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Biological activities of metal oxide nanoparticles modified with hexadeca-substituted cobalt(II) phthalocyanine bearing fluorinated substituents

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Recognition of the flexible structure of phthalocyanines, which can be tailored for specific applications, has attracted growing attention, as these compounds can enhance the efficiency of many technological and scientific systems. This study presents the synthesis of a new phthalonitrile derivative, namely 3,4,5,6-tetrakis(4-(trifluoromethoxy)phenoxy)phthalonitrile and its hexadeca-substituted cobalt(II) phthalocyanine. The newly synthesized phthalocyanine was used to functionalize the surface of alumina (Al_2O_3) and titania (TiO_2) nanoparticles *via* non-bonding interactions for the first time in this study. Additionally, the biological properties of compound (CoPc), unmodified (Al_2O_3 and TiO_2), and modified nanoparticles (Al_2O_3/CoPc and TiO_2/CoPc), including DNA cleavage, antimicrobial, antioxidant, and antidiabetic activities, were examined along with those of the phthalocyanine derivative to compare the effect of the modifying group on the biological properties of the metal oxide nanoparticles. As a result, the modified alumina and titania nanoparticles exhibited higher biological activity and can be considered promising biological alternatives in medicine, pending further clinical research.

Introduction

Metal oxide nanoparticles are usually prepared in the desired shape and size *via* simple synthetic processes. They exhibit high stability, incorporate hydrophobic/hydrophilic moieties, and do not undergo swelling. The negatively charged surface facilitates the functionalization of alumina nanoparticles with a wide range of molecules; therefore, they are considered appropriate nanomaterials for the design of efficient biomedical agents.[1] For instance, aluminum oxide nanoparticles (alumina nanoparticles) exhibit advanced bio/physicochemical properties, arising from their small size (1–100 nm) and high surface-area-to-volume ratio. These unique features make them suitable alternatives for diverse industrial and scientific applications, particularly biomedical ones such as tissue engineering, biomedical imaging, drug delivery, and biosensing systems.[2] Recently, the antimicrobial and antiviral potential of alumina nanoparticles has been studied,[3] as these nano-sized structures can effectively penetrate microorganisms and exhibit high antimicrobial efficacy.[4] Moreover, they can be used in hospital and medical device coatings to reduce the risk of infection by preventing biofilm formation and microbial adhesion. On the other hand, the low cost, chemical stability, and high refractive index of titanium oxide nanoparticles (titania nanoparticles) have attracted considerable interest from researchers. Additionally, high oxidation capacity and oxygen vacancy in their lattice structures make them suitable alternatives for diverse applications.[5] Due to their excellent photochemical properties and high biocompatibility, titanium nanoparticles have shown potential in medical and technological applications, including photodynamic therapy, solar cells, water remediation, and pharmaceutical chemistry, and their potential has been evaluated in recent decades. Particularly, they can generate reactive oxygen species, leading to cell death in tumor tissues.[6]

Metal oxide-containing nanoparticles should exhibit biocompatibility, acceptable stability, solubility, and functionality for most biological applications. To improve their properties, the surface of metal oxide nanoparticles can be modified with various molecules, such as bio- and polymers, dendrimers, and silica. Surface alterations are typically achieved through conjugation, coating, surface encapsulation, self-assembly, and core–shell synthesis.[7] However, only a few studies report the surface functionalization of metal oxide nanoparticles with phthalocyanines.[8,9]

A phthalocyanine ring consists of four indole units, resulting in a planar 18π-electron structure.[10] Due to its excellent electron-transfer capacity, it exhibits high thermal stability, chemical resistance, and optical properties. However, it is insoluble in water and most organic solvents because π–π stacking between phthalocyanine rings leads to aggregation. To improve solubility, various long and/or bulky organic substituents can be introduced at the periphery of the phthalocyanine to prevent aggregation. Additionally, different metal ions, especially metals with oxidation numbers higher than two, can be inserted into the ring center to increase the distance between

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phthalocyanine rings. Although these structural modifications markedly improve solubility, they also lead to distinct behaviors in the phthalocyanine rings.[11–15] Therefore, phthalocyanines are promising materials to design efficient systems for specific applications. Particularly, hexadeca-substituted phthalocyanines, including sixteen substituents on the phthalocyanine ring, can display excellent chemical and physical properties, but their synthesis is hard and requires more effort. Hence, a few studies have presented the synthesis and characterization of hexadeca-substituted phthalocyanines.[16,17]

Fluorine is a highly electronegative small atom that can lead to significant changes in organic structures upon replacement with a hydrogen atom. The organofluorine structures exhibit outstanding bioavailability, lipophilicity, stability, and solubility,[18–20] which are required in life sciences and materials science. For instance, simultaneous modulation of steric, lipophilic, and electronic parameters can be achieved by incorporating fluorine into a drug; therefore, fluorine can play a crucial role in a drug's pharmacokinetic and pharmacodynamic properties.[21] On the other hand, cobalt complexes exhibit a wide range of properties, particularly notable physicochemical features, that can be exploited to develop alternative biological agents for therapeutic goals such as bioactivity enhancement, selective protein inhibition, and bioreductive prodrug activation.[22] Since the biological activity of cobalt derivatives has not been examined as extensively as that of other metals' complexes, investigation of their biological properties can fill this vacancy in the literature.

This study presents novel phthalocyanine-modified metal oxide nanoparticles that are suitable for multidisciplinary biological applications. Therefore, a new phthalonitrile bearing four 4-trifluoromethoxyphenoxy groups and its cobalt(II) phthalocyanine derivative were synthesized and structurally characterized. The resulting phthalocyanine was, for the first time in this study, used to surface-modify alumina and titania nanoparticles. Additionally, the DNA-cleavage, antimicrobial, antioxidant, and antidiabetic activities of the newly synthesized cobalt(II) phthalocyanine, unmodified metal oxide nanoparticles, and phthalocyanine-modified nanoconjugates were examined and compared to evaluate the effect of functionalization on the individual activities of the metal oxide nanoparticles.

