REVIEW

Applications of calixarenes in cancer chemotherapy: facts and perspectives

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Abstract: Research on the therapeutic applications of calixarene derivatives is an emerging area of interest. The anticancer activity of various functionalized calixarenes has been reported by several research groups. Due to their superior geometric shape, calixarenes can accommodate drug molecules by forming inclusion complexes. Controlled release of anticancer drugs by calixarenes might help in targeted chemotherapy. This review summarizes the anticancer potential of the calixarenes and their drug loading properties. The potential use of calixarenes in chemoradiotherapy is also highlighted in brief.

Keywords: cancer, chemotherapy, calixarenes

Introduction

Chemotherapy occupies a pivotal position in the treatment of cancer, and its significance in terms of cure and regression of various types of cancer should not be underestimated.[1] Adjuvant or neoadjuvant and concurrent chemotherapies are usually used along with surgery and radiotherapy.[2] However, one major drawback that limits the effectiveness of cancer chemotherapy is the non-selective nature of the drugs used. The administrated drugs/agents not only target the cancer cells, but also normal healthy cells.[3]

The ability of anticancer drugs to differentiate between cancerous and healthy cells is generally based on the assumption that tumor cells have a higher growth rate when compared with normal cells. As a result, healthy tissues, such as bone marrow, the gastrointestinal mucosa, hair follicles, and skin are directly affected by a chemotherapeutic regimen, even with preliminary chemotherapy. These side effects lead to various complications during and after treatment.[4,5]

Research on designing anticancer agents is still actively ongoing in the quest to introduce more potent anticancer drugs to the market. However, all available and upcoming anticancer drugs face the same problem of non-selectivity. Therefore, from the last three decades, new trends have been developed for targeted chemotherapy, ie, introduction of nanotechnology, use of cancer biomarkers, and exploration of physical parameters (eg, pH, temperature, light) in the treatment of cancer.[6] Likewise, surface modification of polymer drugs and nanoparticles is also used as a tool for more effectively targeted chemotherapy.[7]

Biomedical application of supramolecules

Macromolecules, discovered to have some biomedical applications, behave like drugs, and have shown great potential in drug delivery systems. Among the synthetic macromolecules, crown ether, cyclodextrin, and calixarenes and their derivatives are of great significance. In addition to their unique physicochemical properties, they also serve as hosts that can encapsulate many types of guests.[8]

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Among the macromolecules, calixarene (Figure 1) is considered to represent the third-generation of host-guest supramolecular chemistry. Calixarene is a cyclic oligomer that can be synthesized by the reaction of phenol with formaldehyde in the presence of a catalytic amount of a strong base or acid.[9] Calixarenes already have an established place in the pharmaceutical field (Table 1). The flexible nature of the basic moiety and further modification of their basic core and rims make these cyclic oligomers ideal for use as drugs and drug carriers.[10,11] Calixarene and its derivatives have been reported to have antiviral, antibacterial, antifungal, antitubercular, and anticancer activity.[12–14] Calix[4]arene, bearing methylenebisphosphonic and hydroxymethylenebisphosphonic acid groups, has an inhibitory effect on protein tyrosine phosphatase 1B, which is involved in insulin and leptin signal transduction, and thus has antidiabetic and antiobesity properties.[15]

