Emerging Roles of Green-Synthesized Chalcogen and Chalcogenide Nanoparticles in Cancer Theranostics

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The last few decades have seen an overwhelming increase in the amount of research carried out on the use of inorganic nanoparticles. More fascinating is the tremendous progress made in the use of chalcogen and chalcogenide nanoparticles in cancer theranostics. These nanomaterials, which were initially synthesized through chemical methods, have now been efficiently produced using different plant materials. The paradigm shift towards the biogenic route of nanoparticle synthesis stems from its superior advantages of biosafety, eco-friendliness, and simplicity, among others. Despite a large number of reviews available on inorganic nanoparticle synthesis through green chemistry, there is currently a dearth of information on the green synthesis of chalcogens and chalcogenides for cancer research. Nanoformulations involving chalcogens such as sulfur, selenium, and tellurium and their respective chalcogenides have recently emerged as promising tools in cancer therapeutics and diagnosis. Similar to other inorganic nanoparticles, chalcogens and chalcogenides have been synthesized using plant extracts and their purified biomolecules. In this review, we provide an up-to-date discussion of the recent progress that has been made in the plant-mediated synthesis of chalcogens and chalcogenides with a special focus on their application in cancer theranostics.

1. Introduction

The complexity of successfully eradicating cancer cells without affecting normal cells has been a challenge with cancer chemotherapy. This has resulted in extensive research to develop drug delivery vehicles capable of selectively targeting cancer cells. In the past two decades, tremendous progress has been made in developing nanoparticles (NPs) as delivery agents for anticancer drugs, peptides, nucleotides, antibodies, and proteins in cancer therapy [1–3]. NPs are usually below 100 nm in size and hence possess efficient permeation ability and a large surface area to volume ratio, allowing functionalization with small molecules such as cofactors, metal ions, and polymers [4]. This is critical for their role in drug targeting, gene delivery, cancer diagnosis, and other biomedical applications [5]. NPs display outstanding physical and chemical properties, which vary according to their shape and size. The different forms of NPs synthesized thus far include nanorods, nanoshells, nanocages, nanoprisms, nanoclusters, nanowires, and nanostars. The dimension and morphology of NPs depend on their precursor materials, method, and synthesis conditions, which in turn affect the functionality of the NPs.

NPs can be made from materials such as metals, ceramics, semiconductors, organic polymers, inorganic polymers, and lipid biopolymers. Of particular interest in cancer research are metallic NPs, semimetals, and lipid biopolymers, due to their small size, ease of functionalization, and biocompatibility. While metallic NPs have long been employed in cancer therapy, semiconductors, especially chalcogens and chalcogenides (semiconductor nanocrystal with quantum confinement property), are beginning to gain significant attention [6, 7]. Chalcogens derive their name from the Greek words “chalkos” and “genes,” meaning “ore” and “born,” respectively [8]. They are traditionally associated with elements belonging to group sixteen of the periodic table such as sulfur, oxygen, selenium, tellurium, polonium (a radioactive metalloid), and livermorium (a
synthetic element) [8]. Sulfur, selenium, and tellurium are the most studied of these metalloids as nanocomposites with potential for cancer therapeutics. Sulfur and sulfur-containing compounds have been traditionally used as fertilizers, antimicrobial, and antifungal agents. However, at the nanoscale, sulfur possesses interesting properties such as biodegradability, nontoxicity, and biocompatibility, with significant applications in catalytic bioremediation and as antimicrobial and anticancer agents [9–12]. Selenium is a metalloid with chemopreventive properties that functions physiologically as a micronutrient. Its importance also stems from its ability to participate in the de novo synthesis of selenoproteins and enzymes. So far, selenium nanoparticles have been well studied for their biocompatibility, optical, and photoconductive properties [13]. Tellurium is derived from the Latin word “tellus,” which means Earth. With chemical and structural relationships to those of selenium, tellurium also demonstrates appreciable pharmacological activities; however, unlike selenium, it is not an essential mineral and is not critical for any biological activity in humans. It exists in different oxidation states as telluride (−2), elemental tellurium (0), tellurite (+4), and tellurate (+6) [14].

Chalcogens such as sulfur, selenium, and tellurium combine chemically with other metals at the nanoscale to form alloys known as chalcogenide NPs. They can be grouped as mono-, di-, and polychalcogenides depending on the number of different chalcogens they contain. Chalcogenides have also been grouped as binary or ternary nanocrystals [15]. The former consists of two distinct elements (e.g., Cu2Se and ZnS) combined in the same or different ratio, while the latter is an aggregation of three distinct elements (e.g., CuInSe2 and CuGaSe2). Chalcogenide NPs synthesized thus far include PbS, PbSe, CdS, PbTe, PbSeS, PbSeTe, PbSTe, Ag2S, Ag2Se, Bi2Se3, ZnSe, ZnS, CuS, CdTe, and CdS.

Structurally, chalcogenide nanoassemblies exist in the form of nanoclusters, NPs, and quantum dots. Similar to other nanoassemblies, their properties depend on size and morphology. Generally, these NPs are synthesized using top-down and bottom-up approaches. The former involves reducing a bulky starting material by lithographic techniques into small particles, while the latter involves the assembly of smaller units into nanosized particles either by chemical or biological approaches (biogenic synthesis) [16]. Chemical synthesis of NP includes the dispersion of preformed polymers, polymerization of monomers, and ionic gelation method [17]. These techniques are usually swift and require capping agents to stabilize the NPs. In spite of its efficiency, the toxicity of chemical reductants and their side products generated during the process constitute a challenge for biologic applications, necessitating the use of eco-friendly biological agents for NP synthesis.

While there have been a large number of reviews on NP synthesis via green chemistry, the green synthesis of chalcogens and chalcogenides remains under-reported despite its growing potential in recent years. Over the years, several biogenic agents have been explored to synthesize chalcogens and chalcogenides (Figure 1). This review explores the current knowledge on the green synthesis of chalcogens and chalcogenides with respect to their potential in cancer theranostics.