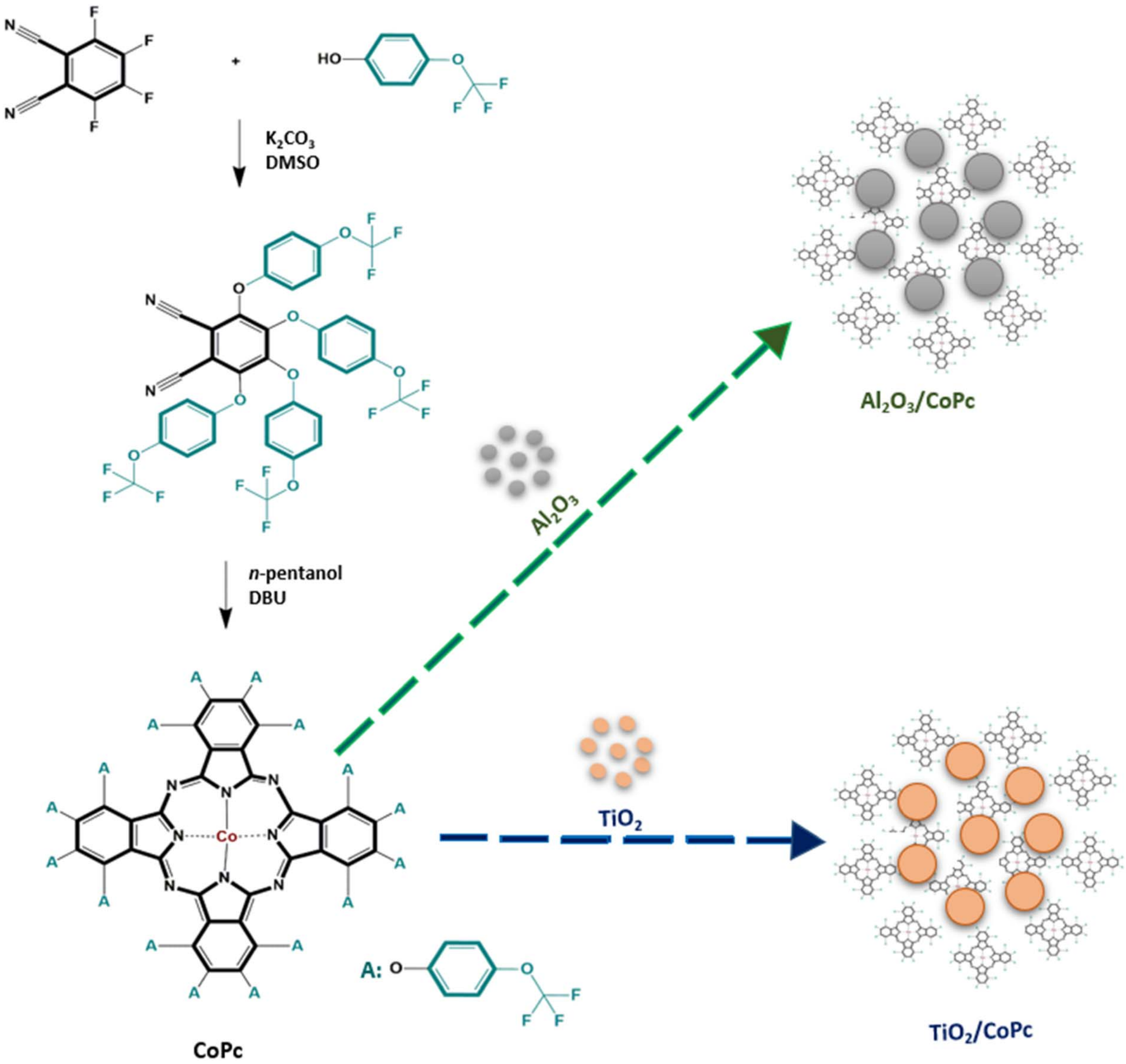
Results and discussion

Synthesis and characterization

The synthetic procedure for the macromolecule (CoPc) is portrayed in Scheme 1. First, a tetra-substituted phthalonitrile derivative was prepared *via* an aromatic substitution reaction, replacing the fluorine atoms with 4-trifluoromethoxyphenoxy groups. The resulting compound was extracted with dichloromethane and characterized by FT-IR, ^{13}C NMR, and ^{1}H NMR spectroscopy. The data were in accordance with the predicted structure. The cyclotetramerization of the newly synthesized compound in the presence of a cobalt ion afforded the macromolecule CoPc *via* a one-step metal-template mechanism. The pure compound was obtained by eluting with THF through a column packed with silica gel. The characterization of the phthalocyanine derivative was carried out using FT-IR, UV-vis, and MALDI-TOF spectroscopies. The difference in the molecular ion peak (fragmentation) of the studied compound may be due to the measurement medium (matrix effects). Generally, the organic matrix absorbs laser energy and facilitates laser desorption/ionization. After laser absorption and self-ionization of the organic matrices, one or more proton transfers occur between the matrix ions and the molecules under analysis. Besides, organic matrices are usually insoluble in water but dissolve in organic solvents such as acetonitrile, ethanol, methanol, acetone, or dimethyl sulfoxide (DMSO). These solvents form adducts, thereby reducing ionizability.[23] The results obtained confirmed the synthesis of the target phthalocyanine. The macromolecule CoPc was used to functionalize alumina and titania nanoparticles. The morphology of the prepared nanoconjugates was analyzed using SEM. The FE-SEM images of the unmodified and modified metal oxide nanoparticles are demonstrated in Fig. 1. The differences confirmed successful coverage of nanoparticle surfaces by the compound CoPc *via* non-bonding interactions (especially H-bonding). The confirmation of oxygen, nitrogen, carbon, fluorine, and cobalt elements in the elemental mapping analysis of the modified nanostructures proved the uniform modification of the metal oxide nanoparticles with compound CoPc.[24] The UV-vis spectra of compound CoPc were studied before and after its use as a modifying agent. The significant changes in the characteristic bands of CoPc (blue shift of the Q-band, decrease in Q-band intensity, and changes in the B-band) confirmed the successful surface modification. Moreover, the FT-IR spectra of unmodified and modified metal oxide nanoparticles were studied. The characteristic bands of compound CoPc were observed in the FT-IR spectra of the metal oxide nanostructures after surface modification. The respective average particle sizes of the aqueous nano alumina and nano titania were approximately 24 and 25 nm. After surface modification, the zeta potential of nano alumina increased from -22.4 ± 1.1 to -6.9 ± 2.2 mV, whereas that of nano titania changed from -18.9 ± 0.9 to 15.6 ± 1.0 mV. The significant shift in zeta potentials after surface functionalization confirmed the successful modification of the metal oxide nanoparticles with compound CoPc.[25]

Biological studies

DNA cleavage activity. DNA is the most essential macromolecule for living organisms; hence, its cleavage can involve enzymatic reactions that are crucial for organisms. In a DNA molecule, phosphodiester bonds link nucleotides; however, these bonds can be cleaved by nucleases. On the other hand, redox-active molecules or metal complexes also act as artificial or chemical nucleases, leading to irreversible cleavage. In general, organisms can remove damaged nucleotides resulting from oxidative stress; therefore, they can repair the DNA helix or halt the cell cycle, inducing apoptosis. Chemical nucleases (drug active ingredients) target DNA, causing damage and leading to cell death. For instance, anticancer agents are compounds designed to target DNA molecules in cancer cells.[26]



Scheme 1 Synthetic route for phthalonitrile derivative, cobalt(II) phthalocyanine (CoPc), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc).

In this study, the DNA cleavage activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were evaluated at three different concentrations, ranging from 50 to 200 mg L^{-1} (Fig. 2). Unmodified nanostructures (Al_2O_3 and TiO_2) induced single-strand breaks at 50, 100, and 200 mg L^{-1}, whereas the compound CoPc and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) induced double-strand breaks at 50 mg L^{-1} and complete DNA fragmentation at 100 and 200 mg L^{-1}. Babonaite *et al.* indicated that Al_2O_3 nanoparticles exhibited a size-dependent DNA-damaging potential.[27] Zhu *et al.* evaluated the interaction of DNA with TiO_2 nanoparticles using a gel-electrophoresis method. The linearised DNA was susceptible to binding by nanotitania.[28] Barut *et al.* synthesized several new silicon(IV) phthalocyanines and investigated their DNA-cleavage activities. They reported that the newly synthesized phthalocyanines cleaved DNA.[29] Çuhadar *et al.* reported the synthesis of some tetra-substituted phthalocyanines bearing dichlorobenzenethiol groups at peripheral or non-peripheral positions and evaluated their DNA-cleavage features. The resulting compounds exhibited moderate DNA-cleavage activity.[30] Compared with the literature, all the nano/structures, particularly compound CoPc and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc), can be considered DNA-targeting agents for anticancer and antimicrobial studies, pending further research.

