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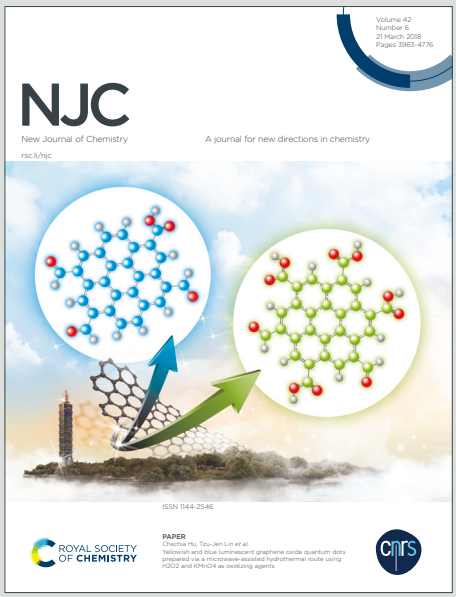
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ARTICLE

Anticancer activity of novel silicon phthalocyanines against colorectal adenocarcinoma cell line (DLD-1)

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In this study, the DNA cleavage activities of a series of new silicon phthalocyanines were studied to measure their potential for cancer treatment. For this purpose, 2-(2,4,6-tris((dimethylamino)methyl)phenoxy)ethan-1-ol (**2**) and 2-(2-(2,4,6-tris((dimethylamino)methyl)phenoxy)ethoxy)ethan-1-ol (**3**) were synthesized and used for the preparation of axially di-substituted silicon phthalocyanines. The resulting phthalocyanines were quaternized in the presence of iodomethane. Characterization of all the newly synthesized compounds was carried out using several spectroscopic approaches including mass, UV-vis, FT-IR, and NMR spectroscopies. Plasmid DNA (pBR322) cleavage, topoisomerase enzyme activity and binding situation with calf thymus DNA (CT-DNA) of the new silicon phthalocyanines were measured using agarose gel electrophoresis assay. **1-QSi** exhibited remarkable cleavage activities on supercoiled plasmid DNA at all the studied concentrations. Compound **3-QSi** displayed significant cleavage activities on supercoiled plasmid DNA only at 200 µg/mL. Besides, compound **3-QSi** exhibited higher human topoisomerase I inhibition activity compared to compound **2-QSi**. Moreover, all the compounds were screened for biological activities. The cytotoxicity and apoptosis activities effects were test against DLD-1 colorectal cancer cell line were studied at different concentrations ranging from 6.25 to 100 µg/mL. The results indicated that the studied compounds can be utilized as anticarcinogenic agents.

Introduction

Understanding the inner workings of Nature is crucial in all the scientific fields as well as in biology. One of the universal properties of life is the cell-to-cell variation that leads to widely biological phenomena and ranges from developmental plasticity to tumor heterogeneity.[1-4] Deoxyribonucleic acid (DNA) is mostly responsible for fundamental variation in living organisms. Indeed, DNA stores and transfers genetic information. Any change in the nucleotide sequence is followed by the change in the genetic information that is stored in DNA. Although a change error rarely occurs in DNA replication, it can lead to a change in the sequence of the nucleotides.[5,6] Mutations take place either by spontaneous agents or artificial ones (mutagens: X-ray, Y-ray, ultraviolet radiation, and chemical agents). All the mutagens alter the base sequence of DNA through different mechanisms.[7] The dynamic state of DNA has been confirmed by studying its replication, recombination, and repair features.[8,9] The number of different damages in DNA tolerate or repair through the ingenious mechanisms devised in living cells. However, these mechanisms can be failed and result in vital disorders including cancer, viral diseases, or cystic fibrosis.[10]