2. Optical and Electronic Properties of Chalcogenide Quantum Dots

Chalcogenide NPs have attracted significant research interest because of their optical and electronic properties. This category includes quantum dots (QDs), which are metalloids with particle size diameters of 1–20 nm, and size-dependent tunable photoluminescence properties [18, 19]. In a semiconductor, at the highest energy level, the electron combines with the hole by Coulomb’s force to form excitons and returns to the ground state [20]. During this process, photoluminescent energy is emitted at an intensity that corresponds to the bandgap energy, confinement energy, and energy of bound excitons (equation (1)). The energy levels can be modelled using a particle in a box [21].

\[
E = E_c + E_b + E_e,
\]

where \( E = \text{total energy} \), \( E_c = \text{energy of confinement} \), \( E_b = \text{bandgap energy} \), and \( E_e = \text{energy of excitons} \). The energy of confinement within a three-dimensional box has been given as follows:

\[
E_c = \frac{\hbar^2 \pi^2}{2 \alpha^2} \left( \frac{1}{m_h} + \frac{1}{m_e} \right),
\]

where \( m_h \), \( m_e \), and \( \alpha \) correspond to the mass of the hole, the mass of free electron, and radius of the quantum dot, respectively. The energy of confinement can be controlled by varying the particle size. At reduced masses, the equation becomes

\[
E_c = \frac{\hbar^2 \pi^2}{2 \mu \alpha^2},
\]

where \( \mu = \text{reduced mass} \).

More so, the energy of the excitons is given as “Coulomb’s energy of electron-hole interaction” [22] expressed by the following equation:

\[
E_e = \frac{1}{E_s} \times \frac{\mu}{m_e} \times R_y
\]

or

\[
-\frac{1.8 e^2}{4 \pi \varepsilon_0 \varepsilon_r}
\]

where \( E_s \) and \( R_y \) connote size-dependent dielectric constant and Rydberg’s energy, respectively.

Combining the equations, the total energy of the quantum dot is given as follows:

\[
E = E_b + \frac{\hbar^2 \pi^2}{2 \alpha^2} \left( \frac{1}{m_h} + \frac{1}{m_e} \right) - \frac{1.8 e^2}{4 \pi \varepsilon_0 \varepsilon_r}
\]

In a three-dimensional quantum box, the band and excitons energy are infinitesimal and can be ignored [23]. The energy of the photon is given as Eigen energy expressed in 3D time-harmonic Schrodinger’s equation as follows:
The above equations are theoretical assumptions that describe the electronic transitions of semiconductor quantum dots. They highlight the role of quantum confinement in the electronic behaviour of quantum dots [22]. Quantum confinement varies inversely with the size of quantum dots; hence, larger particles do not show the quantum confinement effect [24]. The confinement of charge carriers (such as excitons) in a small region of space less than the carrier’s wavelength results in a unique quantization effect [25–27]. Quantum confined NPs show varying photoemission spectra, which are dependent on their size. By tuning their sizes, they can produce fluorescent emissions of different wavelengths (400–700 nm; Figure 2). QD with very small dimensions have the high surface area to volume ratio and reflect a blue photoluminescent shift, while larger QDs show a redshift [28]. It is therefore expedient to ensure a size-controlled synthesis of chalcogenide, which would show desirable optical and electronic properties.

3. Biogenic Synthesis of Chalcogen and Chalcogenide Nanoparticles Using Plant Extracts

This approach involves the use of plant materials in form of plant juice or solvent extracts from the plant material. Plants contain different secondary metabolites that give them the ability to reduce heavy metals and other metallic ions. The use of plant juice and aqueous extracts has several advantages over other conventional biogenic syntheses approaches because it does not require a tedious downstream technique for extraction and purification of the synthesized NPs. The safety and ability to serve as a reducing and a capping agent are added to the list of properties that make this approach a preferred choice [30]. There are many NPs in this category that have found applications in various fields of biotechnology [31].

Different parts of the plant, from the more common leaves, fruit, and bark to the less common buds, seeds, peels,
and pulps, have been used to synthesize NPs [32]. *Capsicum annuum* extracts have been used to reduce selenite to SeNPs [33], while leaf extracts of *Azadirachta indica*, *Catharanthus roseus*, and *Mangifera indica* demonstrated efficacy in the reduction of sulfur ions in sulfur compounds to produce sulfur NPs (SNPs). The underlying reaction appears to proceed via the formation of a stable complex between sulfur ions and the plant phytoconstituents, possibly mediated by electrostatic interaction. Electrons are transferred from the organic molecules to reduce $S^{2-}$ to SNPs [34].

Generally, the suitability of plants as reductants in the synthesis of NPs is a function of the antioxidant content of the plant parts. The first successful plant extract-mediated synthesis of quantum dots was reported by Borovaya et al. (2014), using the *Linaria* spp. root extract to reduce cadmium sulfate to CdS NPs (Figure 3). The CdS nanoparticles produced were stable for about 10 days [35]. Following this study, other chalcogenide quantum dots, such as CdTe, CdSe, and ZnS, were synthesized using plant extracts [36–38]. Recently, a three-step mechanism of CdSe synthesis was proposed: (1) extract-mediated reduction of metallic ion to zero-valent state nanocrystal by intermixing metal salt precursors with plant extracts, (2) spontaneous coalescence between nanocrystals known as Ostwald ripening, and (3) preferential alignment of nanocrystals along regions of phase transformation [39]. While proteins, flavonols, phenols, glycosides, and other phytochemicals are being examined for their critical role in the reduction and capping of the NPs, further studies are necessary to understand the plant-based chalcogenide synthesis process [38, 40].