Antioxidant activity. Instability in free radical production and antioxidant defense mechanisms leads to oxidative stress. Reactive oxygen species (ROS) are oxygen-derived free radicals[31] that are responsible for oxidative stress through their interaction with other molecules in the cell. Free radicals are often generated during mitochondrial metabolism; however, they can also result from external factors, including aging, chemical exposure, inflammation, high oxygen pressure, and radiation.[32] Oxidative stress occurs when the balance between pro-oxidants and antioxidants is disrupted within an organism. Generally,

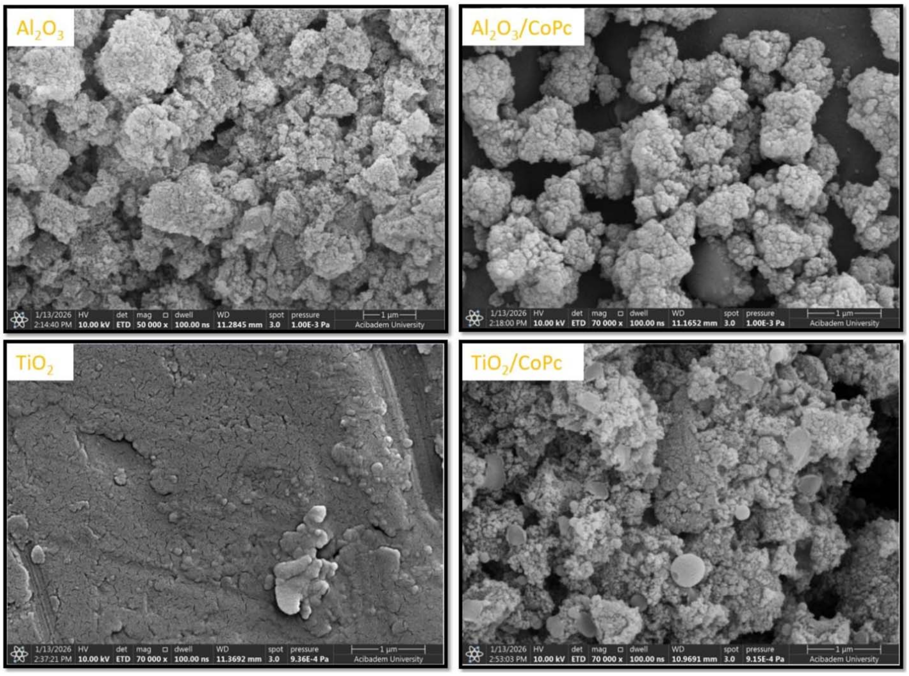


Fig. 1 SEM images of unmodified metal oxide nanoparticles (Al_2O_3 and TiO_2) and metal oxide nanoparticles (Al_2O_3/CoPc and TiO_2/CoPc).

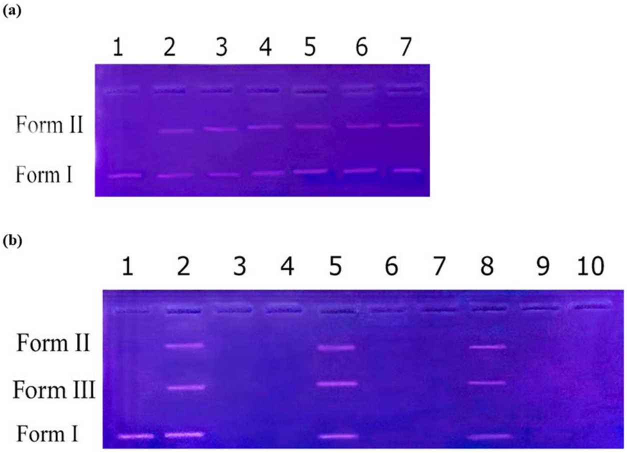


Fig. 2 DNA Cleavage activity of Al_2O_3, TiO_2, CoPc, Al_2O_3/CoPc ve TiO_2/CoPc. (a) Lane 1, pBR 322 DNA + DMSO; Lane 2, pBR 322 DNA + 50 mg L^{-1} of Al_2O_3; Lane 3, pBR 322 DNA + 100 mg L^{-1} of Al_2O_3; Lane 4, pBR 322 DNA + 200 mg L^{-1} of Al_2O_3; Lane 5, pBR 322 DNA + 50 mg L^{-1} of TiO_2; Lane 6, pBR 322 DNA + 100 mg L^{-1} of TiO_2; Lane 7, pBR 322 DNA + 200 mg of TiO_2. (b) Lane 1, pBR 322 DNA + DMSO; Lane 2, pBR 322 DNA + 50 mg L^{-1} of CoPc; Lane 3, pBR 322 DNA + 100 mg L^{-1} of CoPc; Lane 4, pBR 322 DNA + 200 mg L^{-1} of CoPc; Lane 5, pBR 322 DNA + 50 mg L^{-1} of Al_2O_3/CoPc; Lane 6, pBR 322 DNA + 100 mg L^{-1} of Al_2O_3/CoPc; Lane 7, pBR 322 DNA + 200 mg of Al_2O_3/CoPc. Lane 8, pBR 322 DNA + 50 mg L^{-1} of TiO_2/CoPc; Lane 9, pBR 322 DNA + 100 mg L^{-1} of TiO_2/CoPc; Lane 10, pBR 322 DNA + 200 mg of TiO_2/CoPc.

reactive oxygen species (ROS) can damage fundamental cellular molecules, including DNA, lipids, nucleic acids, and proteins, which are associated with serious disorders such as age-related immune deficiency, cancer, hypertension, and neurodegeneration. Because the body's antioxidant defense mechanism may be insufficient to counteract the harmful effects of free radicals, essential alternatives should be developed or identified. Organic or inorganic compounds, that prevent or reduce the formation of free radicals and the resulting biological damage, are defined as antioxidants. Because antioxidants can be added to certain foods to prevent or delay free-radical oxidation caused by environmental factors such as air, light, and temperature,[33] dietary antioxidants and alternative compounds play a key role in health.[34]

In this study, the antioxidant activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were investigated. The results obtained are portrayed in Fig. 3. Accordingly, the antioxidant activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were obtained 38.24%, 31.73%, 24.65%, 39.09%, and 36.63% at 6.25 mg L^{-1}, respectively. As the concentration increased from 12.5 mg L^{-1} to 50 mg L^{-1}, the respective antioxidant activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) increased from 38.24% to 70.25%, from 36.83% to 58.36%, from 27.48% to 40.79%, from 53.82% to 62.89%, and from 38.81% to 53.54%, respectively. Although the highest antioxidant activity (77.62%) was observed for the 100 mg L^{-1} compound CoPc, the nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) exhibited nearly identical antioxidant activities at 100 mg L^{-1}. In general, the antioxidant action mechanism of metal phthalocyanines is related to the resonance occurring in their π-system.[35] Zamani *et al.* studied the antioxidant activity of alumina nanoparticles using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. The high potential for the

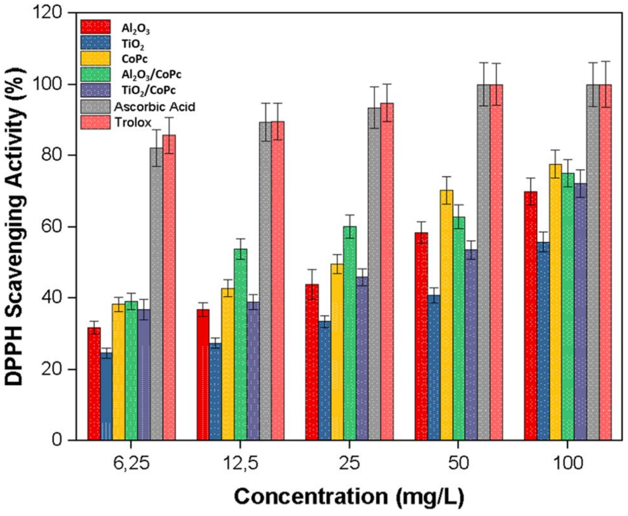


Fig. 3 Antioxidant activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc).

