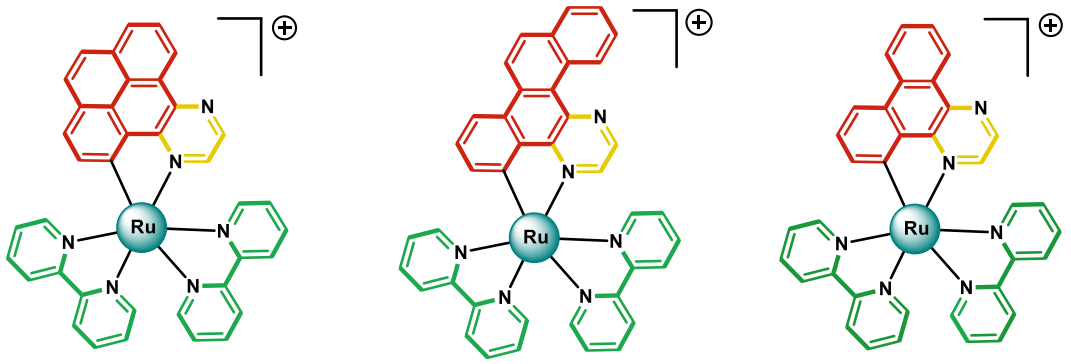

Figure 27. Structures of glutathione-depleting anti-proliferative Ru(II) complexes.

GSH depletion and simultaneous retention of the drug. To achieve their goal, they synthesized a Ru(II)−arene complex, [(η^6-arene)Ru(azpy)I], and screened its anticancer potential against A549 and A2780 cancer cell lines, observing IC_{50} values from 2 to 6 μM. It was very interesting that the substitution of iodide ligand by chloride ligand vividly diminished the potential of the concerned Ru(II)−arene complex. Of note, the Ru(II) iodide complexes acted as a catalyst and took part in the oxidation reaction successfully with GSH (γ-L-Glu-L-Cys-Gly), converting the few millimolar GSH to GSSG upon treatment with micromolar concentrations of the Ru(II)−arene complex (Figure 28).[215]

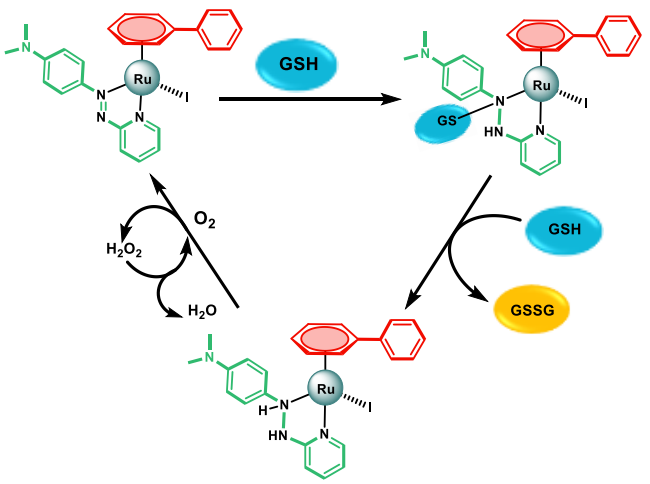

Figure 28. Catalytic action of glutathione-depleting Ru(II) complex.

7.3.5. Iridium Complexes, Glutathione Depletion and Anticancer Activity. In recent times, the importance of iridium metal in cancer therapy is receiving much more attention due to its exceptional chemical and photophysical properties. As the high intracellular GSH level is a major obstruction for cancer therapeutics, the ability of iridium complex to deplete the GSH level has become an interest of current studies. Among various cell death mechanisms, ferroptosis is now considered one of the significant mechanisms, which is a result of two salient factors such as lipid and iron metabolisms. It has been found that excessive iron can generate profuse ROS in cells through the Fenton reaction or by the activation of certain enzymes to bring about lipid peroxidation. The activation of cytochrome P450 oxidoreductase (POR), lipoxygenases (LOXs) and NADPH oxidases (NOXs) has been regarded as a way to enhance the ferroptosis process through lipid peroxidation, whereas inhibition of polyunsaturated fatty acids (PUFAs) synthesis restricts ferroptosis. It was already mentioned that GPX4 can directly transform phospholipid hydroperoxide to hydroxyphospholipid and thus suppress the ferroptosis. On the other hand, expression of GPX4 in the cellular medium depends on GSH. Therefore, anticancer agents having the potential to deplete the GSH level can downregulate the expression of GPX4 and thereby facilitate the ferroptosis process. According to the literature survey by Guo et al., Wang et al. developed the first anticancer iridium(III) complex that was able to cause ferroptosis. The mitochondria-targeting iridium(III) complex **IrL2** (Figure 29) showed

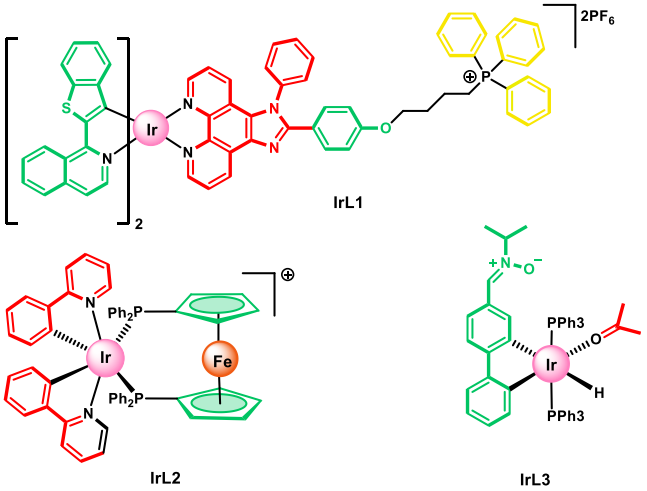

Figure 29. GSH-depleting iridium complexes causing cell death through ferroptosis.