### 4. Green Synthesized Chalcogens and Chalcogenides with Anticancer Potential

Several chalcogens and chalcogenides have shown outstanding physicochemical and pharmacological properties crucial for cancer prevention and chemotherapy. While these properties have been attributed to the role of the metalloid constituents, the morphology, and the size of the particles, it should be noted that the green syntheses of the NPs confer additional advantages, since the metabolites from the plants may sometimes also act as capping agents [41]. We will look further at some of the potential anticancer applications of these green synthesized NPs (Table 1).

#### 4.1. Green Synthesized Chalcogens

Chalcogens, predominantly sulfur, selenium, and tellurium, have been synthesized through green chemistry. Although they have certain similarities in their electronic structure, these elements have distinctive properties that confer selective advantages in cancer chemotherapy.

##### 4.1.1. Selenium

Green SeNPs have demonstrated antimicrobial, photocatalytic, antioxidant, and anticancer activities. Their antioxidant effects are linked to their sequestration capacity of Se at the reactive oxygen species (ROS) release site, thus preventing the release of free radicals that mediate DNA oxidative damage [13]. The significant role of NP size in free radical scavenging suggested that SeNPs with smaller sizes possess better scavenging activity compared to larger NPs [52]. The anticancer mechanism of SeNPs has been linked to their ability to bind to metal ions and intracellular proteins. For instance, SeNPs bind to Cu$^{2+}$ and DNA forming a ternary complex leading to the reduction of Cu$^{2+}$ to Cu$^{+}$, which is later reoxidized to generate reactive oxygen species and induce cell death. This cell death mechanism has been described as cancer-specific since copper (Cu) ions (which are more abundant in cancer cells) are critical for generating the free radicals responsible for oxidative damage [53]. SeNPs also interact with intracellular proteins involved in glycolysis and mitochondrial activity, as cellular oxidative stress and mitochondria dysfunction were observed prior to apoptosis induction in human melanoma (A375) cells following exposure to SeNPs [54]. SeNPs also
upregulated proapoptotic Bcl-2 and downregulated anti-apoptotic Bcl-2 expression in HepG2 cells [55]. These observations suggest that the anticancer role of SeNPs is via ROS-dependent mitochondrial-mediated intrinsic apoptosis [41]. SeNPs also stimulate p53 release and induce caspase-8 expression, indicating activation of extrinsic apoptosis. Furthermore, SeNPs also mediate tumor cell necrosis and autophagy [56, 57]. In addition to their cytotoxic activity, selenium nanoparticles also displayed a chemopreventive action by inducing cell cycle arrest in human umbilical vein endothelial cells [58]. Biogenic SeNPs have also been reported to possess immune-stimulatory functions [59]. The exposure to SeNPs promoted the inhibition of cancer cell migration via inhibition of annexin A2, a pleiotropic protein involved in cancer cell motility. They also inhibited the expression of matrix metalloproteinase, which is involved in tumor metastasis [60]. The anticancer activity of SeNPs is summarized in Figure 4.

4.1.2. Tellurium. Te and its compounds (including inorganic tellurides, organo-Te, and Te complex) have shown significant antimicrobial and anticancer activities [61]. Te has been synthesized as nanorods, nanodots, nanowires, and nanocubes [62–64]. These NPs have demonstrated antioxidant, antimicrobial, and anticancer activities similar to those of the element. The use of biological materials such as microorganisms and plants in the synthesis of Te nanoparticles is gaining attention. Besides these favorable biological activities, evidence has suggested that the NPs synthesized through biogenic routes offer the additional advantage of selective toxicity compared to their chemically synthesized counterparts [42, 65, 66]. Similar to Se, Te also serves as a free radical scavenger [67]. However, tellurides have shown more antioxidant activities than Se [68]. Their low electronegative potential, ability to donate electrons, radical trapping tendencies, and glutathione peroxidase-mimicry nature are some of the likely reasons for its antioxidant function [69, 70].

The mechanism of TeNP cytotoxicity has not been fully unravelled, but studies indicate that it might be related to its ability to bind cellular protein and DNA, causing oxidative and DNA damage leading to cell death via mitochondrial-dependent pathways [71, 72] (Figure 5). Also, it is suggested that the biocompatibility of biogenic TeNPs might be dependent on the nature of capping biomolecules. This was elaborated in a comparative study involving TeNPs synthesized with lemon (LEM-TeNPs), lime (LIM-TeNPs), and orange extract (OR-TeNPs) [42]. Although the available research on the anticancer potential of biogenic TeNPs is still very limited to draw any conclusions, they show promising prospects worthy of exploration.

In spite of these pharmacological activities, the use of Te and its compounds has not been fully explored in drug design due to their toxicity [73, 74]. They can interact strongly with mitochondrial enzymes and proteins through the sulfhydryl group of cysteine, causing neurodegeneration [75]. Te, due to its similarity to Se, also interacts with...
Sulfur. Sulfur (S) has long been employed as an antimicrobial for treating different bacterial and fungal infections and in agriculture to produce fertilizers, pesticides, and fungicides. The emergence of SNPs has led to the discovery of additional properties apart from that inherent in sulfur. SNPs possess exceptional reactivity and properties such as antibacterial, antifungal, antioxidant, anticancer, and wound healing. Most reports of SNPs synthesis have however focused on the use of chemical methods such as membrane assisted precipitation, sodium polysulfide hydrolysis, ultrasonication, supersaturated solvent methods, electrochemical methods, eggshell membrane template methods, liquid-phase chemical precipitation, surfactant-assisted method, microemulsion method, solvent-free method, organic and inorganic polysulfide decomposition method, PEG-600, ethanolamine-assisted method, and PEG-200 in the sublimed sulfur technique [78–80]. These methods have achieved excellent SNP yield, controlled size and shape, and high NP purity in recent years [78–80].

Table 1: Green synthesized chalcogen and chalcogenide nanoparticles with anticancer properties.