Calixarenes as anticancer drugs

The anticancer activity of calixarene-based compounds has been investigated by several research groups. Baggetto et al[16] synthesized and studied the effect of calix[4]arene derivatives on various tumor cells (MU2, MU2F, HT1080, SP6.5, 1PC227, Jurkat, MEWO, Hl-60, Huh7, Hep-G2, MEWO, DLM.1) and compared the activity of these compounds with standard anticancer drugs. They discovered that these functionalized calix[4]arene derivatives are potent anticancer agents, particularly in lymphoblastic leukemia and melanoma cell lines. Likewise, Nasuhi Pur and Dilmaghani[17] synthesized calix[4]arene functionalized with four platinum (II) centers and reported that it was a prospective anticancer agent. When compared with a chemotherapeutic agent, carboplatin, the newly synthesized compound showed better activity against non-small cell lung cancer, hepatocellular cancer, and breast cancer. By using colorimetric cytotoxicity tests, its median inhibitory concentration (IC_{50}) in a lung cancer cell line was found to be 2.6 µM. In another study,
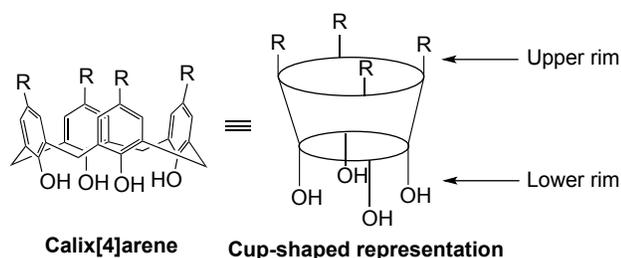
the in vivo effect of glycoconjugates of calix[4]arene in a mouse melanoma model was reported to include a substantial reduction in tumor growth within 2 weeks.[18]

Clinical trial evidence for calixarene-based anticancer agents is still scanty. To date, the US clinical trials database shows only one Phase I study of OTX008, a calixarene-based compound and galectin-1 inhibitor with potential antiangiogenic and antineoplastic activity. The drug functions through downregulation of a multifunctional carbohydrate binding protein, galectin-1, in patients with advanced solid tumors. The study has been ongoing since 2012, with no results reported as yet.

Mechanisms of action of calixarene-based anticancer agents
Enzyme inhibition

Enzyme inhibition is a common mode of action for some chemotherapeutic drugs. Several calixarenes derivatives have been studied and reported to act as potent enzyme inhibitors. For instance, Cherenok et al[19] studied calix[4]arenes coupled with phosphonic, aminophosphonic, and methylenebisphosphonic acid residues and found them to be active inhibitors of some phosphatases such as alkaline phosphatase and tyrosine phosphatase. The same group also reported that hydroxymethylphosphonic acid calixarenes inhibited glutathione-S-transferase, a key enzyme in the xenobiotic detoxification process in cells, thereby contributing toward the multidrug resistance observed in many types of tumors.[20] Consoli et al[21] reported inhibition of DNA replication of a *Penicillium digitatum* fragment in their study of calix[4]arenes, which were modified with thymine/adenine 2′-deoxynucleotide moieties via polymerase chain reaction.

Angiogenesis antagonists

Survival of tumor cells depends on a sufficient supply of nutrients and oxygen, and waste removal via blood vessels. Hence, the presence of new blood vessels within the tumor is essential for their growth. Platelet-derived growth factor (PDGF) plays an important role in angiogenesis by interaction with the PDGF receptor.[22] The synthesized calixarene derivatives interrupt the interaction of PDGF with its receptor, resulting in prevention of phosphorylation of the receptor, thus acting as antiangiogenic agents.[22] In an in vitro study using proliferation of endothelial cells, Dings et al[23] also revealed that calixarenes possessing tetra-amine, methyl, and diguanidino groups on the lower rim with *tert*-butyl, isopentyl, isobutyl and propyl groups on the upper rim are potent angiogenesis inhibitors. Later, in vivo studies demonstrated

Figure 1 Cup-shaped representation of calixarene macrocyle with calix[4]arene as an example.

