Cyclotriphenaphazene-based Derivatives for Antibacterial Applications: An Update on Recent Advances

XiQi Su¹, Le Wang¹*, JingHua Xie¹, XiaoHui Liu¹ and Helena Tomás²

Abstract: As a phosphorus scaffold, hexachlorocyclotriphenaphazene (HCCP) is widely used for the synthesis of a variety of derivatives, including metal-binding complexes and several unique organometallic compounds, which exhibit potential catalytic, flame retardant and biological activities. Some metal-binding HCCP derivatives have shown antibacterial activities as free ligands and metal complexes. These derivatives can also serve as building blocks for the formation of antibacterial metal-containing polymers. This mini-review is focused on the design and development of HCCP derivatives as potential antibacterial agents with representative examples as well as antibacterial mechanisms from recent years.

Keywords: Hexachlorocyclophenaphazene (HCCP), antibacterial, antibacterial mechanism, metal, ligand, polymer.

1. INTRODUCTION

Bacterial infections are considered serious public health concerns by several international organizations and local agencies, as they may lead to high mortality [1-3] and have been well illustrated by the few pandemic plagues in history. Infections by some common Gram-positive pathogenic bacteria (e.g., Staphylococcus, Streptococcus, and Enterococci) are still the main causes of human morbidity and death worldwide [4, 5], especially those antibiotic resistant ones such as MRSA (methicillin-resistant Staphylococcus) and VRE (vancomycin-resistant Enterococci), although some other common ones can also cause serious illness such as tetanus-causing Clostridium difficile; whereas the widely distributed Gram-negative bacteria can be opportunistic such as Pseudomonas aeruginosa, pathogenic such as the plague-causing Yersinia pestis, or even life-supporting ones such as certain types of the very common gut bacterium Escherichia coli. The emergence of antibiotics revolutionized modern medicine, which has been commonly used for the prevention and treatment of bacterial infections, including the disastrous plague. However, continuous extensive use of antibiotics, and especially misuse, has resulted in bacterial resistance [6, 7] and increased the number of multi-drug resistant bacteria worldwide, namely MRSA and VRE. The lack of effective last-resort treatments has resulted in high mortality [8, 9]. Based on this fact, the World Health Organization (WHO) claims that it is a priority to develop novel antibacterial drugs for the treatment of multidrug-resistant infections [10].

In addition to further discover new antibiotics, natural strategies have also been developed for combating infectious microorganisms, including the use of phages, antimicrobial peptides, bacteriocins, probiotics, fecal transplants, predatory bacteria, antibodies, and therapeutics produced with biotechnology (such as the use of genetically modified bacteriophages, lymphs, and the CRISPR-Cas9 system) [11-13]. However, synthetic approaches are still preferable for potential production of effective bactericides that are immune tolerable and relatively less expensive [14]. Moreover, synthetic analogs can mimic natural antibiotic actions with potentially improved antibacterial efficacy, and can possibly afford new antibiotic molecules against drug-resistant bacteria. Since bacteria can form biofilms which render those bacteria relatively more resistant to bactericides [15, 16], the development of coating materials resistant to microbial adhesion, capable of killing bacteria, and/or as matrices for controlled release of antibacterial drugs has also been an area of intense research.

Hexachlorocyclophenaphazene (NiP₃Cl₆, HCCP) is a well-known phosphorus-containing cyclic compound that contains six reactive P-Cl bonds (Fig. 1). Since its first synthesis in the 19th century (1834s), cyclopropaphenazene has been an essential phosphorus scaffold in phosphorus chemistry for the synthesis of a great variety of derivatives [17-19]. As its chemical and physical properties are influenced by the connected substituents [20], many HCCP derivatives with specific substituents have been prepared for broad applications, such as the use as flame retardant additives [21-23], fluorescent materials [24, 25], and liquid crystals [26]. Recently, their use in biomedical fields has attracted particular attention, for instance, as anti-HIV, anticaner, and anti-neurodegenerative agents [27-30]. In this review, we highlight the latest antibacterial research and future perspectives of HCCP-based derivatives and their complexes and composite materials, as well as their use as drug delivery systems.