<table>
<thead>
<tr>
<th>Plant and extract</th>
<th>Nanoparticle</th>
<th>Size (nm)</th>
<th>Shape</th>
<th>Activity</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange – Citric juice</td>
<td>TeNPs</td>
<td>2–15</td>
<td>Rods</td>
<td>Anticancer effect in human melanoma cells (≤50 μg/ml)</td>
<td>[42]</td>
</tr>
<tr>
<td>Lemon – citric juice</td>
<td>TeNPs</td>
<td>100–200</td>
<td>Cubic</td>
<td>Anticancer effect in human melanoma cells (≤50 μg/ml)</td>
<td>[42]</td>
</tr>
<tr>
<td>Lime – citric juice</td>
<td>TeNPs</td>
<td>100–200</td>
<td>Cubic</td>
<td>Anticancer effect in human melanoma cells (≤50 μg/ml)</td>
<td>[42]</td>
</tr>
<tr>
<td>Fenugreek – seed extract</td>
<td>SeNPs</td>
<td>50–150</td>
<td>Oval</td>
<td>Dose-dependent (25–100 μg/ml) cytotoxicity in human breast-cancer cells (MCF-7)</td>
<td>[43]</td>
</tr>
<tr>
<td><em>Allium sativum</em> – garlic clove extract</td>
<td>SeNPs</td>
<td>40–100</td>
<td>Spherical</td>
<td>Better biocompatibility in Vero cells than chemically synthesized SeNPs</td>
<td>[44]</td>
</tr>
<tr>
<td>Hawthorn – fruit extract</td>
<td>SeNPs</td>
<td>113</td>
<td>Spherical</td>
<td>Antitumor activity in HepG2 cells Induced intracellular oxidative stress and mitochondrial dysfunction to initiate apoptosis via the mitochondrial pathway</td>
<td>[41]</td>
</tr>
<tr>
<td><em>Cereopsis bulbosa</em> – tuber extract</td>
<td>SeNPs</td>
<td>52.5 ± 3</td>
<td>Spherical</td>
<td>Higher cytotoxicity in MDA-MB-231 cells compared to normal breast cells</td>
<td>[45]</td>
</tr>
<tr>
<td>Arabinogalactan from <em>Larix principis-rupprechtii</em></td>
<td>SeNPs</td>
<td>94–156</td>
<td>Spherical</td>
<td>Significant dose-dependent inhibitory activity (10–100 μg/ml) in A549, HepG-2, and MCF-7 cells via apoptosis</td>
<td>[46]</td>
</tr>
<tr>
<td>Drumstick – leaf extracts</td>
<td>SeNPs</td>
<td>23–35</td>
<td>Spherical</td>
<td>Highest cell inhibition in A549 cells</td>
<td>[47]</td>
</tr>
<tr>
<td><em>Urtica dioica</em> – leaf extract</td>
<td>SeNPs</td>
<td>21.7–83.6</td>
<td>Spherical</td>
<td>Effective against Caco-2, HepG2, and MCF-7 cells</td>
<td>[48]</td>
</tr>
<tr>
<td><em>Aloe vera</em> – leaf extract</td>
<td>TeNPs</td>
<td>20–60</td>
<td>Spherical</td>
<td>Significant anticancer activity against HepG2 cells Nontoxic to Vero cells</td>
<td>[49]</td>
</tr>
<tr>
<td><em>Octumb tenusflorum</em> – inflorescence extract</td>
<td>Ag@SeNPs</td>
<td>33.1 ± 2.7</td>
<td>Spherical</td>
<td>Cytotoxicity in HDF and melanoma cells</td>
<td>[50]</td>
</tr>
<tr>
<td><em>Stevia rebaudiana</em> – leaf extract</td>
<td>ZnS NPs</td>
<td>8.35</td>
<td>Spherical</td>
<td>Dose-dependent cytotoxicity in HEK293 and MCF7 cells</td>
<td>[37]</td>
</tr>
<tr>
<td><em>Camellia sinensis</em> – leaf extract</td>
<td>CdS</td>
<td>2–5</td>
<td>Spherical</td>
<td>Arrested A549 cell growth at the 5 phase of the cell cycle</td>
<td>[51]</td>
</tr>
</tbody>
</table>

selenoenzymes such as glutathione peroxidase, thus lowering cellular redox modulatory activities and causing conditions of oxidative stress that mediate cellular damage [76]. Te intake in patients has also been associated with nausea, vomiting, and bad breath [77]. However, their synthesis via green chemistry offers NPs with lower toxicity and interesting anticancer properties that should be explored in future studies.
Furthermore, Cu acts as a cofactor for mitogen-activated protein kinases (MAPK) and facilitates the phosphorylation of the extracellular-signal-regulated kinases (ERK) in the MEK/ERK signalling pathway, leading to the activation of downstream effector proteins such as CREB, JNK, fos, myc, C-Jun, and Ets, which are mediators of cell proliferation. The binding and sequestration of Cu by SNPs impact Cu availability, essentially inhibiting cancer cell proliferation via the MEK/ERK signalling pathway [12, 89].

SNPs also inhibit cell proliferation by mediating cell cycle arrest by inhibiting cyclin-dependent kinases. Although further research is required to understand the underlying mechanism, a link between the CDK inhibitory activity of SNP and the inhibitory effects of p53 and p21 on cyclin E/CDK2, and the resulting replicative senescence has

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**Figure 4: Anticancer mechanisms of selenium nanoparticles.**

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**Figure 5: Anticancer mechanism of tellurium nanoparticles.**
been reported. This pathway has also been linked to the apoptotic mechanism of SNPs [88]. Another possible cytotoxic mode of SNP may through its interaction with intracellular and membrane proteins that results in altered membrane permeability leading to cell death [12, 62]. This protein binding can be achieved either by direct interaction of SNPs with the sulphydryl group of proteins that form hydrogen sulfide [63] or by complexation of SNPs to form polysulfides that bind to the sulphydryl groups of proteins and modulate their structure and activity [12].