Table 1 Biomedical applications of calixarenes

Type	Modification(s)	Activity	Mechanism of action	Reference
Calix[4]arene	Methylenebisphosphonic and hydroxymethylenebis phosphonic acid groups	Antiobesity, antidiabetic	Inhibitory quality on protein tyrosine phosphatase 1B	15
	Functionalized with four platinum (II) centers	Anticancer	Growth inhibitory	17
	Glycoconjugation	Anticancer	Reduction in tumor growth	18
	Phosphonic, aminophosphonic, and methylenebisphosphonic acid residues	Anticancer	Inhibit phosphatases such as alkaline phosphatase and tyrosine phosphatase	19
	Hydroxymethylphosphonic acids	Anticancer	Inhibit glutathione S-transferase and prevent multidrug resistance of cancer cells	20
	Thymine/adenine 2′-deoxynucleotide moieties	Anticancer, antibacterial	Inhibition of DNA replication	21
	p-sulfonated	Anticancer	Prevent interaction of platelet-derived growth factor and its receptor to prevent angiogenesis	22,23
	Tetra-amines, methyl and diguanidino groups on the lower rim with *tert*-butyl, isopentyl, isobutyl and propyl on the upper rim	Anticancer	Angiogenesis inhibitors	24
	Four Tn antigen glycomimetic units (S-Tn) and an immunoadjuvant moiety (tripalmitoyl-S-glycerylcysteinylserine)	Anticancer	Immune system stimulator against cancer	29
	Dinuclear platinum compounds	Anticancer	Inhibit tumor growth	32
	Polyethylene glycol	Anticancer	PDT	46
Calix[6]arene	Polycationic modification	Anticancer	Inhibits growth	25
	Imidazole moiety	Anticancer	Upregulates tumor suppressor gene, p53 gene	26
	No modification	Anticancer	Stop signal transduction to AXL and Mer tyrosine kinase receptors overexpressed in pancreatic cancer; it also inhibited the pathway of PI3K/mTOR	27
	p-sulfonated	Anticancer	Photodynamic therapy	51
Calix[8]arene	Glycoxylation	Anticancer	Prevent tumor migration and proliferation	30
	TLR2 ligands	Anticancer	Stimulate B lymphocytes for antibody production	31
	p-sulfonated	Anticancer	PDT via initiation of the apoptotic caspase system, and destruction of the membrane and mitochondria	51

Abbreviations: PDT, photodynamic therapy; TLR2, Toll-like receptor-2; PI3K, phosphatidylinositide 3-kinase; mTOR, mammalian target of rapamycin.

that a polycation-modified member of these compounds had improved efficacy against MA-148 ovarian cancer cells and B16 mouse melanoma cells.[23,24]

Suppression of oncogenes and upregulation of tumor suppressor genes

An important gene in the etiology of cancer is the tumor suppressor (p53) gene. Li–Fraumeni syndrome, an hereditary disorder, results from mutated p53 tumor suppressor protein. The transcriptional activity of the Li–Fraumeni p53 mutant was found to be improved by imidazole-calix[6]arene moiety through stabilization of oligomer formation.[25]

Pelizzaro-Rocha et al[26] investigated the effect and mechanism of calixarenes in a pancreatic carcinoma cell line overexpressing the AXL and Mer tyrosine kinase receptors. Calix[6]arene without any modification was found to be more effective than the commonly administered anticancer drug, 5-fluorouracil. The compound successfully abolished signal transduction of these two receptors and inhibited the phosphatidylinositide 3-kinase/mammalian target of rapamycin pathway. Calix[6]arene was believed to reduce levels of Mer and AXL by stimulating degradation of these two receptors. However, hydrophobic modification of calix[6]arene at the upper rim removed its anticancer potential.

Modulation of innate immunity

Some anticancer drugs work by stimulating and/or strengthening the ability of the host immune system to combat cancer cells, as achieved by photodynamic therapy (PDT). Likewise, antibody-based chemotherapeutic drugs potently target host immunity components or receptors that enhance proliferation, survival, dedifferentiation, and migration of cancer cells. Examples of such drugs include rituximab (Rituxan®), trastuzumab (Herceptin®), alemtuzumab (Campath®), bevacizumab (Avastin®), cetuximab (Erbitux®), gemtuzumab ozogamicin (Mylotarg®), calicheamicin, ibritumomab tiuxetan (Zevalin®), and tositumomab (Bexxar®).[27] In this respect, calixarenes have also been found useful. A calix[4]arene scaffold linked with four Tn antigen glycomimetic units (S-Tn) and an immunoadjuvant moiety (tripalmitoyl-S-glycerylcysteinylserine) was able to produce better immune-stimulating activity than its corresponding monovalent analogs, thus serving as a novel synthetic cancer vaccine candidate when tested by immunization in mice.[28] Further investigations showed that glycocalix[8]arenes inhibited migration and proliferation of rat C6 glioma cells in a scratch wound model.[29]