adsorption of DPPH radical on the alumina nanoparticles occurred *via* the interaction of radical nitrogen N$^\bullet$ and NO_2 groups of DPPH with the acidic and basic sites of the nano alumina surface.[36] Santhoshkumar *et al.* prepared titania nanoparticles using aqueous leaf extract of *Psidium guajava* and examined their antioxidant properties. They exhibited higher antioxidant activity than ascorbic acid.[37] Çuhadar *et al.* (2023) prepared two dichlorophenylthio-substituted phthalonitrile derivatives. They used them for the preparation of novel non-peripherally tetra-substituted metal phthalocyanines {ZnPc (**3**), CuPc (**4**), and MnPc (**5**)} and peripherally tetra-substituted metal phthalocyanines {ZnPc (**6**) and CuPc (**7**)}. The antioxidant activities were 83.96% for **3**, 78.43% for **4**, 61.78% for **5**, 64.87% for **6**, and 61.72% for **7** at 100 mg L^{-1}. Compound **3** demonstrated the highest DPPH scavenging activity (99.42% at 200 mg L^{-1}).[30] Korkut *et al.* reported the highest DPPH radical inhibition activity of 66.77% for 500 mg L^{-1} of the newly synthesized np-TEMPO-ZnPc.[38] Sajjadifard *et al.* examined the antioxidant activities of some new metal phthalocyanines (Zn and InCl) bearing (9,9-bis(5-hydroxypentyl)-9H-fluoren-2-yl) ethynyl groups on peripheral positions. Antioxidant activities ranged from 57.4% to 72.6% at 100 mg L^{-1}.[39] Compared with the literature, compound CoPc and its nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) may serve as alternative agents to synthetic antioxidants, warranting further study.

Amylolytic activity. Diabetes mellitus (DM) is a chronic metabolic disease that is characterized by hyperglycemia. As this disease is often associated with other serious disorders, including cardiovascular, hyperlipidemia, hypertension, and obesity, it is well-known as one of the vital growing global health problems.[40] Although metformin and sulfonylurea derivatives are some common antihyperglycemic drugs to control diabetes along with insulin therapy, they are expensive and cannot completely control complications. Also, some side effects, consisting of abdominal pain, bloating, diarrhea, fatigue, hepatotoxicity, hypoglycemia, lactic acidosis, vascular complications, weight gain, and weakness.[41] To reduce potential complications, prevalence, and costs, efficient population health strategies are needed as alternatives to traditional insulin therapies.[42] Alpha-amylase (1-4-α-glucan glucanohydrolase) is a valuable industrial enzyme, since it is used extensively in detergent production, food, fruit juice, pharmaceutical, and the textile industries.[43] Additionally, this enzyme is widely found in animals, bacteria, fungi, and plants, which consume polysaccharides. This hydrolase cleaves polysaccharides such as glycogen and starch at their alpha-(1,4)-glycosidic bonds, producing oligosaccharides of varying lengths.

In this study, different concentrations of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were prepared, and their antidiabetic properties were evaluated using α-amylase enzyme (Fig. 4). Accordingly, the α-amylase activity was inhibited 23.71%, 14.66%, and 14.53% by compound CoPc, unmodified nanoparticles (Al_2O_3), and nanoconjugate (Al_2O_3/CoPc) at 50 mg L^{-1}, respectively. The highest enzyme inhibition was observed at 100 mg L^{-1}, with 36.89% for CoPc, 31.29% for Al_2O_3 nanoparticles, and 41.79% for the nanoconjugate (Al_2O_3/CoPc). However, TiO_2 nanoparticles and nanoconjugate (TiO_2/CoPc) inhibited enzyme activity 26.56% and 21.14% at 100 mg L^{-1}, respectively. These results are similar to the literature. Biyiklioglu *et al.* investigated the α-glucosidase inhibitory activities of some new water-soluble metal phthalocyanines (Co and Cu). The cobalt phthalocyanine displayed the highest inhibitory activity.[44] Saka *et al.* prepared two novel water-soluble Co(II) and Zn(II) phthalocyanines and evaluated their α-amylase inhibitory activities. The inhibition rates were 10.1% and 64.2% for 450 μM Co(II) phthalocyanine and 300 μM Zn(II) phthalocyanine, respectively.[45] Çelik *et al.* synthesized two new silicon(IV) phthalocyanines bearing halogen substituents at the axial positions and evaluated their inhibitory activities against α-glucosidase and α-amylase. The inhibitory activities ranged

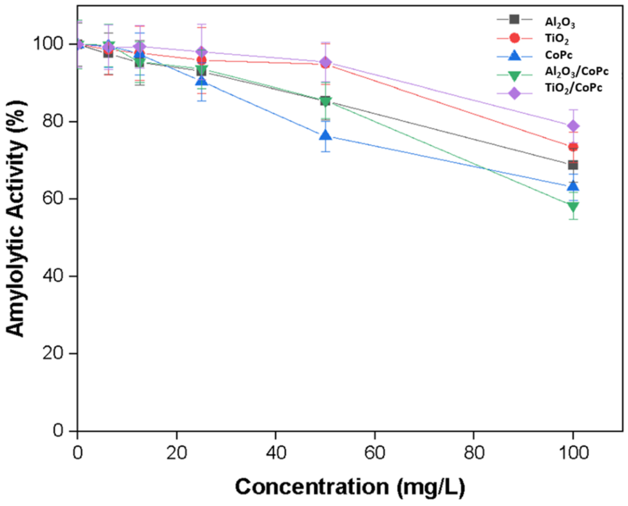


Fig. 4 Antidiabetic activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc).

from 9.2% to 63.8%.[46] Compared with the literature, 100 mg L^{-1} of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) exhibited acceptable antidiabetic activity and could be considered alternative agents in the healthcare industry after further research.

Antimicrobial activity. Antibiotics have been used to treat bacterial diseases for many years. However, overuse and misuse of antibiotics have led to a significant increase in antimicrobial resistance, along with a failure to monitor their residual effects and environmental contamination.[47–51] Subsequently, the number of effective antibiotics has gradually declined as the number of resistant pathogenic bacteria has increased.[47] Therefore, an urgent action is required to design or discover new efficient antimicrobial agents, and the number of studies in this field is steadily increasing.[52,53]