4.2. Green Synthesized Chalcogenides. Chalcogenides synthesized via the green approach have demonstrated significant anticancer activity in in vitro and in vivo experimental models. Silver-selenium bimetallic NPs (Ag-Se) synthesized by green chemistry using gallic acid and quercetin showed significant antioxidant and anticancer potential [90]. The mechanism of cancer cell death although not fully studied has been related to the antioxidant and anticancer potential of the respective biological reductants, since chemically synthesized Ag-selenide NPs showed no cytotoxicity [64, 91]. Similarly, green synthesized ZnSNPs were cytotoxic to MCF-7 cells, with no toxicity in normal cells, but with significant antioxidant activities [92].

Nanocrystals of the semiconductor chalcogenides known as QDs or quantum rods are distinguished for their quantum confinement properties, which confer characteristic optical attributes. Although QDs are mostly synthesized for drug delivery and biosensing, there have been several reports on the anticancer activity of green synthesized chalcogenide QDs. The bioreduction of cadmium sulfate and sodium sulfide by tea leaf extracts (Camellia sinensis) produced cadmium sulfide QDs (CdS QDs) that demonstrated significant cytotoxicity in lung cancer (A549) cells (Figure 3). This cytotoxicity involved induction of apoptosis that leads to arrest in the S phase of the cell cycle, with good biocompatibility in normal cells [51]. The QDs of CdS synthesized using the hairy roots of Raphanus sativus showed greater cytotoxicity in the human breast cancer cells (MCF-7) than in the gastric adenocarcinoma cells (AGS), with apoptosis identified as the mechanism of cell death [93].

The effect of CdS QDs has been mainly linked to cellular oxidative stress [94, 95], with different mechanisms being suggested (Figure 7). Subsequent to photodynamic therapy in CdS-QDs-treated cancer cells, Cd$^{2+}$ is released from the core of the nanoshell [96, 97] and combines with molecular oxygen generating superoxide anion radicals and unpaired QDs [96, 98]. The high concentration of oxidizing species results in oxidative stress that causes damage to DNA and other intracellular proteins and eventually leads to mitochondrial-dependent apoptosis [96, 99–101].

Similarly, other Cd-based chalcogenides, such as CdSe and CdTe, have also demonstrated significant anticancer activity with mechanisms similar to those of CdS. In an investigation of the cytotoxicity of CdTe QDs in HEK293 and HeLa cells, it was observed that the QDs bound to serum proteins and crossed the cellular membrane via clathrin-mediated endocytosis. Upon incorporation into the cells, they were degraded in the lysosomes, and the Cd$^{2+}$ was released leading to ROS causing mitochondria swelling and cell necrosis [102]. Similarly, CdTe QDs also mediate cell death in cancer cells via intrinsic and extrinsic apoptosis [103]. It was further reported that ROS-induced DNA damage leading to cell cycle arrest and cell necrosis were the mechanisms behind the cytotoxic effect of CdSe QD in A549 cells [104].

QDs have also been used in photodynamic therapy (PDT) in cancers. QDs absorb photons of light energy of a specific wavelength to produce electron holes known as excitons. This energy is then transferred from excitons to surrounding species or directly to molecular oxygen to form singlet oxygen radicals that mediated cellular damage [105].
However, the success of this therapeutic approach depends on the efficiency of the QD internalization by tumor cells. It should be noted that not all QDs are capable of demonstrating anticancer activity. Some chalcogenide quantum dots, such as zinc oxysulfide QDs, are biocompatible; do not pose a risk of cytotoxicity; and are synthesized primarily for use as biosensors [106]. Biocompatible QDs conjugated via carboxymethylcellulose (Zn_xCd_{1-x}QD) were reported to demonstrate tremendous fluorescence activity [107]. Biocompatible nanochalcopyrites, including CuInS_2, AgInS_2, and ZnInS, have also been synthesized and have been applied as biosensors in cancer therapy [108–110].

NPs have been used to deliver drugs due to their enhanced permeability effect and tumor site-directed drug bioaccumulation. Furthermore, they have been shown to improve drug solubility and facilitate drug transport across membrane barriers. Earlier studies on the use of NPs in the delivery of chemotherapeutic agents focused on the use of organic polymers; however, the last two decades have seen a significant rise in the use of inorganic NPs for drug delivery. Like NPs generated via chemical synthesis, biogenic NPs are engineered to counter the challenges of systemic toxicity, stability, intracellular distribution, and the ability to navigate biological barriers for efficient delivery. We will focus on the current progress made in the use of chalcogens and chalcogenide NPs in drug delivery.

5. Chalcogen and Chalcogenide Nanoparticles: Potential in Biomedicine

The advances made over the years in nanomedicine have seen the development of a variety of nanodelivery platforms such as lipid-based NPs (liposomes and micelles), organic and inorganic polymers (chitosan, polyethylenimine, poly (lactic-coglycolic acid), and polyethylene glycol), synthesized as single-unit NPs or as copolymers. Others include metalloids, metal oxides, and metallic NPs such as selenium, ferrite, silver, and gold NPs, which in several designs have had their versatility enhanced through surface functionalization with polymers. Hence, they have been vigorously studied because of their higher cargo capacity and optical properties.

5.1. Chalcogens as Delivery Tools. The sulfur-based chalcogen NPs nano-S has been studied for the efficient delivery of cisplatin in cancer cells. In addition to this primary role, an interesting observation was the ability of nano-S to also sequester Cu ions in cancer cells, blocking the MEK/ERK pathway [89]. It has also been observed that functionalizing the surface of organic polymer with sulfur can increase the selectivity of the transmembrane protein, p-selectin, and cell adhesion molecule involved in tumor metastasis [111].