The cancer term is referred to a number of diverse diseases that arise from abnormal growths of cells. Genetic cancers often originate from abnormal recombination (e.g. chromosomal translocations).[11] This uncontrolled growth can spread to other parts of the body, as well. Albeit radiotherapy[12] and chemotherapy[13] have been mostly applied for the treatment of cancer diseases, the toxicity of cancer drugs and drug resistance of cancerous cells are not ignorable.[14,15] As a result, the identification of the chemical structure of genomic DNA can play an essential role in the design of new drugs since genomic DNA is located in the cell nucleus and includes critical functions. Uncontrolled proliferation of cancer cells arises from increased DNA replication.[16] Therefore, sufficient recognition of chemical structures can help decrease or halt replication. Small organic molecules, inorganic compounds, and peptides usually bind to DNA through several processes encompassing replication and transcription.[6,17] Low selectivity of medicinal drugs against tumor cells becomes the essential problem of modern chemotherapy.[18] Photodynamic therapy (PDT) is well-known as an efficient alternative for these traditional approaches. Photosensitizer, light, and oxygen are the prominent elements of this method. Generally, photosensitizing agent activated by exposure to light converts triplet oxygen molecules to the singlet oxygen molecules that in turn destruct the lesion tissue[19] Low toxicity, high photosensitivity, stable chemical composition, and high clearance rate from normal tissues are necessary features of a suitable photosensitizer.[20,21] The

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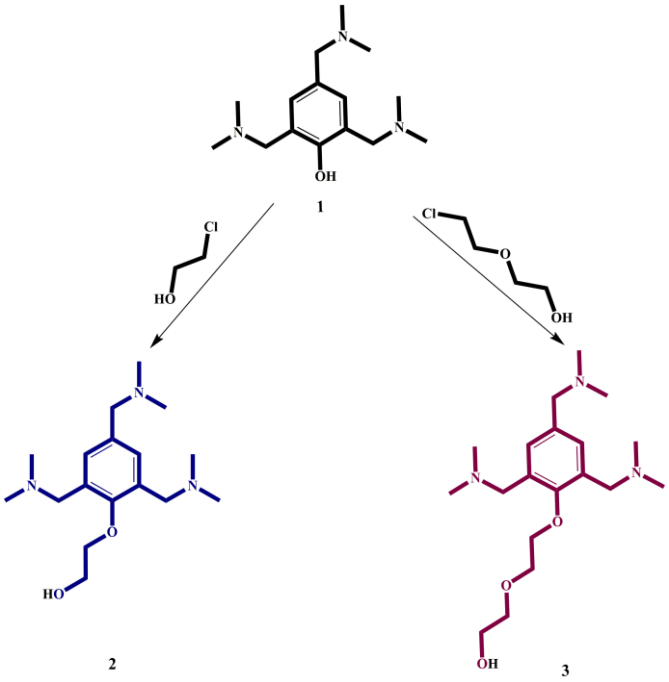
interaction of DNA with metal complexes has attracted attention since these compounds especially aromatic ligand-containing complexes exhibit excellent chemical and physical properties that make them appropriate probes of DNA structure.[22] Metal complexes can bind to DNA through diverse interactions owing to their extraordinary chemical, photochemical, and electrical reactivity.[23] As the metal-based drug may be non-toxic and highly anti-proliferative, anti-cancerous research has focused on the potential of metal complexes as DNA targeting drugs.[24] Among the metal complexes, metallophthalocyanines have been interested in the treatment of cancer since they exhibit high absorptions and fluorescence at longer wavelengths as well as high singlet oxygen quantum yields which are vital for the destruction of cancerous lesions.[25]

Phthalocyanines and their derivatives have been utilized as traditional dyes for many years. However, the strong electron transferring abilities that originate from their planar aromatic conjugated structure result in wonderful electrical and optical characteristics.[26,27] The potent utility of phthalocyanines has been explored in the well-known scientific and technological fields that encompass electronics, sensors, medicine, and nanotechnology.[28-37] Since most of these applications request soluble materials, insolubility of unsubstituted phthalocyanines in common organic and aqueous media is known as an essential limitation for their use in these fields. Introduction of bulky or long substituents on the periphery of phthalocyanines and insertion of metal cations into the phthalocyanine cavity increase the distance between the phthalocyanine rings and decrease the intermolecular interactions. Therefore, substituted metal phthalocyanines bearing organic or quaternized organic groups exhibit higher solubility in organic solvents or water.[26-38] In comparison to non-peripheral and peripheral substituents, axial ligands cause more distance between phthalocyanine rings. As a result, the π-π interaction between the rings highly diminishes and the solubility of phthalocyanines increases. Also, the presence of axial ligand on the metal core shifts ultraviolet-visible spectra of phthalocyanines especially the Q-band to the longer wavelengths and make the phthalocyanines functional materials for near-infrared and infrared applications such as photocatalytic systems, photodynamic therapy, photovoltaic cells, and electrochromic devices.[39-41] The alteration of the phthalocyanines' periphery can change the electronic absorption spectra whereas the substitution of different axial ligands on the metal ion can lead to a slight change in the spectra.[42] Among the metal phthalocyanines, silicon phthalocyanines include hexa-coordinated silicon (IV) and exhibit extraordinary Si-N bonds. The binding of C-connected, thio-, or oxo- ligands with silicon induces the red-shift of the ultraviolet-visible spectra. Additionally, some literature has been reported high anticancer properties of silicon phthalocyanines against breast, human colon, and ovarian cancers.[43-45] Accordingly, this study aims to design efficient multidisciplinary anticancer drugs for diverse anticancer therapeutic applications. The synthesis and characterization of three silicon phthalocyanines that differ in the length of ligand are presented in this study. Also, their DNA cleavage, binding, topoisomerase enzyme activities, and anti-cancer properties were studied.