In this study, the antimicrobial activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were evaluated against a series of microorganisms. The corresponding MIC values are listed in Table 1. Accordingly, the lowest MIC values were obtained for nanoconjugate TiO_2/CoPc, which exhibited the highest activity. The MIC value of 64 mg L^{-1} was obtained for TiO_2/Co-Pc against all Gram-negative strains and assigned to a 2–4 fold higher antimicrobial activity of nanoconjugate TiO_2/CoPc compared to antimicrobial activities of other studied nano/structures obtained between 128 and 256 mg L^{-1} against Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *L.* pneumophila). Compound CoPc, unmodified nanoparticles (TiO_2 and Al_2O_3), and nanoconjugate Al_2O_3/CoPc exhibited moderate antimicrobial activity (MIC = 256 mg L^{-1}) against *E. coli*. These nano/structures demonstrated similar antimicrobial activities against Gram-positive bacteria (*B. subtilis*, *E. faecalis*, *S. aureus*). Given the growing concern over antibiotic-resistant *Enterococcus* infections, these results suggest that phthalocyanines may serve as promising candidates for novel antimicrobial strategies.[54] Compound CoPc and Al_2O_3 nanoparticles exhibited a moderate activity (MIC = 256 mg L^{-1}) against all Gram-positive bacteria, whereas nanoconjugate TiO_2/CoPc displayed good antimicrobial activity (MICs = 64 mg L^{-1}). The respective MIC values of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were obtained 64 mg L^{-1}, 256 mg L^{-1}, 128 mg L^{-1}, 64 mg L^{-1}, and 128 mg L^{-1} against the fungal strain of *C. albicans*. While the MICs of 256 mg L^{-1} were determined for the metal oxide nanoparticles (Al_2O_3 and TiO_2), the MIC value of 128 mg L^{-1} was obtained for CoPc and nanoconjugates (TiO_2/CoPc and Al_2O_3/CoPc) against *C. glabrata*. Studies in the literature also supported these findings. Baghdadi *et al.* synthesized Al_2O_3 nanoparticles from aluminum wastes. They examined their antimicrobial activities against a series of Gram-negative (*E. coli* ATCC25922, *S. Typhimurium* ATCC14028, *Pseudomonas aeruginosa* ATCC27853, *Alcaligenes aquatilis*) and Gram-positive (*Staphylococcus aureus* ATCCBAA977 and *Streptococcus pneumoniae* ATCC49619) bacteria, as well as the fungi *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium* sp. Significant results were obtained against *S. Typhimurium*, *S. Aureus*, and *S. pneumoniae*.[55] Santhoshkumar *et al.* investigated the antibacterial activity of biosynthesized titania nanoparticles against *S. aureus* and *E. coli*. They displayed higher antibacterial activities than tetracycline.[37] Omeiri *et al.* prepared Al_2O_3, $CoAl_2O_4$, and $Zn0.9Al_{0.1}O$ nanoparticles and examined their antibacterial activities against *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *S. aureus*. The minimum inhibitory concentration values for the nanoparticles ranged from 4 mg mL^{-1} to 16 mg mL^{-1}.[56] Ağırtaş *et al.* prepared a new zinc phthalocyanine bearing 2-(3,4,5-trimethoxybenzyl)oxy)phenoxy groups on peripheral positions, and they exhibited strong antimicrobial activities against multiple microorganisms.[57] In our previous study, a series of new phthalocyanine-functionalized gold and silver nanostructures was prepared, and their antimicrobial features were examined. All nanomaterials, particularly modified silver nanoparticles, exhibited high antimicrobial activity (MIC values: 2–64 mg L^{-1}).[58] Unluer *et al.* synthesized some new 2-methoxy-4-{(*Z*) [(4-morpholin-4-ylphenyl)imino]methyl} phenoxy-substituted metal phthalocyanines exhibiting notable antimicrobial activities, particularly against *E. coli* and *S. typhimurium* (MIC: 625 mg mL^{-1}). However, they exhibited lower activity against *Y. enterocolitica* and *S. aureus*.[59] As a result, the nanoconjugate TiO_2/CoPc exhibited the most effective and broad-spectrum antimicrobial profile against Gram-negative, Gram-positive, and fungal strains; however, the other studied nanostructures displayed selective antifungal or moderate antibacterial activity. Therefore, the TiO_2/CoPc nanoconjugate is a highly promising antimicrobial agent for future therapeutic or materials-based applications and warrants further investigation.

Table 1 Antimicrobial activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc)

	MICs (mg L^{-1})				
Microorganisms	Al_2O_3	TiO_2	CoPc	TiO_2/CoPc	Al_2O_3/CoPc
E. coli	256	256	256	64	256
P. aeruginosa	256	128	128	64	256
L. pneumophila	256	128	128	64	256
B. subtilis	256	256	256	64	128
E. faecalis	256	256	256	64	256
S. aureus	256	128	256	64	128
C. albicans	256	128	64	64	128
C. glabrata	256	256	128	128	128

Biofilm inhibition

A polymeric gel-like layer is a polysaccharide-based network produced by bacterial cells, referred to as an "extracellular polymeric structure," "exopolysaccharide," or "exopolymer (EPS)". The extracellular matrix is composed of polysaccharides, proteins, deoxyribonucleic acid (DNA), and water. This matrix provides adhesion sites for biofilm cells and protects bacteria against environmental factors, including ultraviolet radiation, pH changes, osmotic pressure, water loss, and antibiotics.[60]

Microorganisms that live within this gel-like layer form biofilm communities by adhering to a surface.[61] Antibiotics and surfactants are antimicrobial agents that can cause environmental damage. Although using their lowest concentration was considered an effective method to control biofilm formation for many years, it has become insufficient to destroy the biofilm structure in recent years. Indeed, the exopolysaccharide in biofilms reduces antibiotic penetration, preventing antibiotics from reaching the bacteria. Hence, the number of infections caused by antibiotic-resistant microorganisms has increased in recent decades.[62]

In this study, the antibiofilm activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were evaluated against *S. aureus* and *P. aeruginosa* at different concentrations ranging from 50 to 200 mg L^{-1}. The results are depicted in Fig. 5. As the concentration increased, all the studied nanostructures markedly inhibited *S. aureus* biofilm formation (Fig. 5a). Although the biofilm–inhibitory activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) increased in a concentration-dependent manner, inhibition levels varied considerably with the chemical nature. The highest inhibitory activities were observed for the unmodified metal nanoparticles (TiO_2: 55.99% and Al_2O_3: 53.61%) at 50 mg L^{-1}, as these nanostructures exhibit inherent surface reactivity and antibacterial properties, thereby dominating the early-stage inhibition response. Additionally, compound CoPc (46.97%) and modified nanoconjugates (Al_2O_3/CoPc: 48.27% and TiO_2/CoPc: 47.96%) exhibited good antibiofilm activities at 50 mg L^{-1}. The highest inhibitory activity was obtained 76.16% for TiO_2 nanoparticles at 100 mg L^{-1}. Additionally, the antibiofilm activities of unmodified and modified Al_2O_3 nanoparticles were 71.87% and 74.71% at 100 mg L^{-1}, respectively. Although compound CoPc displayed the lowest inhibitory activity (68.49%) at 100 mg L^{-1}, the improved performance of the modified alumina nanoparticles may result from the synergistic effect of compound CoPc at the studied concentration. Nanoconjugates displayed the highest antibiofilm activities (Al_2O_3/CoPc: 92.75% and TiO_2/CoPc: 90.62%) at 200 mg L^{-1}. These results clearly confirmed the improving effect of CoPc on the antibiofilm performance of the nanostructures. The biofilm inhibitory activities of compound CoPc and unmodified metal oxide nanoparticles (TiO_2 and Al_2O_3) were obtained 78.76%, 91.21%, and 89.75% at 200 mg L^{-1}, respectively. Fig. 5b demonstrates the antibiofilm activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) against *P. aeruginosa* at different concentrations (50–200 mg L^{-1}). The analysis of the inhibition data showed that all studied nanostructures exerted progressively stronger inhibitory effects on *P. aeruginosa* biofilm formation as concentration increased. The respective antibiofilm activities of unmodified metal oxide nanoparticles (TiO_2 and Al_2O_3) and nanoconjugate (TiO_2/CoPc) were obtained 52.18%, 50.44%, and 57.75% at 50 mg L^{-1}. Compound CoPc and nanoconjugate (Al_2O_3/CoPc) exhibited comparatively lower activity at 50 mg L^{-1}. A greater increase in activity was observed at 100 mg L^{-1}. Although compound CoPc and unmodified Al_2O_3 nanoparticles displayed considerable inhibitory activities at 100 mg L^{-1}, unmodified titania nanoparticles (TiO_2: 71.72%) and nanoconjugate (TiO_2/CoPc: 69.08%) exhibited the highest performance at the same concentration. The highest suppression rate of 84.49% was achieved for the nanoconjugate (TiO_2/CoPc) at 200 mg L^{-1}, attributed to the strong synergistic effect of phthalocyanine and metal oxide nanoparticles. Unmodified metal oxide nanoparticles (TiO_2 and Al_2O_3) and nanoconjugate (Al_2O_3/CoPc) demonstrated substantial activities, while CoPc consistently remained the least effective at all the studied concentrations. The enhancement observed at higher concentrations, particularly in the nanoconjugates, confirmed the beneficial effect of surface functionalization of metal oxide nanoparticles with the compound CoPc.