2. HCCP-BASED DERIVATIVES FOR ANTIBACTERIAL APPLICATIONS

With six chemically active chlorine atoms, the cyclophenaphazene core can undergo substitution reactions to yield various derivatives [31, 32]. The synthesis of HCCP-based antibacterial derivatives is mainly focused on nucleophilic substitution reactions
with antibacterial active moieties, such as alkoxy, phenoxyl, thiol, and amido groups. In this review, two preparation methods are described: a) direct linking of active groups to HCCP to form novel antibacterial molecules or ligands for complexation with metal ions and b) the utilization of HCCP as a core to form polymers and antibacterial composites. These general methods are illustrated in Fig. (1) whereas Table I summarizes relevant information regarding the examples presented in the review, namely the type of HCCP derivatives, the synthetic approaches used in the preparations, the bacterial strains that are sensitive to the compounds, and the Minimum Inhibitory Concentration (MIC) of the compounds.

Although cyclotriphosphazene itself is an active antimicrobial agent due to the bactericidal effect of the phosphazene group [33], the modification of the cyclotriphosphazene core can be considered a significant strategy to synthesize novel antibacterial drugs. This section is thus organized into three parts: 1) cyclotriphosphazene-based derivatives with antibacterial activity; 2) metal complexes of cyclotriphosphazene-based derivatives with antibacterial activity; and 3) polymeric cyclotriphosphazene-based derivatives as antibacterial agents or as carriers for antibacterial drugs.

2.1. Cyclotriphosphazene-based Derivatives with Antibacterial Activities

As the Schif base imine group (C=N) may promote antibacterial activity [34-36], its oxime derivatives (C=N-OR) have been introduced into HCCP-based systems and their biological action investigated. A series of oxime-phosphazene derivatives were reported (1a-j, Fig. 2), and their antibacterial and antifungal properties verified with the agar-well diffusion method [37] to exhibit

Fig. (1). Schematic procedures for the preparation of HCCP-based antibacterial derivatives. Method a: direct linking of AG (active groups) to form novel antibacterial molecules or ligands for metal binding; Method b: utilization of HCCP as a core to link macromolecules to form antibacterial polymers.

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$^{a}$Not determined; $^{b}$MICs were transformed by us from micromolars to micrograms per milliliter.
various antibacterial activities (MIC = 100–25 µg/mL) against two Gram-positive bacteria (S. aureus and E. faecalis) and two Gram-negative bacteria (E. coli and K. pneumonia). Among them, compounds 1g, 1h, 1j with benzoyl, and 1i with thiophene have a positive effect on these four bacterial pathogens and showed inhibitory zones of 23–30 mm with MIC=25 µg/mL. Compounds 1d-f with a carbonyl group have only moderate activities against these four microorganisms with inhibitory zones of 17–21 mm and MIC of 50 µg/mL. Although the authors have not further discussed the antibacterial mechanism of the oxime phosphazene derivatives, it may be suspected that the imine group in the compound promotes antibacterial activity as previously observed. In addition, the results showed that aromatic rings such as benzoyl (1g, 1h, 1j) and thiophene(1i) show higher antibacterial activities than other substituents on the oxime moieties. This implies that the introduction of benzoyl or thiophene ring on the oxime phosphazene backbone can significantly improve the antimicrobial activity of the compounds. These results provide new strategies for the preparation of cyclotriphosphazene-based derivatives bearing antimicrobial activity.

Similarly, some other aromatic ring-linked imine phosphazene derivatives were prepared and investigated [38]. A series of aromatic ring-linked imine phosphazene derivatives were synthesized (2a-j, Fig. 3), and their antibacterial was screened. These compounds show mild to good antibacterial activities (MIC=240–0.94 µg/mL) against either of the two Gram-positive/negative bacteria. Among compounds 2a-j, 2b with a chlorophenol group shows an excellent antimicrobial activity toward the Gram-positive S. aureus and Gram-negative P. aeruginosa and E. coli with MIC in the range of 3.75–0.94 µg/mL, better than the standard antibacterial drugs ciprofloxacin and gatifloxacin with MIC values of 15–1.875 µg/mL. Moreover, compound 2d with only the phenol group exhibits a favorable activity against the Gram-negative bacteria E. coli ATCC 25922 with a MIC of 15 µg/mL and an acceptable antibacterial activity against K. pneumonia CIP 53153 with MIC=30 µg/mL. The CN- and CO-containing 2j shows promising antimicrobial activity against the Gram-positive bacterium S. aureus ATCC 25923 and the Gram-negative bacteria P. aeruginosa ATCC 27853 and E. coli ATCC 25922 with a MIC about 15 µg/mL. It seems that the aromatic ring-linked imine phosphazene derivatives that contain OH, Cl, and/or CN groups can promote antibacterial activity. These aromatic ring-linked imine derivatives were suspected to disrupt the cell wall, change the permeability of the plasma membrane, and affect protein synthesis of the microbes, causing bacterial death [39].