Geraci et al[30] also explored the immunotherapeutic anticancer activity of calixarene. They focused on MUC-1 protein, overexpression of which is associated with human epithelial carcinomas. Specifically modified calix[4]arene and calix[8]arene platforms with Toll-like receptor-2 ligands were developed as novel anticancer vaccines. The resulting complex potently stimulated B lymphocytes to produce an antibody specific for MUC-1, denoted as MUC-1 immunoglobulin G antibody.

Calixarenes as anticancer drug carriers: drug-loading capacity

Due to their distinctive geometry, cup-shaped calixarenes (Table 1) have the ability to accommodate different chemical compounds by forming inclusion complexes through noncovalent bonding, van der Waal's interactions, and hydrogen bonding. Brown et al[31] studied the host-guest chemistry of anticancer drug, dinuclear platinum complexes, with *para*-sulfonato-calix[4]arene. Based on spectroscopic results, it was revealed that, in addition to intramolecular and hydrophobic interactions, four intermolecular hydrogen bonds between calix[4]arenes and the dinuclear platinum compounds significantly enhanced the stability of the host-guest complexes. Their anticancer activity was explored in vitro and the complexes were found to be active against A2780 ovarian cancer cells, and particularly against A2780cp70, a cisplatin-resistant subline.

Imatinib is one of the anticancer drugs approved by the US Food and Drug Administration and is known to be a potent tyrosine kinase III inhibitor. Galindo-Murillo et al[32] investigated the interaction between calixarene derivatives and imatinib. In silico study of these complexes was done through density functional theory (DFT) and molecular dynamics (MD) simulation that explained the controlled delivery of anticancer drug into the tumor cells.[33] Based on calculation of interaction energies, they found that the cavity size of calixarene and the nature of the attached functional groups played a vital role in the stability of imatinib-calixarene host-guest chemistry. Hydrogen bond donor functional groups were found to be more effective than others. Among the 18 selected calixarene derivatives, they found that calix[6]arene, functionalized with SO_3H and $(CH_2)_2OH$ groups at the upper rim, formed more stable complexes with imatinib.[32] Galindo-Murillo et al also investigated inclusion complexes of an anticancer drug, 3-phenyl-1H-[1]benzofuro[3,2-c]pyrazole (a tyrosine kinase inhibitor) and calix[n]arenes (n=4, 5, 6, 8) functionalized with sulfonic acid and ethoxyl groups. They reported that calix[8]arenes and calix[6]arenes, bearing

an SO_3H group at the upper rim, formed relatively more stable complexes with 3-phenyl-1H-[1]benzofuro[3,2-c]pyrazole. Again, the large cavity size of these calixarenes and the hydrogen bond donor ability of SO_3H were considered to be possible reasons for this stability.[32]

Mechanism of drug release

Optimal conditions are needed for controlled release of drugs from the cavity of a host molecule. One of the mechanisms via which drugs are released from the cavity of calixarene involves a pH-triggered mechanism. The tumor microenvironment has been shown to have pH values in the range of 5.7–7.8, whereas healthy tissue has a pH of 7.4.[34,35] The pH difference between normal cells and tumor cells increases further at the subcellular level, and is useful for controlled delivery of anticancer drugs.[36] The role of pH in targeted chemotherapy has been clarified further by Vivek et al[37] who investigated pH-responsive delivery of oxaliplatin from a chitosan nanocarrier. Oxaliplatin was loaded onto polysaccharide chitosan, and was released from the carrier more rapidly at pH 4.5 than at pH 7.4. These authors reported inhibition of Bcl-2 and survivin in MCF-7 breast cancer cells by the action of these nanoparticles through pH-triggered mechanism.