Results and discussion

Synthesis and characterization

The synthetic pathways for compounds (**2** and **3**) are portrayed in Scheme 1. Compound **1** and the related alcohol [2-chloroethanol or 2-(2-chloroethoxy)ethanol] were combined in ethanol through the nucleophilic substitution reaction. Sodium hydroxide catalysed the reaction. The crude products were crystallized from methanol. Compounds (**2** and **3**) were characterized by applying FT-IR and ^{1}H NMR spectroscopies. All the data proved the successful synthesis of the prposed structures. In the FT-IR spectra of compounds (**2** and **3**), vibration bands of the CH_2-OH appeared at around 3400 cm^{-1}. In the ^{1}H NMR spectra of compounds (**2** and **3**), the protons of OH appeared at 4.81 and 5.75 ppm, respectively.



Scheme 1 The synthetic pathway for compounds 2-3. NaOH, EtOH, 70°C.

The synthetic procedures for compounds (**1-3Si** and **1-3-QSi**) are demonstrated in Scheme 2. Silicon phthalocyanines (**1-3-Si**) were synthesized by replacement of chlorine atoms of axially substituted silicon phthalocyanine dichloride with the related compounds (**1-3**) in toluene. Sodium hydride was used as a catalyzed base. Compounds (**1-Si** and **1-QSi**) were prepared as described in Ref. 73 with some modifications. Characterization of compounds (**2-Si**, **3-Si**, **2-QSi**, and **3-QSi**) was carried out using FT-IR, ^{1}H NMR, Mass, and UV-vis spectroscopies. All the data were in agreement with the target structures. In the FT-IR spectra of the newly synthesized phthalocyanines, the disappearance of the CH_2-OH vibration bands confirmed the