Recently, Lakshmikandhan et al. [40] prepared a phenol-linked imine phosphazene derivative (compound 3) (Fig. 4) aimed to use as an antibacterial additive. Compound 3 was added to epoxy nanocomposites with various weight percentages (1 wt%, 3 wt%, 5 wt%, and 7 wt%) to determine the optimal antibacterial activity. The results showed that the reinforced epoxy nanocomposite could im-

Fig. (2). Chemical structure of oxime phosphazene derivatives with antibacterial activities (compounds 1a-k). Adapted from reference [37].

Fig. (3). Chemical structures of aromatic ring-linked imine phosphazene derivatives (compounds 2a-j). Adapted from reference [38].
prove antimicrobial activity against the growth of *B. subtilis* and *P. aeruginosa*. The antimicrobial activity of the epoxy nanocomposites was enhanced with increasing amounts of 3 and reached the highest antibacterial activity at 7 wt%, showing inhibition zones for these two bacteria of 18 and 17 mm, respectively. However, whether or not the antibacterial activity of the nanocomposite is further enhanced with higher amounts of 3 was not conducted and could not be determined. The enhanced antibacterial activity of phenol-linked imine phosphazene/epoxy nanocomposites is presumably related to the lipophilicity of the nanocomposites. The delocalization of p-electrons increases the lipophilicity of the composites, which in turn promotes the decomposition of the bacterial cell wall, resulting in bacterial death. Furthermore, compound 3 can interfere with the permeability of cells, causing the outflow of electrolytes, nutrients, proteins, and genetic material, leading to cell death [41-43].

![Chemical structure of an imine-phosphazene derivative (compound 3). Adapted from reference [40].](image)

Some fluorophores with excellent photophysical properties (such as BODIPY and naphthalimide), can also be introduced into the HCCP-derived system and used for antibacterial applications. Some studies have pointed out that BODIPY can kill bacteria by producing singlet oxygen, which can initiate a non-specific killing of bacteria, without inducing resistance [44, 45]. Naphthalimide, a cyclic imide with a strong hydrophobicity and a large p-conjugated skeleton, shows antibacterial activities by interacting with target molecules in bacteria (e.g., DNA) through non-covalent forces (such as p-p stacking) [46]. Based on these research results, a cyclotriphosphazene cored naphthalimide-BODIPY dendrimeric system was developed (Fig. 5) for antibacterial research [47]. The antibacterial activities of mono- and di-aryl-BODIPY substituted cyclotriphosphazene derivatives (compounds 4a,b) against Gram-positive *S. aureus* and Gram-negative *E. coli* were tested by the use of the Kirby-Bauer disk diffusion method, showing inhibition zones of 16 to 23 mm against *S. aureus* respectively, but without significant effect on *E. coli* (at a concentration of 10\(^{5}\) CFU/mL). These findings suggest that the naphthalimide-BODIPY derivatives of cyclotriphosphazene may be more selective against Gram-positive bacteria and can have specific antibacterial applications.

The authors stated that the good antibacterial activity of derivatives 4 could be attributed to the presence of the BODIPY moieties. The BODIPY moieties have a photo-sterilization effect that can produce singlet oxygen to kill bacteria [45, 48]. Moreover, the interaction between the BODIPY moieties in the phosphazene derivative and the cell wall increases permeability of bacterial cells, which can also enhance their bactericidal activities [44]. The authors believe that the bactericidal effects of BODIPY can overcome the resistance of a variety of bacteria and that the naphthalimide-BODIPY-cyclotriphosphazene compounds can be successfully applied to defeat them if they are proven safe at clinical doses and demonstrated specific in vivo. Regardless, this design strategy for killing bacteria, which uses photosensitizers for the generation of singlet oxygen, is a potential direction for producing promising novel antibiotic agents.