Xue et al[38] similarly reported that the drug-releasing ability of calix[8]arene was pH-dependent. They successfully loaded an antimicrobial drug (ciprofloxacin) onto functionalized water-soluble calix[8]arene and found that the drug was successfully released from the cavity of calix[8]arene via a pH-triggered mechanism. Drug release was found to be 38%–42% with pH 7.4 buffer, 86% with pH 5.0 buffer, and 82% with pH 8.5 buffer.

A review by Liu et al[39] summarizes the contribution of the different research groups with regard to drug delivery using pH-sensitive based nanosystems. They detailed the role of the pH difference between normal cells and cancer cells and discussed the mechanism of anticancer drug release and absorption using various organic and inorganic nanosystems. These efforts are helping to overcome the problem of non-selectivity when using chemotherapy for the treatment of cancer.

Photodynamic therapy

PDT, photoradiation therapy, and photochemotherapy are terms that are used interchangeably to describe a treatment whereby light-sensitive chemicals known as photosensitizers and light radiation are used to combat cancer and other forms of malignancy.[40] The light is usually in the visible

and near-infrared range (400–800 nm). Photosensitizers preferentially remain in tumor cells whereas normal cells eliminate them rapidly.[41] Therefore, photosensitizers can serve a dual purpose, ie, they serve as both tumor-detecting and tumor-treating agents.[42,43] The photosensitizer is allowed to distribute around the body and selectively accumulate in cancer cells for few hours or days, depending on the types of photosensitizer. Subsequently, the photosensitizer is activated by irradiation (5–20 minutes) at a wavelength corresponding to the absorbance range of the photosensitizer.[40] The light is used to excite photosensitizers that triggers non-reversible destruction of tumor cells by stimulating numerous apoptotic pathways, necrosis, or autophagy-mediated cell death.[40–42] This non-reversible destruction is achieved either by direct interaction of emitted fluorescence energy of excited photosensitizer with cellular biological components (type I) or by transfer of its energy to an innate biological triplet oxygen in order to generate cytotoxic free radical singlet oxygen ($^1O_2$) (type II).[41,44,45] These generated free radicals can kill cancer cells in various ways, such as by reacting with and destroying vital cell organelles[46] and by triggering cell-death proteins in the cytoplasm.[43] Since clinically approved photosensitizers do not accumulate in DNA, the probability of free radical singlet oxygen causing DNA mutation is reduced.[40] In addition, photosensitizers can destroy blood vessels supplying the tumor, which starves the cancer cells of both nutrients and oxygen until death.[43] The singlet oxygen generated also helps to overcome the problem of selectivity as it only exerts its cytotoxic action on the tumor cells where it is generated. Due to its very short life span (10–320 nanoseconds), the singlet oxygen cannot diffuse to other surrounding normal cells.[40] Furthermore, it can also stimulate the immune response to fight cancer cells and this method is advantageous for successful treatment of metastatic cancers.[40,42]

Hematoporphyrin derivatives such as porfimer sodium (Photofrin®), aminolevulinic acid and its methylesters are the most popular and clinically approved photosensitizers used during PDT.[44] Preclinical and clinical studies supporting the efficacy of porphyrin photosensitizers for PDT in various precancerous or cancerous growths of the bladder, esophagus, lungs, breast, and skin, as well as non-cancerous diseases have been extensively reviewed by Dougherty et al,[41] Dougherty,[47] Brown et al,[43] and Agostinis et al.[40] Although convincingly potent, porphyrin photosensitizers have some major drawbacks, in particular, their drug-to-light interval (time between administration of the photosensitizer and light irradiation) during which the patient has to be protected from light is quite long (24–72 hours).[42,43] Photosensitizers with an absorbance