promising cytotoxicity against A2780 cells, triggering heme oxygenase-1 (HMOX1)-dependent ferroptosis. Higher expression of HMOX1 in the cellular medium accelerated the heme metabolism, which increased the level of intracellular iron and enhanced the ferritin production. As a result, a large amount of ROS accumulated in cells, leading to lipid peroxidation. It has also been well studied that iridium complexes also induce ferroptosis through PDT. For instance, Yuan et al. synthesized two iridium photosensitizers, viz. **IrL1** and **IrL3**, which were used as ferroptosis inducers. It was recorded that both of them generated $OH^{\bullet}$ and $O_2^{-\bullet}$ radicals upon irradiation with light, following a type I photodynamic process along with the capability of GSH depletion. Therefore, the amount of ROS liberated by these complexes was exhausted by GSH, which initiated the lipid peroxidation and ultimately caused

https://doi.org/10.1021/acsomega.3c08890
ACS Omega 2024, 9, 20670−20701

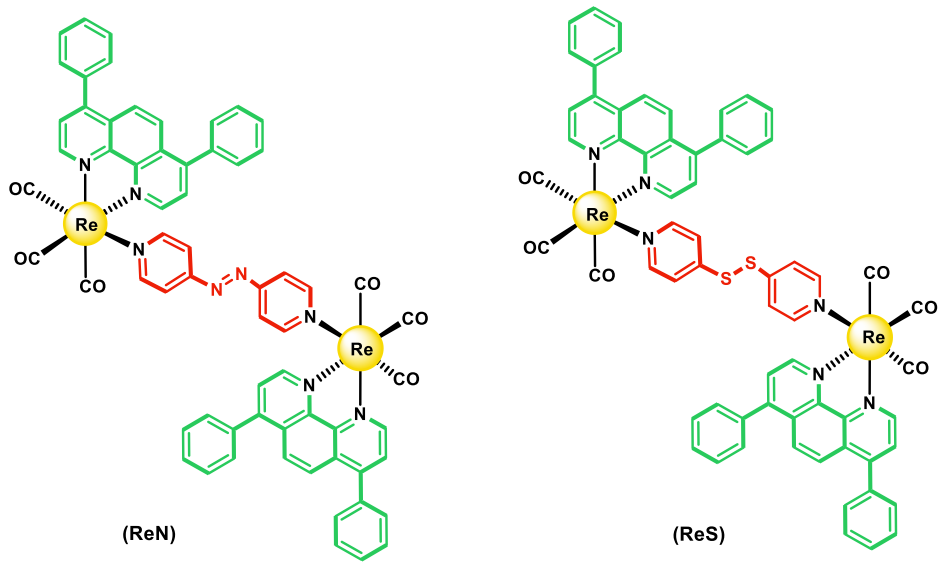

Figure 30. Structures of mitochondria-targeting, glutathione-depleting and SOD-deactivating Re(I) complexes.

ferroptosis. Moreover, the mitochondria-targeting **IrL1** complex was competent to create synergism of apoptosis and ferroptosis, exposing a substantial inhibition effect toward apoptosis-resistant cancer cell lines (Figure 29).[216]

7.3.6. Rhenium Complexes, Glutathione Depletion and Anticancer Activity. Rhenium complexes are now emerging as very active anticancer agents. These types of complexes are now well established to exhibit their anticancer potential by triggering any of the cell death mechanisms. But the capability of rhenium complexes to deplete the intracellular GSH is not explored clearly. Therefore, very few examples are there in this regard. Mao and co-workers developed mitochondria-targeting rhenium complexes, **ReN** and **ReS** (Figure 30), which showed excellent anticancer activities against various cancer lines. They induced the cell death through necroptosis along with caspase-dependent apoptosis by deactivating the role of SOD. These complexes created irreversible oxidative stress and disturbed the GSH metabolism process.[217]

8. CONCLUSION AND FUTURE PERSPECTIVE

We have endeavored to unveil the role of glutathione in maintaining the cellular redox balance and its importance in regulating cellular physiological events. As in cancer cells its concentration is exceptionally high, this situation makes the potential of many anticancer drugs very pitiable. Therefore, the depletion of intracellular GSH levels has now been considered to augment the therapeutic potential of anticancer metallodrugs which are being utilized for ROS-based cancer therapy. But the effects of GSH depletion in cancer therapy have not been explored so far. Therefore, we have tried to uncover the role of GSH depletion in cancer therapy by the disruption of the function of the most important antioxidant, GSH, as it is the chief regulator in maintaining the redox balance in the cell. Herein, various cell death mechanisms related to GSH depletion and the importance of GSH depletion in cancer therapy have been described. It is hoped that this Review provides the deep insight needed for designing anticancer drugs which will be boosted with the capability of both GSH depletion and ROS generation to annihilate cancer cells in the body and therefore that this informative Review will help to accelerate studies of the anticancer potential of metal-based cytoselective anticancer drugs in the near future.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Science and Technology, Government of India, for supporting the work through the DST-SERB CRG project grant (CRG/2021/002267). The authors thank Vellore Institute of Technology for arranging VIT SEED funding.

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