The spirocyclic phosphazenes have attracted much attention for antibacterial applications, where the chiral cyclotriphosphazenes possess stereoisomeric properties [49]. Therein, bifunctional nucleophilic aromatic diamines are introduced into the HCCP-based system to produce spiro-cyclotriphosphazene derivatives [50, 51]. Akbas et al. [52] firstly introduced N-alkylmonom (4-fluorobenzyl) diamine into the HCCP-based system to produce spirophosphazene, aiming to develop antimicrobial drugs against certain bacteria and fungi. This newly synthesized spirophosphazene was fully substituted by pyrroldinyldi or piperidino to form tetrapyrophosphazene 5a-c or tetrapiperidino 6a-c phosphazene derivatives (Fig. 6). The antibacterial and antifungal activities of these compounds assessed by the use of the agar well diffusion method showed an inhibition zone of 11 to 20 mm toward the Gram-positive *B. subtilis* and the fungi *Candida tropicalis* and *Candida albicans* at 2500 µM with MIC values ranging from 625 to 9.8 µM.

Among all compounds, compounds 5a-c had good activity against *B. subtilis*, with the inhibition zone ranging from 11 to 16 mm. While compounds 6a-c exhibited strong antifungal properties against *C. tropicalis* and *C. albicans*, and 6c was the strongest antifungal compound against these two strains (inhibition zone: 20–18 mm versus 17–13 mm for 6a and 17–12 mm for 6b). The results indicated that this antimicrobial activity might be dependent on the chain length of the alkane diamine precursor and substituents (pyrrolidinyl or piperidino groups) in compounds 5 and 6, i.e., the longer the chain length of the diamine precursor, the better the liposolubility and the more obvious the antibacterial activity [53]. It seems that the size of the substituted heterocyclic might also have some influence on the selectivity of antibacterial pathogens. For example, compounds 5 had high antibacterial activities toward Gram-positive bacteria, whereas compounds 6 were active against fungi.

Similarly, a selection of spiro-bino-spiro cyclotriphosphazene derivatives has been prepared with octapyrophosphazene, morpholine, and 1, 4-dioxy-hetero-8-azaspiro (DASD) for antimicrobial research [54]. The with antibacterial activities of the derivatives 7a-c (Fig. 7) were screened with the agar well diffusion method, showing different antibacterial activities against Gram-positive *B. cereus*, *B. subtilis*, and *E. faecalis* and Gram-negative *P. vulgaris* with inhibition zones of 10–26 mm at high concentration of 5 mM. Compound 7a with octapyrophosphazene had antibacterial activities against *B. cereus*, *B. subtilis*, and *P. vulgaris* with inhibition zone diameters of 22–26 mm. It is worth noting that these values are close to those of standard drugs such as ampicillin (inhibition zone diameters: 10–35 mm) and chloramphenicol (inhibition zone diameters: 23–31 mm) under the same conditions. We speculate that octapyrophosphazene may facilitate the antimicrobial activity of compound 7a.

Fluorenyl can be used as a protective group to form bridged(bino) phosphazene derivatives. A few fluorenylidene-bridged cyclotriphosphazenes 8a-c (Fig. 8) have been reported to possess antibacterial activity against some Gram-positive and Gram-negative bacteria [55]. The derivatives 8b and 8c exhibit good antibacterial activities against *S. aureus* ATCC25923, *E. coli*
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Fig. (5). The chemical structure of the naphthalimide-BODIPY-cyclotriphosphazene triads (compounds 4a, b). Adapted from reference [47].

Fig. (6). The chemical structure of the tetrapyrrolidino spirocyclotriphosphazenes (compounds 5a-c) and the tetrapiperidino spirocyclotriphosphazenes (compounds 6a-c). Adapted from reference [52].

Fig. (7). The chemical structure of the spiro-bino-spiro cyclotriphosphazene derivatives 7 (general chemical structure) and compounds 7a-c. Adapted from reference [54].
ATCC35218, and P. aeruginosa ATCC27853 with inhibition zones ranging from 25 to 37 mm. The compound 8a showed milder antibacterial activities against S. aureus ATCC25923 and P. aeruginosa ATCC27853 with inhibition zones of 10–35 mm. Specifically, the phosphonitrile derivatives (8b and 8c) with two fluorene groups show better antibacterial activities than the phosphonitrile derivative 8a with only one fluorene group. Thereby, we suspect that the presence of the fluorene group may favor the antibacterial effect. However, fluorenylidene-based antimicrobial agents are rarely reported in the literature, the mechanism of the antimicrobial activity of these derivatives still needs further investigation and confirmation.