wavelength within the near infrared region (<700 nm) penetrate deeper into the inner organs, which results in better activation of the photosensitizer in those organs and better PDT.[40] However, a photosensitizer such as porfimer sodium absorbs at 630 nm and has a low molar absorption coefficient (1,170 M/cm) and thus a large concentration and intensity of light are required for sufficient activation.[42] In addition, patients experience long-lasting photosensitivity and skin reactions to sunlight after treatment with porphyrin.[48] Although verteporfin successfully overcomes the long drug-to-light interval problem faced by its counterparts because irradiation is applied 15 minutes after its administration, the other aforementioned challenges still remain.[42] Hence, there is still a need to develop second-generation photosensitizers that overcome a tangible portion of the setbacks faced by the existing photosensitizers.

Calixarenes are also being explored in the search for better photosensitizers in PDT. For example, two synthetic calixarenes (p-sulfonato-calix[6]arene and p-sulfonato-calix[8] arene) were found to possess antitumor activity when tested in a K562 myelogenous leukemia cell line using mercury light at 430 nm.[49] The mechanism of this cytotoxic effect was discovered through apoptotic activation via caspase system, membrane integrity disruption and mitochondria damage. Another very promising novel study by Cakmak et al[44] reported the synthesis of PEGylated bodipycalix(4) arene through a Knoevenagel reaction. Poly(ethylene glycol) (PEG) was added to each bodipyl complex to confer hydrophilic properties on the complex. The resulting compound was reported to absorb light at 725 nm (near-infrared region) and generate more singlet oxygens which could translate into more cytotoxicity in tumor cells.

Challenges
In the past, low solubility of calixarenes was considered an obstacle to their application. Incorporation of hydrophilic moieties into the basic core has made them easily soluble in common organic solvents, and the synthesis of water-soluble calixarenes is now common practice.[50] Different conformations of calixarene can affect its physical and chemical behavior and researchers are able to control this by using various synthetic methods.[51] Calixarene as a drug, however, does not follow Lipinski's rule of five, especially when bulky groups are introduced at both the upper and lower rims.[52] Furthermore, the drug carrier potential of calixarene is still not adequate for use in the complex human biosystem. The stability of host-guest chemistry and exact "switch on" release of drug from the cavity of calixarene still needs more investigation.

Conclusion
Calixarenes possess both drug-like and drug-loading properties. Functionalization at the lower or/and upper rim could enhance their therapeutic value and also solve the problem of their low solubility in water and common organic solvents. The anticancer activity of calixarenes is an emerging area of research which further increases their therapeutical significance. Due to their special geometric shape and flexibility, drugs can easily be incorporated into the cavity of these cyclic oligomers. The release of drugs from the cavity depends on the external factors employed. pH-triggered drug release has been developed by several research groups to some extent, but is still not refined. The narrow difference in pH range of normal and cancer tissues makes it difficult to specify the target. Radiation-based drug release from the cavity of amphiphilic calixarenes has not been given much consideration. For this purpose, appropriate functionalized calixarenes are being developed and studied. For targeted chemotherapy, research on calixarenes loaded with anticancer drugs and their controlled release upon radiation will provide a new area of interest. Introduction of chemoradiotherapy will minimize the adverse effects of anticancer drugs on normal cells. Thus the administered drugs, though circulating through the whole body, will only be activated/released in the targeted cancer cells through preoptimized radiation. This mechanism seems somewhat different and more sophisticated than that observed in hypoxia-activated prodrugs. Computational study will further help us to understand calixarene-drug stability for auspicious inclusion complexes. Chemotherapy-based radiotherapy is a step toward safer treatment of solid cancers and may be a useful approach to overcome the multidrug-resistant nature of tumor tissue.

In conclusion, the search for new potent anticancer drugs that can only target cancer cells, rather than affecting normal tissues is very much commendable. Calixarene is a highly promising candidate in this regard, and could be modified and appropriately used for targeted chemotherapy. Incorporation of clinically approved active drugs into the basic moiety could enhance the biologically active portion of calixarene.

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