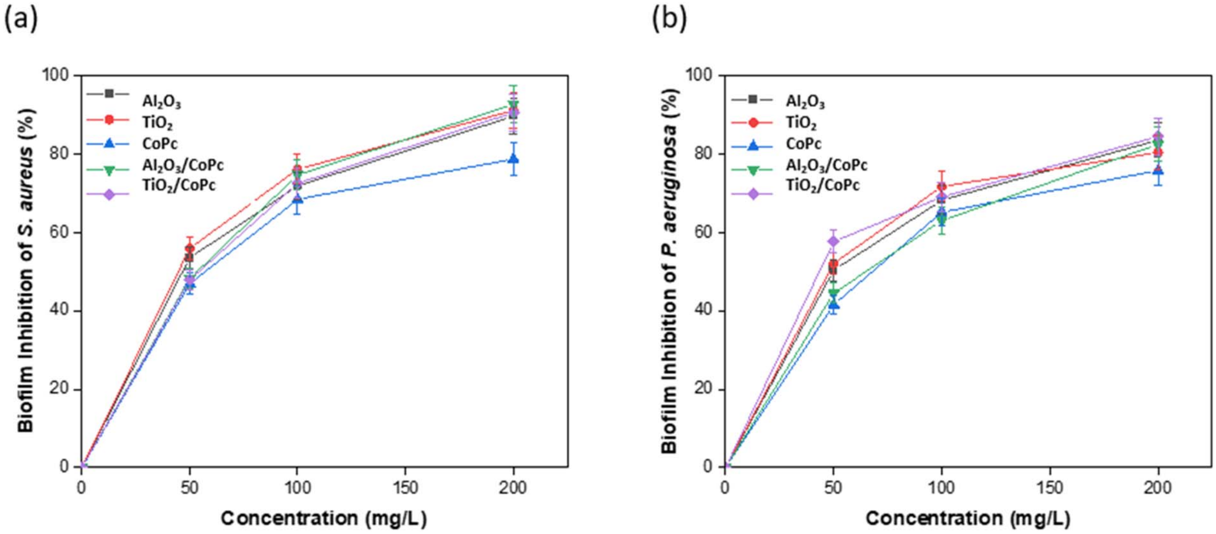


Fig. 5 Biofilm inhibitory activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) against *S. aureus* (a) and against *P. aeruginosa* (b).

Ansari *et al.* synthesized electrospun TiO_2 nanofibers and tested their antibacterial and antibiofilm activities against *P. aeruginosa* and *S. aureus*. The nanoparticles exhibited more effect against *P. aeruginosa*. They also indicated that the biofilm inhibition by TiO_2 nanofibers was dose-dependent.[63] Chrzanowska and Załęska-Radziwiłł reported that aluminum oxide and zirconium oxide nanoparticles were more damaging to *Pseudomonas putida* and *Aeromonas hydrophila* than their bulk counterparts (aluminum and zirconium oxides). Aluminum oxide nanoparticles were also more toxic than zirconium oxide nanoparticles against both bacterial species.[64] Magadla *et al.* reported that silica nanoparticles effectively disrupted bacterial biofilms when a water-soluble Zn(II) phthalocyanine was incorporated into functional nanocarriers. The modified silica nanoparticles exhibited strong inhibitory activities against *S. aureus* and *E. coli*.[65] Celep *et al.* prepared Schiff-base-substituted Zn(II) and In(III) phthalocyanines, exhibiting high antibiofilm activities against *S. aureus* (74.51–87.99%) and *P. aeruginosa* (69.83–84.61%) at 15 mg L^{-1}.[66] Cavalcante *et al.* found that chloroaluminium phthalocyanine (ClAlPc)-loaded chitosan nanoparticles displayed significant antibacterial and antibiofilm activities through effective photosensitizing delivery and disruption of biofilm structures.[67] Trigo-Gutierrez *et al.* reported that cobalt(II) phthalocyanine–modified metal oxide coatings exhibited strong antibiofilm activity, particularly under light exposure, and supported the utility of phthalocyanine-functionalized surfaces for biofilm control.[68] Compared with the literature, nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) can be considered advanced antibiofilm nanomaterials for biomedical and antimicrobial surface applications, provided further research is conducted.

Microbial cell viability inhibition with and without photodynamic therapy

Control or prevention of microbial growth is a crucial issue in many fields, particularly food processing/preparation, healthcare, and materials preservation. Generally, the growth control can be achieved using chemical agents that eliminate microorganisms or inhibit their further growth. The most fundamental drawback of long-term, high-dose antibiotic use is the emergence of antibiotic resistance among microorganisms, which makes antibiotic use undesirable in clinical practice.[69] On the other hand, pathogenic agents can cause serious infectious diseases; therefore, their multiplication must be prevented by eliminating them or inhibiting their growth. As a result, novel efficient materials and techniques are required to overcome these problems. Photodynamic therapy (PDT) is considered a therapeutic modality with minimal side effects, either alone or in combination with conventional antimicrobial therapy (aPDT).[70,71] The three main elements of this method are light, a photosensitizer, and an oxygen molecule.[72,73] Photosensitizer molecules are excited by a light source at a certain wavelength. Subsequently, the interaction of the light-activated compound with an oxygen molecule generates highly toxic singlet oxygen species in target cells, which, in turn, induce cell death *via* oxidative damage.[74,75]

In this study, the *E. coli* cell growth inhibition activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were examined. The results are depicted in Fig. 6a. The inhibitory activity of titania nanoparticles (TiO_2) was obtained 85.56% and closely followed by the inhibitory activities of nanoconjugate (Al_2O_3/CoPc: 85.62%), compound CoPc (80.67%), and alumina nanoparticles (Al_2O_3: 76.85%) at 50 mg L^{-1}. The TiO_2/Co-Pc composite exhibited the highest inhibition of around 90% at this concentration. As the concentration increased from 50 to 100 mg L^{-1}, the *E. coli* inhibitory activities of titania nanoparticles (TiO_2) and compound (CoPc) reached 94% inhibition, while those of nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) reached 98.27% and 100%, respectively. 200 mg L^{-1} of each nano/structures (CoPc, TiO_2, Al_2O_3/CoPc, and TiO_2/CoPc) inhibited 100% *E. coli* cell growth, whereas alumina nanoparticles (Al_2O_3) exhibited an inhibitory activity of 98.37%.