2.2. Antibacterial Metal Complexes of Cyclotriphosphazene-based Ligands

Many organometallic macromolecules have shown significant activities against diverse cellular forms, including cancer/tumor cells, bacteria, and fungi, through various mechanisms such as their binding to nucleic acids and other biomolecules [56-60]. In this section, the chemistry and activities of a series of metal-binding HCCP-based derivatives and their metal complexes are discussed.

Owing to their high Lewis basicity, nitrogen- and oxygen-containing moieties have been introduced into HCCP-based system for the preparation of various metal-binding ligands. As shown in Fig. (9), N, O-aminoethanol was linked to the HCCP cycle to form O-aminoethanol tetracyclochlorotrifluorophosphine (compound 9) [61]. It was claimed that this ligand could complex with Cr(III), Fe(III), Cu(II) and Mn(II) transition metal ions, and that majority of the complexes displayed better antibacterial effect than the ligand. However, the structures of any of the complexes were not conclusively determined by means of structure-specific spectroscopic methods. The antimicrobial activity was evaluated based on the diffusion agar assay. The findings presented that the Fe(III) complex had significant activity on P. aeruginosa, which was superior to some known antibiotics such as metilmicine and gentamicin (inhibition zone diameter: ~17 mm versus ~14 mm for metilmicine and gentamicin). Furthermore, the antibacterial activity of Fe(III) complex against P. aeruginosa was comparable to the standard antibiotic amikacin (inhibition zone diameter: ~21 mm). It may be attributed to the interference of iron in the replication process of the genetic material occurring in microbes or disturbance of iron homeostasis [62], which needs to be further confirmed.

![Chemical structure of fluorenylidene-bridged cyclophosphazenes (compounds 8a-c)](image_url)

**Fig. (8).** Chemical structure of fluorenylidene-bridged cyclophosphazenes (compounds 8a-c) with antibacterial activity. Adapted from reference [55].

![Chemical structure of the ligand N, O-aminoethanol tetrachlorocyclophosphazene (compound 9)](image_url)

**Fig. (9).** The chemical structure of the ligand N, O-aminoethanol tetrachlorocyclophosphazene (compound 9). Adapted from reference [61].

Furthermore, ferrocene is an essential organometallic compound with a very unique sandwich-like structure and diverse chemical reactivity. Along with other "metalloenes", ferrocene and derivatives have been investigated for their pharmaceutical properties, including anticancer, antibacterial, antifungal, and antimalarial activities [63]. A few ferrocenyl and tetrpyrrolidino-HCCP derivatives have been synthesized (10, Fig. 10) and their antibacterial activities have been determined [64]. Results showed that compounds 10 had potential antimicrobial activity towards Gram-positive B. cereus and antituberculosis activity towards M. tubercu-
lossis H37Rv (ATCC 27294). Compared with the HCCP-based core modified by the heterocyclic ring alone, these new compounds showed significantly better antibacterial activities, reflecting possible roles ferrocene plays. Whether it is due to the redox property of ferrocene or its possible specific interactions awaits future explorations.

Fig. (10). The chemical structure of the ferrocenyl bisspirophosphazene derivatives 10 (n = 0, 1, 2). Adapted from reference [64].

The same research group further developed another ferrocene-containing phosphazene derivatives (11 and 12, Fig. 11) [65]. These compounds showed varying degree antibacterial activities against bacterial strains with an inhibition zone of 8 to 18 mm at 200 µM. It is shown that compound 12c had the obvious antibacterial activity against E. coli ATCC35218 (inhibition zone, ~14 mm), almost the same as that of the standard drug chloramphenicol (inhibition zone, ~8 mm). Along with studies of other metalloccenes, the results herein suggest that ferrocene phosphazene derivatives may be potential antibacterial agents. To reveal the possible antibacterial mechanism of these compounds, the researchers also conducted DNA interaction studies, which can be a potential intracellular target. The results showed that compounds 11 and 12 can affect in vivo DNA synthesis, suggesting that ferrocenyl phosphazene derivatives can interact with genetic materials and hinder their replication [66, 67].