SeNPs have also been employed to deliver anticancer drugs due to their biocompatibility, intrinsic anticancer activity, excellent pharmacokinetics, and efficient drug loading capacity due to their tunable and polyvalent surface...
mortality. Four forms of SeNPs have generally been employed in targeted drug delivery, including nude SeNPs, surface-functionalized SeNPs (using polymer, ligand, and peptide), mesoporous SeNPs, and block polymer encapsulated SeNPs. Most studies on drug loading using Se involved conjugation of the drug to the surface of the SeNPs. The ease of drug loading in SeNPs is due to unreacted molecular ions and stabilizing agents from biogenic sources that form a stable interaction with a pharmaceutical agent through their amino, carboxyl, and amide groups [112]. Recently, an eco-friendly synthesis of a noisome-Se nanohybrid drug delivery vehicle with anticancer activity and good biocompatibility has been reported [113]. The intriguing feature of this nonviral drug delivery vehicle was its potential inhibition of multidrug-resistant protein. Furthermore, mesoporous SeNPs have also been synthesized to facilitate doxorubicin delivery [114]. The NPs showed a significant high drug-loading capacity and redox responsive drug release and were nontoxic to critical organs in an in vivo nude mouse model. SeNPs synthesized by fenugreek seed extract were also shown to work synergistically with doxorubicin to increase cytotoxicity in MCF-7 cells [43]. SeNPs synthesized using Spermacoce hispida aqueous leaf extract was shown to efficiently deliver S-allyl glutathione into HepG2 cells. They initiated cell death through cell cycle arrest, DNA fragmentation, and mitochondrial-dependent apoptosis [115].

Targeted bioaccumulation of NPs such as Se has proven to be highly effective in cancer therapy over the years. Hence, SeNPs have been surface modified to facilitate targeted delivery to the required site of action. This was achieved either through a ligand receptor-targeted release or stimulus-responsive mechanism, which mostly involved the attachment of ligands or stimuli-responsive components to side chains via covalent or noncovalent interactions [116]. An interesting example of this surface modification includes the functionalization with epidermal growth factor receptor peptide to facilitate targeted delivery to the tumor microenvironment [117]. SeNPs functionalized with polyethylene glycol (PEG) and polyethyleneimine (PEI) have been used to selectively deliver epirubicin (an anticancer drug) and NAS-24 aptamer (apoptosis induction agent) to MCF-7 cells [118]. This PEG-PEI functionalization improved drug circulation, increased NP stability, improved drug loading, and increased selectivity via toll-like receptor targeting of PEI [119]. It was also noted that conjugating galactose to the surface of SeNPs increased in vitro cellular uptake by hepatocellular carcinoma cells via clathrin-mediated endocytosis [120]. A concurrent in vivo study showed hyperaccumulation of the NPs in tumors without damage to the heart, liver, spleen, lung, and kidney. Apart from galactose, glucose is another aldohexose employed in the surface functionalization of SeNPs. These NPs also showed good biocompatibility and induced apoptosis through intrinsic and extrinsic pathways [121]. These aldose-hexoses’ cell target activity can be linked to carbohydrate metabolism aberration in cancer cells as described by Otto Warburg [122]. This results in increased selective uptake of the drug-loaded NPs by cancer cells. It was also reported that folate functionalized mesoporous silica-SeNPs were able to bind to the folate receptor of cancer cells upon acid cleavage to facilitate the targeted release of doxorubicin [123]. In a study involving chemo- and photothermal therapy, SeNPs were covalently attached to RC-12 and PG-6 peptides to deliver indocyanine green and doxorubicin [124]. The peptides stimulated drug uptake via lipid raft endocytosis and clathrin-mediated endocytosis in cancer cells, which were then released after irradiation by a NIR laser to mediate ROS-induced cell death and apoptosis. The incorporation of SeNPs into a block polymer was the final approach described for drug-loaded SeNPs. Although this is still in its early stages, it has shown promising potential to deliver anticancer drugs efficiently with good biocompatibility [116, 125].

TeNPs have been incorporated into polymers and biofilms to selectively deliver chemotherapeutic agents. Te was first reported as a drug delivery vehicle in 2014 when Wei Cao and colleagues demonstrated the successful use of Te to deliver cisplatin [126]. The authors later demonstrated the efficient synthesis of a biofilm containing Te, which showed a significant potential for cisplatin drug loading and an ion-responsive drug release [127]. The use of Te to deliver cisplatin is linked to the coordination chemistry of Te and platinum [126]. The attractive property of this nanoassembly was the possibility of controlled drug release kinetics. When competitive ligand biomolecules such as mercapto ligands and spermine were introduced, they could control the release kinetics of a cisplatin conjugated Te-containing polymer. In this way, TeNPs have been used to deliver and control the release of platinum-containing drugs successfully.

Although TeNPs could scavenge ROS, due to their low electronegativity, they are also easily oxidized by ROS [128]. The oxidized Te can in turn be liberated from its coordination complex with platinum-based drugs leading to drug release at a specific target site. This approach was recently used to selectively target tumors using Te-conjugated cantharadin in combination therapy [129]. Radiations within near-infrared regions, gamma radiations, and light radiations have been used to generate this ROS for the stimuli-responsive drug release in Te-drug conjugates [130–132]. This mechanism, in turn, provides a synergistic cell-killing effect in cancer cells.

5.2 Chalcogenides in TheraNoxics and Bioimaging

Chalcogenides, because of their optical properties, near-infrared (NIR) absorption properties, and proficient photothermal conversion efficiency, are mostly studied for their application in photothermal ablation, bioimaging, and diagnostics in cancer therapy. The options they provide in theranostics have encouraged studies in targeted systems for drug and gene delivery. In this section, we highlight the contributions of chalcogenide NPs in biomedicine, especially as delivery tools.