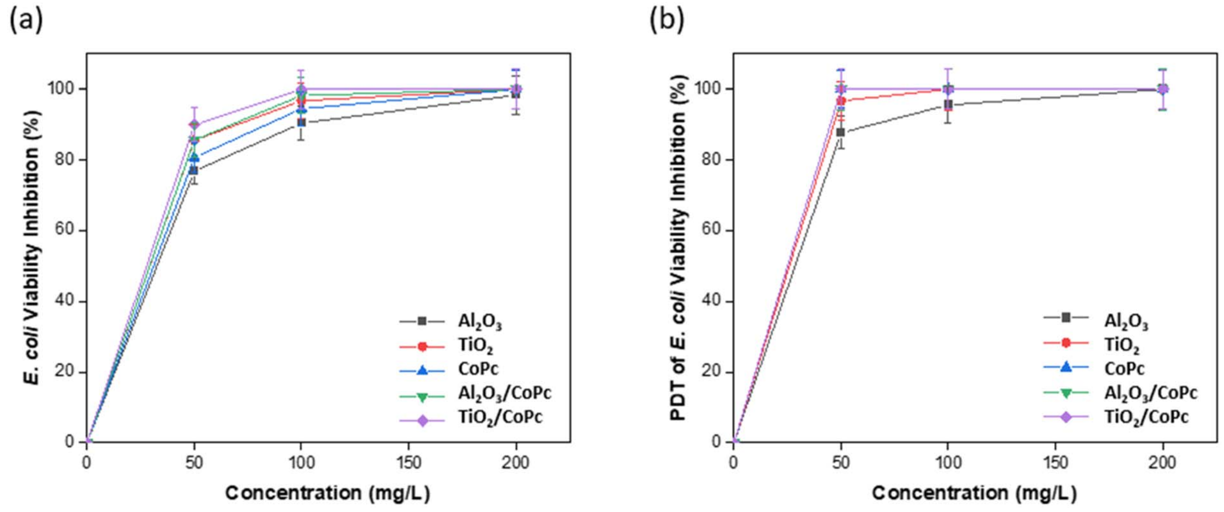


Fig. 6 Microbial cell viability inhibitory activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) against *E. coli*; without irradiation (a) and with irradiation (b).

Sadiq *et al.* investigated the growth-inhibitory effect of alumina nanoparticles on *Escherichia coli*. They suggested that alumina nanoparticles may exhibit only mild toxicity toward microorganisms.[76] Kumar *et al.* examined the mechanisms of toxicity of ZnO- and TiO_2-engineered nanoparticles against *E. coli*. Both metal oxide nanoparticles exhibited a statistically significant, concentration-dependent decrease in *E. coli* cell viability.[77] Güleç *et al.* prepared benzylidene-4-oxo-2-thioxothiazolidin-3-yl substituted metal-free and metallophthalocyanines [H_2Pc, ZnPc, CuPc, CoPc, MnPc, and GaPc] and studied their biological properties comprehensively. The antimicrobial activities of the resulting phthalocyanines ranged from 7.8 to >500 $\mu g\ mL^{-1}$ against Gram-positive, Gram-negative bacteria, and yeast.[75] Similarly, Sajjadifard *et al.* synthesized a fluorene-based phthalonitrile derivative and its Zn(II) and In(III) phthalocyanine derivatives. The resultant phthalocyanines exhibited pronounced antimicrobial activity, with strong concentration-dependent effects on *E. coli* cell viability.[39] Compared with the literature, the newly prepared phthalocyanine-functionalized metal oxide nanoparticles are effective antimicrobial agents owing to the synergistic effect between the CoPc compound and the metal oxide nanoparticles (Al_2O_3 and TiO_2).

Fig. 6b depicts the microbial cell viability inhibitory activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) after exposure to light. As the concentration increased, the inhibitory activities of alumina nanoparticles (Al_2O_3) were 87.71% (at 50 mg L^{-1}), 95.58% (at 100 $\mu g\ mL^{-1}$), and 100% (at 200 mg L^{-1}) against *E. coli* cell growth. Although titania nanoparticles (TiO_2) inhibited 96.78% of the cell growth, 100 and 200 mg L^{-1} of these nanoparticles inhibited 100% of *E. Coli* growth. Additionally, the compound CoPc and the nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) exhibited 100% inhibitory activity at all tested concentrations. Therefore, the synergic effect of compound CoPc and metal oxide nanoparticles (Al_2O_3 and TiO_2) on their individual biological properties was negligible after irradiation. Sen and Nyokong reported that a Pd(II)-complex-substituted silicon(IV) phthalocyanine achieved a 99.94% reduction in viable bacterial cells and exhibited exceptional photodynamic antibacterial activity in photodynamic antimicrobial chemotherapy (PACT) assays against *S. aureus*.[78] Dlugaszewska *et al.* prepared a cationic magnesium(II) phthalocyanine derivative that exhibited robust, dose-dependent photodynamic bactericidal activity against both Gram-positive and Gram-negative bacteria. A 10 M solution of magnesium(II) phthalocyanine inactivated microorganisms almost completely under irradiation.[79] In comparison to the literature, the evaluation of the antimicrobial photodynamic activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) clearly demonstrated that compound CoPc and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) delivered the most potent antimicrobial photodynamic responses by the inhibitory activities of 100% at all tested concentrations.

Experimental

Synthesis and characterization

Tetra-substituted phthalonitrile derivative. Tetrafluorophthalonitrile (0.500 g, 2.50 mmol) and 4-trifluoromethoxyphenol (1.80 g, 10.11 mmol) were dissolved in dimethyl sulfoxide (15 mL). After the addition of potassium carbonate (1.21 g, 8.75 mmol), the reaction content was stirred at 80 °C for 48 hours under an inert atmosphere. The mixture was cooled to room temperature, poured into iced water (100 mL), and stirred for 2 hours. The pure product was obtained by extracting with dichloromethane. Molecular formula: $C_{36}H_{16}F_{12}N_2O_8$. Yield: 1.36 g (65%). ^1H NMR (500 MHz; DMSO-d_6): δ (ppm) 9.26–9.13 (d, 4H), 8.78–8.64 (d, 4H), 7.89–7.77 (d, 4H), 7.56–7.49 (d, 4H). ^{13}C NMR (126 MHz; DMSO-d_6): δ, ppm 152.44, 151.34, 146.47, 146.46, 123.45, 123.28, 122.82, 121.57, 121.40, 120.70, 119.35, 117.30, 114.60, 111.36. FT-IR υ (cm^{-1}): 3064 (aromatic C–H), 2231 (nitrile C≡N), 1243 (C–F), 1098 (C–O–C).

Hexadeca-substituted cobalt(II) phthalocyanine (CoPc). The resultant phthalonitrile derivative (0.100 g, 0.120 mmol) and cobalt chloride (0.006 mg, 0.046 mmol) were dissolved in *n*-pentanol (3 mL). The reaction mixture was catalyzed with an excess of DBU and stirred at reflux for 24 hours. After cooling to room temperature, the content was treated with a water: methanol mixture (1 : 1) and stirred for 2 hours. The crude product was purified by column chromatography on silica gel using THF as the eluent. Molecular formula: $C_{144}H_{64}CoF_{48}N_8O_{32}$. Yield: 0.052 g (51%). FT-IR υ (cm^{-1}): 3078 (C–H aromatic), 1257 (CF_3), 1100 (Ar–O–Ar). UV-vis (DMSO): λ_{max} nm (log ε) 313 (5.00), 709 (5.49). MS *m*/*z* calcd 3388.94 $[M]^+$ found 3369.340 $[M-F+H_2O]^+$, 3296.75 $[M-CF_3+2H_2O]^+$, 3266.58 $[M-OCF_3+H_2O+2H]^+$, 3211.22 $[M-C_7H_4F_3O_2]^+$, 3124.141 $[M-2OCF_3+H_2O+2H]^+$, 3037.61 $[M-2C_7H_4F_3O_2]^+$.

Preparation of CoPc-modified metal oxide nanoparticles. Alumina nanoparticles and titania nanoparticles were synthesized as described extensively in the literature with some modifications.[3,80] Each metal oxide nanoparticle (0.050 mg) was dissolved in distilled water, and the surface of the nanoparticles was modified by adding CoPc solution (10 mg in excess amount of DMSO). The modified nanoparticles were collected by centrifugation, washed several times with distilled water, and redispersed in distilled water. The obtained nanoconjugates were characterized using FE-SEM, UV-vis, and FT-IR techniques.