Phosphazene derivatives can also complex with Ru(II) ion for antibacterial applications, such as the one shown in Fig. (12) [68]. Their antibacterial and antifungal activities were screened with a disc diffusion and a dilution assay. The Ru(II) complex 13 showed stronger antibacterial activities against some Gram-positive and negative bacteria and yeast cultures than the free ligand. The antibacterial activities of the Ru(II) complex 13 were determined to be better than the standard drug gentamycin against S. aureus (MIC:~12.5 mg/mL versus 25 mg/mL for gentamycin), and achieving gentamycin against E. coli and K. pneumonia with MIC ~ 6.25 mg/mL. These antibacterial results suggest that these compounds deserve further investigation in view of their application as effective antibacterial agents.

The authors pointed out that the antibacterial activity of the Ru(II) complex 13 can be elucidated by the chelation theory of Tweedy [69]. The binding of the ligand with Ru(II) may reduce the polarity of the metal ion and enhance lipophilicity throughout the delocalization of pi electrons. Along with the lipophilic enhancement of the Ru(II) complex 13, the complex may into the lipid membrane showing potential interactions with some biomolecules on microbial enzymes[70]. In addition, this group also claimed that these compounds may interfere with the cellular respiration/redox process, preventing protein synthesis, which limits further bacteria growth, which was attributed to a dependency on the properties of the bacteria membrane and the difference of ribosomes in microbial cells [71].

Fig. (11). The chemical structure of the dispirocyclic ferrocene phosphazene derivatives (compounds 11a-c and 12a-e). Adapted from reference [65].
aureus, E. coli, and P. aeruginosa and are also active against Mycobacteria strains (M. tuberculosis H37Rv and M. Bovis BCG Pasteur) with MIC values in a range of 0.12–15.6 µM. Their antibacterial activities are significantly better than that of AgNO3 (MIC: ~31.25–15.6 µM), silver sulfadiazine (AgSD) (MIC: ~90–30 µM), and the phosphazene ligands (MIC: ~250–15.6 µM). The results showed most of the complexes were effective against S. aureus and E. coli with MIC among 7.8 and 0.12 µM. In particular, the complexes 13a, 13b, and 13f exhibit the best antibacterial effect on S. aureus and E. coli with MIC values of 0.97–0.12 µM.

The broad-spectrum antibacterial activity of complexes 14 was suspected to be related to weak Ag-N bonds, which make the ligands easily replaced by sulfur or nitrogen donor sites of proteins and nucleotides in bacteria [75]. Alternatively, binding silver ions with mercapto groups in proteins [76] including membrane proteins can denature or inactivate those proteins and eventually cause bacteria death [77, 78]. Owing to their low toxicity and strong and broad-spectrum antibacterial activities, these Ag(I) metallophosphazenes complexes and analogues warrant further investigation.

3. ANTIBACTERIAL CYCLOTRIPHOSPHAZENE-BASED POLYMERS

On account of their excellent antibacterial properties, such as relatively long antibacterial action, diverse antibacterial polymers have attracted much attention [79-81]. Cyclophosphazene-based polymers, for example, may possess antibacterial properties per se or may be used as carriers for antibacterial drugs, and have thus been previously evaluated and highlighted herein.

3.1. Cyclophosphazene-based Polymers with Antibacterial Properties

A polymer composite of cyclophosphazene(CP) with blended benzoazine(Bz) and bismaleimide(Bmi) has been synthesized (Cp-Bz-Bmi 15, Fig. 14) and its applicability as an antibacterial agent investigated [82]. Different compositions of cyclophosphazene (5 wt%, 10 wt%, and 15 wt%) in the Bz-bmi copolymer exhibit higher antibacterial activities than pure Bz-bmi copolymer. As the weight percentage of cyclophosphazene increases, the inhibitory effect on bacteria becomes more pronounced. The Cp(15 wt%)-Bz-Bmi composite restraints the growth of S. aureus and E. coli, showing 11 and 10 mm in inhibition diameter, respectively. The antibacterial mechanism of 15 seems to be related to the amount of cyclophosphazene present in the composite, and the authors stated that the van der Waals linked with the polymer and the bacteria [83]. However, this proposal needs to be further investigated clearly for their potential use as antibacterial coating materials.