The immense potential of chalcogenides as drug delivery platforms in cancer is evident in the impressive number of research publications in this area. The recent surge in the study of chalcogenides for NP design for drug delivery lies in
the options they offer in combination therapy, specifically, through photothermal ablation [133]. A recent report described a dual responsive system involving the dichalcogenide MoS₂ NPs optimized for folate targeting and loaded with the anthracycline doxorubicin [134]. Functionalized with liposac-acid-polyethyleneimine (LA-PEI) and liposac-acid-polyethylene glycol (LA-PEG) copolymers, the FA-BSA-PEI-LA-MoS₂-LA-PEG (FBPMP) nanocomposite showed sensitivity to changes in pH and NIR irradiation in the release of its doxorubicin payload in folate-positive MDA-MB-231 breast cancer cells. Furthermore, exposure of the nanocomposite to NIR irradiation at 808 nm and 0.5 W cm⁻² for 5 minutes induced heat and affected ablation in cancer cells, acting in synergy with doxorubicin for a marked impact on cell viability [134].

The investigation of cobalt-selenide (Co₉Se₈) nanoplates, for photoacoustic imaging, photothermal ablation, and drug delivery, emphasizes the versatility of chalcogenide nanoconstrusts. Biocompatible Co₉Se₈ nanoplates functionalized with polyacrylic acid responded to changes in pH and NIR in doxorubicin release, with amplitude cytotoxic effects observed in in vitro and in vivo models of liver cancer, especially in the induction of hypothermia by exposure to NIR [135]. Besides their anticipated photoacoustic property, Co₉Se₈ nanoplates markedly enhanced MRI contrast of the tumor area in hepatic (HepG2) tumor bearing mice. Negative enhancements observed in the T₂-weight images revealed the potential of Co₉Se₈ nanoplates as contrasting agents in clinical diagnostics, which in combination with its photoacoustic properties produces a multimodal imaging tool with higher sensitivity and precision [135, 136].

A similar multimodal imaging-guided delivery of doxorubicin by pegylated bismuth sulfide nanourchins was reported. The chalcogenide NPs had a loading capacity of 37.9% and displayed pH and thermal sensitive drug release, while the photothermal property of the construct acted in concert with the chemotherapeutic to significantly inhibit cervical tumor growth in a Balb/c mouse model [167]. Moreover, the study described the strong X-ray attenuation feature of Bi₃S₂-PEG nanourchins and suggested their potential application as a computed tomography (CT) contrast agent, which, with their innate photoacoustic and infrared thermal imaging properties, offer an invaluable possibility for monitoring the therapeutic impact of treatments in real time [137, 138]. In a recent study, a ternary Cu-Fe-Se chalcogenide nanosheet demonstrated a significant chemo-thermal effect in mouse mammary tumors, similar to the examples already described. Furthermore, with its inherent multimodal imaging properties, the pharmacokinetics of the anticancer drug and nanosheets were elucidated, thus highlighting another application of chalcogenide NPs in nanomedicine [139].

In a related study describing the mitigation of hydrophobicity and toxicity in Cu₉S₅ nanocrystals by coating with mesoporous silica (mSiO₂) to yield Cu₉S₅@mSiO₂, following PEG functionalization, Cu₉S₅@mSiO₂-PEG nanocomposite showed efficient doxorubicin encapsulation efficiency (63.8%), and a large amplitude drug release at pH 4.8, suggesting pH sensitivity. Upon exposure to NIR at 980 nm and 0.78 W cm⁻² for 10 minutes, Cu₉S₅@mSiO₂-PEG-DOX had a marked impact on the viability of human colorectal cancer cells in vitro, while the mean tumor weight was reduced by a significant 83% [140]. A different design, but of similar composition, drug-loaded Cu₉S₅@mSiO₂ nanofibers were studied for their efficacy in mouse hepatoma [141]. In another system involving a combination of silica and copper sulfide, silica nanorods were decorated with CuS NPs and then functionalized with lactobionic acid—an agonist of asialoglycoprotein receptors for targeted delivery of doxorubicin to HepG2 cells. As expected, in conjunction with the pH-dependent release of doxorubicin from the SNT-LA-CuS-PEG nanocomposite, exposure to NIR radiation enhanced the drug release profile, with the thermal energy generated improving tumor inhibition in vivo [142, 143]. In a similar report, Zhang and coworkers described a novel nanocomposite consisting of a mesoporous silica scaffold, to which CuS nanospheres were conjugated via two complementary DNA sequences (MSN-DNA-CuS) [144]. This unique design employing the oligonucleotides and CuS nanospheres as gatekeepers was a thermally regulated drug delivery system allowing for a reversible opening and closing of the drug release portals of the drug-loaded MSN. On exposure to NIR and the consequent hypothermia brought about by the CuS, the oligonucleotides dehybridized, permitting the outward movement of the drug. At the same time, detached CuS nanospheres contributed to cancer cell death through thermal ablation, acting in synergy with the released drug [144].

Most studies involving nucleic acid-based functionalization of chalcogenides are generally on the development of molecular probes for diagnostics and imaging; however, chalcogenides have also been applied in nucleic acid delivery [145, 146]. In an interesting study, Guo and coworkers described a dual photothermal-immunotherapy strategy that involved the chalcogenide CuS NPs as a delivery platform and photo-absorber, with oligodeoxynucleotides rich in cytosine-guanine (CpG) motifs as an immunologic adjuvant [147, 148]. The idea was to target primary tumors with photothermal ablation and to initiate antitumor immunity against metastasized tumor cells. The study, conducted in a mouse model of breast cancer, involved the intratumoral injection of hollow chitosan-functionalized CuS NPs (Chi-HCuSNPs) loaded with a CpG-rich oligonucleotide and elicited a significant inhibition of tumor growth by 68% and 86% in primary and distant tumors over 12 days and 24 days, respectively. Mechanistically, upon treatment with NIR laser, primary tumor cells were disrupted by photothermal ablation, and hollow CuS NPs disintegrated into nanocrystals, releasing their oligonucleotide cargo, which was taken up by the plasmacytoid dendritic cells in the tumor microenvironment. Plasmacytoid dendritic cells then secreted the cytokine IFN, which on the one hand activates innate immunity by activation of natural killer cells (NK) and on the other hand initiates the activity of antigen-specific CD8⁺ T cells by orchestrating the conversion of activated myeloid dendritic cells to antigen-presenting professional dendritic cells [148]. The synergistic
impact of the chalcogenide CuS NPs and their cargo underlines the immense clinical potential of this class of NPs.