Biological studies

DNA cleavage activity. DNA cleavage activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were examined using an agarose gel electrophoresis assay. A mixture of each studied nanostructure (15 μL) and pBR322 plasmid DNA (5 μL) was prepared in a PCR tube and incubated for 2 hours at 37 °C in the dark. A 1% agarose gel was prepared and placed in an electrophoresis tank filled with Tris-acetate-EDTA (TAE) buffer. Each sample was mixed with loading dye and carefully loaded into the gel wells. Electrophoresis was then carried out at 100 V for 1 hour. Finally, the gel was removed and visualized using a UV transilluminator.

Antioxidant activity. The antioxidant activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were evaluated using the DPPH stable free radical method, with slight modifications.[3,58] Due to its paramagnetic nature and a single unpaired electron, DPPH behaves like a stable radical. As its absorption decreases after pairing its single electron with a free radical scavenger, the color of the DPPH solution changes from dark purple to light yellow. Samples of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were prepared at concentrations ranging from 6.25 to 100 mg L^{-1}. 1 mL of each sample and 4 mL of the DPPH solution were added to a test tube, mixed, and shaken vigorously. The mixture was kept at room temperature in the dark for 30 minutes. The absorbance was then measured at 517 nm using a UV-visible spectrophotometer. Trolox and ascorbic acid (AA) were preferred as controls in our study. The percentage value of the DPPH free radical scavenging ability was calculated by applying eqn (1).

$$\text{DPPH inhibition (\%)} = ((A_{\text{bs}}(\text{control}) - A_{\text{bs}}(\text{sample}))/A_{\text{bs}}(\text{control})) \times 100 \quad (1)$$

Amylolytic activity. To evaluate antidiabetic activity, different concentrations (6.25, 12.5, 25, 50, and 100 mg L^{-1}) of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were prepared. After adding phosphate buffer and α-amylase to each sample, the mixture was incubated for 15 minutes at 37 °C. Upon addition of 1% potato starch solution (0.2 mL), hydrolysis began, and the sample was incubated for 20 minutes at 37 °C. The enzymatic reaction was stopped by adding 0.4 mL of 3.5% dinitrosalicylic acid (DNS). The mixture was heated in a bain-marie bath for 5 minutes. After cooling to room temperature, distilled water (3 mL) was added to each tube, and the absorbance was measured at 540 nm using a spectrophotometer. The sample not containing the studied nano/structures eas used as the control. The amylolytic activity of each sample was calculated using eqn (2).

$$\text{Antidiabetic activity (\%)} = 100 - (\text{Control}_{\text{abs}} - \text{Sample}_{\text{abs}})/\text{Control}_{\text{abs}} \times 100 \quad (2)$$

Antimicrobial activity. The antimicrobial properties of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were investigated using the microdilution method against *Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis*, and *Legionella pneumophila* subsp. *Pneumocystis jirovecii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans, and Candida glabrata* were used to assess their antimicrobial activities. Fresh cultures were prepared one day before testing. The 1:1-fold dilution series of the studied nanostructures was prepared in 96-well microplates. After incubation for 24 hours at 37 °C, the minimum inhibitory concentration (MIC) was defined as the lowest concentration required to completely inhibit microbial growth.

Biofilm inhibition activity. The antibiofilm activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were tested at different concentrations (50, 100, 200 mg L^{-1}) against *S. aureus* and *P. aeruginosa* in 24-well plates. Each sample was added to a well containing Nutrient Broth (NB). All plates were inoculated with freshly prepared bacterial suspensions and incubated for 72 hours at 37.5 °C to allow biofilm formation. The wells were gently rinsed twice with PBS (200 μL) to remove unattached cells, then air-dried for 30 minutes. The biofilms were stained with 200 μL of 1% crystal violet for 60 minutes. Excess dye was washed away with PBS, whereas the bound dye was eluted with ethanol. The absorbance of the eluted dye was measured at 595 nm after 15 minutes at room temperature. The biofilm inhibition was calculated using eqn (3).

$$\text{Biofilm inhibition (\%)} = ((A_{\text{bs}}(\text{control}) - A_{\text{bs}}(\text{sample}))/A_{\text{bs}}(\text{control})) \times 100 \quad (3)$$

Microbial cell viability inhibition with and without photodynamic therapy. The microbial viability-inhibiting activities of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc) were examined as described in the literature, with some modifications.[81] *E. coli* (ATCC 25922) was used as a microorganism to test the antimicrobial activity of these compounds. First, bacterial cultures were grown in Nutrient Broth (NB) by shaking at 150 rpm at 37.5 °C for 24 hours. The grown cells were collected by centrifugation, washed twice with sterile distilled water, and exposed to different concentrations of the studied nanostructures. They were incubated at 37.5 °C for 90 minutes, serially diluted, and then plated on Nutrient Agar. The contents were incubated for an additional 24 hours at 37.5 °C. To evaluate the photodynamic therapeutic effect, each nanostructure was exposed to LED light for 30 minutes before bacterial treatment. The colony counts were determined, and microbial viability was calculated using eqn (4).

$$\text{Cell viability inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100 \quad (4)$$

Conclusion

In this study, a new tetrasubstituted phthalonitrile derivative and its cobalt(II) phthalocyanine were successfully synthesized. The resulting phthalocyanine was used to surface-modify alumina and titania nanoparticles. The biological features (DNA cleavage, antioxidant, antidiabetic, and antimicrobial properties) of the phthalocyanine, unmodified metal oxide nanoparticles, and phthalocyanine-modified metal oxide nanoparticles were examined at different concentrations. A single-strand breakage occurred in the presence of unmodified metal oxide nanoparticles at all the studied concentrations. In contrast, double-strand breakage and complete fragmentation were observed in the presence of the phthalocyanine and nanoconjugates. The antioxidant activities of the metal oxide nanoparticles were increased significantly after modification with the cobalt(II) phthalocyanine. All the studied nano/structures exhibited moderate antidiabetic activities. Moreover, the antimicrobial activities of all the nano/materials increased with increasing concentration. This manner confirmed the concentration-dependent behavior of the studied nano/structures. The comparative evaluation of CoPc, unmodified metal oxide nanoparticles, and nanoconjugates indicated that unmodified nanoparticles exhibited strong inhibition at low concentrations, whereas the nanoconjugates showed higher efficacy at higher concentrations. This concentration-driven shift confirmed that the compound CoPc acted as a catalytic enhancer within the oxide matrix, thereby amplifying biofilm suppression under suitable conditions. Compared to the literature, the newly synthesized phthalocyanine-based nanoconjugates exhibited strong antibacterial and antifungal activities against various microbial species. In particular, the nanoconjugate (TiO_2/CoPc) is a promising candidate for preventing robust biofilm formation by *Pseudomonas* species.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary information (SI): S1. ^{1}H NMR spectrum of the compound tetra-substituted phthalonitrile derivative. S2. ^{13}C NMR spectrum of compound tetra-substituted phthalonitrile derivative. S3. MALDI-TOF spectrum of macromolecule CoPc. S4. UV-vis spectra of compound CoPc and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc). S5. Particle size distribution curve of unmodified alumina nanoparticles. S6. Particle size distribution curve of unmodified titania nanoparticles. S7. FT-IR spectra of compound CoPc, unmodified nanostructures (Al_2O_3 and TiO_2), and nanoconjugates (Al_2O_3/CoPc and TiO_2/CoPc). S8. Elemental mapping analysis of unmodified nanostructures (Al_2O_3) and nanoconjugate (Al_2O_3/CoPc). S9. Elemental mapping analysis of unmodified nanostructures (TiO_2) and nanoconjugates (TiO_2/CoPc). See DOI: https://doi.org/10.1039/d6ra01414c.

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