A covalent composite of aminophenol-cyclophosphazene (ATCP) and functionalized TiO2 nanoparticles (FTiO2) with benzoazine (Bz) cyanate ester (CE) was synthesized and evaluated as a coating material which shows anti-corrosion and antibacterial activities against S. aureus and E. coli (16, Fig. 15) [84]. The weight percentages of ATCP (5, 10, and 15 wt%) and FTiO2 nanoparticles (1, 3, and 5 wt%) were varied to reveal optimal antibacterial activities. The results showed that the 15 wt% ATCP incorporated Bz-CE composite exhibits better antibacterial activity than the original Bz-CE polymer, and the composite ATCP(15 wt%)/FTiO2(5 wt%)Bz-CE showed considerable antibacterial

Fig. (13). The chemical structures of two series of silver(I) metallophosphazenes (14a-i). Adapted from reference [74].

Fig. (14). Chemical structure of cyclophosphazene (Cp) blended with benzoazine (Bz) and bismaleimide (Bmi) polymer composite (Cp-Bz-Bmi, 15). Adapted from reference [82].
properties against *S. aureus* and *E. coli* with suppression zones of 21 and 20 mm, respectively. The unique photocatalytic activity of the FTiO$_2$ nanoparticles may also contribute to the antibacterial effect as reported elsewhere [85]. Since UV-irradiated TiO$_2$ nanoparticles can produce the powerful oxidation agent singlet oxygen, which can degrade most organic compounds in bacterial cells [86], TiO$_2$ nanoparticles thus can be expected to contribute to the antibacterial effect of 16. Thus, this material warrants further development for antibacterial applications in medical facilities.

A polyhedral oligomeric silsesquioxane (POSS)-crosslinked phosphazene polyimide (PZI) nanocomposites (POSS/PZI nanocomposites) was synthesized (17, Fig. 16), and its antibacterial activities against *S. aureus* G(+) and *E. coli* G(-) investigated [87]. The antibacterial activity of 17 was detected to be better (with an inhibition zone in well diffusion assay of 13-15 mm) than that of the PZI matrix (11 mm). Nanocomposites of different concentrations of POSS in the PZI matrix (1, 5 and 10 wt% POSS/PZI) show antimicrobial activities against *E. coli* and *S. aureus* with the POSS(10 wt%)/PZI nanocomposite exhibiting the best antimicrobial effect (15 and 14 mm, respectively). The results suggest that the silica constituent POSS may be the key factor in this antibacterial nanocomposite [88]. It is also probable that silica nanoparticles increase the contact area between the composite and the bacteria, rendering a higher interaction with the PZI matrix, which improves antibacterial efficiency [89].

A cyclotriphosphazene-containing antibacterial polymer based on positively charged polyethyleneimine (PEI/HCCP) has recently been synthesized (18, Fig. 17) to serve as an antibacterial coating.

**Fig. (15).** Chemical structure of the covalent composite of aminophenol-cyclophosphazene (ATCP) and functionalized TiO$_2$ nanoparticles (FTiO$_2$) with benzoxazine (Bz) cyanate ester (CE) polymer (16). Adapted from reference [84].

**Fig. (16).** Chemical structure of polyhedral oligomeric silsesquioxane (POSS)-crosslinked phosphazene polyimide (PZI) nanocomposites (17). Adapted from reference [87].
over a polysulfone substrate and for the preparation of nanofiltra-
mation membranes [90]. Its antibacterial activity against Gram-
positive S. aureus and Gram-negative E. coli was screened with
colony-count method, showing nearly 100% bacterial inhibition
rate against S. aureus and E. coli (at 10^7 CFU/mL). Therein, the
bacteria at 10^7 CFU/mL were successfully killed in less than 20
minutes. The combination of PEI with HCCP clearly resulted in an
obviously effective antibacterial material.

Owing to their polycationic structure, polyethyleneimines (PEI)
are potent antibacterial moieties [91-93]. The antibacterial activities
of 18 can be attributed to the cationic charges of PEI, which pro-
motes bacteria attachment through electrostatic attraction and
interferes with bacteria viability [94] and also to the presence of HCCP,
which renders the polymer with higher hydrophobicity. The mecha-
nisms for cationic moiety-caused bacteria death are not very well
understood, but likely associated with cell wall and membrane dis-
ruption [60]. At the same time, the hydrophobic interaction of the
HCCP moieties with bacteria may render the formation of bacterial
biofilms more difficult and impact bacterial growth [95, 96].