In another report, CdTe/CdS core/shell QDs were employed as a scaffold and tracker for the thymidine kinase (HSV-TK) gene of the suicide herpes simplex virus. The HSV-TK gene belongs to the class of therapeutic genes whose gene products convert prodrugs to their cytotoxic metabolite and has been used extensively in chemotherapy [149, 150]. The HSV-TK gene conjugated to the QD via an EDC/NHS (ethyl (dimethylaminopropyl) carbodiimide/N-hydroxy succinimide) coupling was efficiently trafficked to the nucleus of treated cervical cancer cells, with the QD providing the option of tracking the nanocomplex. As expected, the addition of the prodrug ganciclovir significantly reduced viability in treated cells due to the conversion to active ganciclovir triphosphate, a DNA polymerase inhibitor [146].

QDs have evolved as one of the most sought-after nanomaterial in drug delivery. Similar to NPs, their small size allows for an increase in permeation retention capacity and provides a large surface area for drug conjugation. Their superinfinitesimal dimension confers additional properties including ease of shape and size adjustment and the facility of doping and pliability associated with drug functionalization [151]. QDs can be conjugated with drugs via electrostatic interaction, covalent bonding, or surface adsorption. Extensive studies on the use of QDs as a drug delivery vehicle have been carried out. This is because of the deep interest in their amenability to stimuli-responsive drug release. QDs have been applied in photothermal therapy to facilitate subcutaneous drug uptake after irradiation. NIR irradiation has also been used to stimulate drug and thermal energy release in camptothecin-loaded CuS QD, causing cancer cell death [152].

6. Limitations and Future Perspective

The theranostic potential of chalcogenide NPs is largely responsible for their growing popularity. Most theranostic nanoconstructs comprise a scaffold and a tethered diagnostic component and are characterized by complex synthesis procedures and the challenge of achieving a balance between diagnostic and therapeutic components for effective impact. However, chalcogenide NPs are a unique nanoplatform where both diagnostic and therapeutic properties are contained in a single NP unit, and alongside chalcogen NPs, such as SeNPs and SNPs, which have demonstrated bioactivity under certain conditions, their delivery capacity positions them as viable candidates in the development of multifunctional nanomedicines for clinical applications. Equally attractive is the biogenic synthesis advantage of this class of NPs, with evidence associating the capping of biogenic chalcogen and chalcogenide NPs with their diverse therapeutic properties and capacity to mitigate debilitating pathological conditions. Besides their ease of synthesis and stability, the biogenic synthesis of chalcogen and chalcogenide NPs significantly improves biocompatibility, alleviating concerns associated with the application of inorganic NPs in clinical scenarios.

While studies focusing on the preparation of inorganic NPs for clinical applications are in their advanced stages, research on certain chalcogen and most chalcogenide NPs are still quite elementary. Of the 75 NPs currently in clinical trials, liposomal and polymeric NP-based nanomedicines are in the majority, while only 5 involve metallic NPs, with no chalcogen or chalcogenide included. At any rate, the progressive cultivation of ideas centred on the development of chalcogen and chalcogenide nanomedicines should produce candidates for clinical testing in the near future.

However, the existing challenges and limitations require due attention. For instance, the tunability of NP size and shape, which is achievable through adjustments of one or more physical factors such as temperature or pH in chemical synthesis procedures, is not feasible in certain biogenic processes, especially where living organisms are involved.

Another limitation is the synthesis time and yield, which are lower in most cases compared to what is obtainable by chemical synthesis procedures. Attempts to improve NP yield in some of these organisms may require alterations at the genetic level, which features complex procedures with significant cost implications.

Moreover, there is a paucity of information on the clearance properties of most chalcogenide nanoformulations. The general reservation about inorganic NPs is their tendency to accumulate in vital organs and tissues where they pose significant toxicity risk. Generally, the size and the charge of inorganic nanoparticles influence their fate in vivo. NPs with hydrodynamic sizes <6 nm can easily scale the filtration-size threshold of the glomerulus. NPs with hydrodynamics sizes between 6 and 8 nm are more affected by surface charge with cationic nanoparticles more likely to transverse the glomerular basement membrane due to its highly negative charge, while NPs >8 nm are taken up by the reticuloendothelial system (RES) and stored in the lungs, liver, spleen, bone marrow, and the lymphatic system. Aside from the possible toxicity implications of prolonged storage in the organs and tissues making up the RES, reports have suggested that the prolonged accumulation of NPs with optical properties such as chalcogenides may interfere with diagnostics and bioimaging. For instance, QDs have been reported to remain optically responsive for more than 100 days in vivo. This necessitates more studies on the impact of long-term accumulation and the potential approaches to modulating clearance in vivo.

So far, the vast potential application of chalcogen and chalcogenide NPs, which includes their use in biosensor design, as theranostic agents with application in photothermal and photodynamic therapies, and in bio-imaging applications such as X-ray, NIR fluorescent imaging, photoacoustic imaging, and multimodality imaging, positions this class of NPs as veritable tools in cancer management, subject to advances in their development. Further incursions into their performance in vivo involving various preclinical models may assuage questions on toxicity and the side effects of long-term accumulation and facilitate their progress to clinical applications. In addition, given the advantages of biogenic synthesis highlighted in this review, the development of cost-effective methods to improve the quantum
yield of biogenic synthesis, which is a disadvantage, will improve the chance of large-scale production for future clinical use.

Going forward, we anticipate that the surging interest in this class of NPs, initiated by their significant biocompatibility and versatility in diagnostics, therapy, and imaging, will drive the improvement of existing biogenic and characterization procedures toward the production of chalcogens and chalcogenide NPs with broader applicability in medicine.

Data Availability
This is a review paper, and no data are available.

Conflicts of Interest
The authors declare that there are no potential conflicts of interest.

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