3.2. Cyclotriphosphazene-based Polymers as Antibacterial Drug
Carriers

Some specific cyclotriphosphazene derivatives can form hydro-
gels for the delivery of antibacterial drugs. As shown in Fig. (18), a
novel biodegradable hydrogel was synthesized by crosslinking
formyl-phosphazene with chitosan via imine bond formation be-
tween the formyl group and the amino moiety (19) which can serve
as a carrier for the widely used effective β-lactam antibiotic amox-
icillin (AMX) as a model drug [97]. Through intermolecular forces
(H-bonding and van der Waals forces), AMX can be loaded into
and retained by this hydrogel 19. The antibacterial activities of
AMX-19 against S. aureus, B. subtilis, and E. coli were determined
with the disk diffusion method, showing positive antibacterial ef-
ects on S. aureus and B. subtilis G(+), yet not against E. coli G(-)
which is consistent with E. coli resistance to AMX. Moreover, the
hydrogel drug carrier is biodegradable, biocompatible, and capable
of responding to environmental stimuli, such as pH and the salinity.

The cyclotriphosphazene ring can be opened to form linear
polymers which can also be used as antibacterial drug carriers [98-
100]. Polyphosphazenes are heteropolymers consisting of alter-
ating phosphorous and nitrogen atoms, where the phosphorous atom
is usually connected to two side groups. Cyclotriphosphazene can
be thermally opened to form poly(dichlorophosphazene) at 250 °C,
and then substituted by various nucleophiles to form polyphos-
phazenes (20, Fig. 19) [101-105]. For example, the phosphoramide
linear polymer can be grafted with fluoroquinolones (e.g., cipro-
floxacine or norfloxacine) and ethyl amino acids to form a derivative
20 for antibacterial applications.

In order to increase the solubility of the polymeric polyphos-
phazene derivatives and control the release rate of antibiotics, the
authors attached multiple amino acid ethyl esters (glycine, alanine,
and phenylalanine) as co-substituents connected to the polyphos-
phazene skeleton. The in vitro antibacterial experiments showed that
ciprofloxacin- and norfloxacine-incorporated 20 had good anti-
bacterial effects on E. coli with MIC values of 0.45 µg/mL and 0.58
µg/mL, respectively. These antibacterial activities are equivalent to
those of the antibiotics ciprofloxacin and norfloxacine. Meanwhile,
an amount of 4–30% antibiotic release was observed in a six-week
hydrolysis study at 37 °C, indicating possible localized drug release
for prolonged periods from the polymer. The antibiotic release pro-
files of 20 make it a promising material as a new type of wound
dressings.

CONCLUSION

The research progress in the past 10 years of cyclotriphos-
phazene derivatives with antibacterial properties is reviewed in this
report with representative examples which show that these deriv-
aives, their metal complexes, and polymeric structures with these
derivatives as building blocks can all be used in antibacterial appli-
cations. Several of these derivatives show good antibacterial activi-
ties against a variety of bacteria or can serve as carrier matrix for

Fig. (17). Chemical structure of polyethyleneimine/hexachlorocyclotriphosphazene (PEI/HCCP) polymer (18). Adapted from reference [90].

Fig. (18). Chemical structure of chitosan hydrogel crosslinked with formyl-
phosphazene (19). Adapted from reference [97].
The delivery of existing antibiotics. These examples point out the opportunities for phosphorous chemistry to take challenging problems in antimicrobial research, such as bacterial resistance. Moreover, the study of the mechanisms for the antibiotic actions of these cyclotriphosphazene derivatives regarding their structure-function relationship will provide valuable knowledge complementary to the existing mechanisms for the action and resistance of better-studies antibiotics.

**LIST OF ABBREVIATIONS**

- **HCCP** = Hexachlorocyclotriphosphazene
- **MRSA** = Methicillin-resistant *Staphylococcus* 
- **VRE** = Vancomycin-resistant *Enterococci*
- **AG** = Active Groups
- **MIC** = Minimum inhibitory concentration
- **CP/BZ/Bmi** = Cyclophosphazene/Benzoazine/Bismaleimide
- **ATCP/FTiO2/Bz-CE** = Amine Terminated Cyclophosphazene/Functionalized TiO2/Benzoazine based Cyanate Ester
- **POSS/PZI** = Polyhedral Oligomeric Silsesquioxane/Phosphazene Polymide
- **PEI/HCCP** = Polyethyleneimine/Cyclotriphosphazene
- **AMX** = Amoxicillin
- **CPFX** = Ciprofloxacin
- **NFX** = Norfloxacin

**CONSENT FOR PUBLICATION**

Not applicable.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

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**REFERENCES**


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