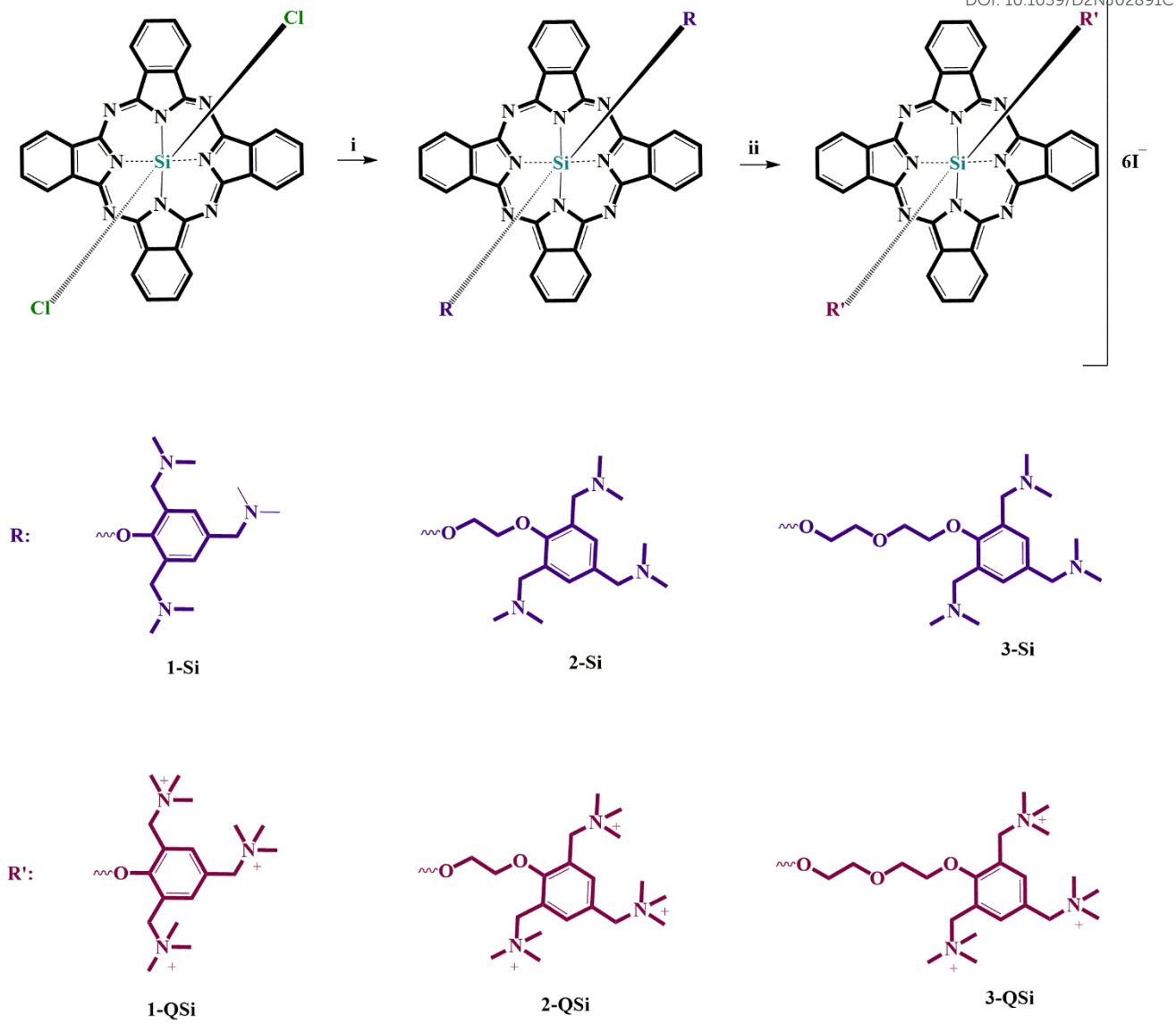
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Scheme 2 The synthetic pathway for compounds **1-3-Si** and **1-3-QSi**. (i) Compound **1**, **2**, or **3**, NaH, toluene, 130°C, (ii) Iodomethane, chloroform, room temperature.

formation of the Si-O-CH_2 bonds. In the ^{1}H NMR spectra of compounds (**2-Si**, **3-Si**, **2-QSi**, and **3-QSi**), the aromatic protons appeared between 9.66 and 7.10 ppm as broadening singlets or multiplets. Due to the magnetic anisotropy of phthalocyanine rings, the aliphatic protons of the chains that were connected to silicon shifted to the strong magnetic fields. Therefore, the related bands appeared between 0.65 and – 1.71. In the mass spectra of compounds (**2-Si**, **3-Si**, **2-QSi**, and **3-QSi**), molecular ion peaks were obtained 1113.32 $[M-3CH_3]^+$, 1248.77 $[M+3H]^+$, 1669.81 $[M-3I+Na+H_2O+H]^+$, and 1651.60 $[M-4I+2Na+OH]^+$, respectively.

UV-vis spectra

High electron transfers of the planar phthalocyanine ring results in excellent electronic and optical behaviours of phthalocyanines. Study of their ground state electronic absorption can help follow the changes in the electronic arrangement of phthalocyanines. The UV-vis spectra of phthalocyanines consist of two strong bands called B-band and Q-band. The Q-band is observed at the visible region (around 700 nm) and assigned to π-π* transitions from HOMO to LUMO. The B-band appeared at the ultraviolet region (around 350 nm) and corresponds to π-π* transitions from deeper π-orbitals to can shift by changing solvent nature, the substituent (type and position), temperature, and metal ions.[26,27,42]

As the biological properties of the newly synthesized phthalocyanines were studied in dimethyl sulfoxide (DMSO), the UV-vis spectra of compounds (**1-3-Si** and **1-3-QSi**) were examined in DMSO at room temperature (Fig. 1) in this study. The electronic absorption of macromolecules (**2-Si** and **3-Si**) shifted to shorter wavelengths compared with that of compound **1-Si**. However, the UV-vis spectra of the quaternized silicon phthalocyanines were obtained approximately as the same as the related compounds.[35] All the obtained data are

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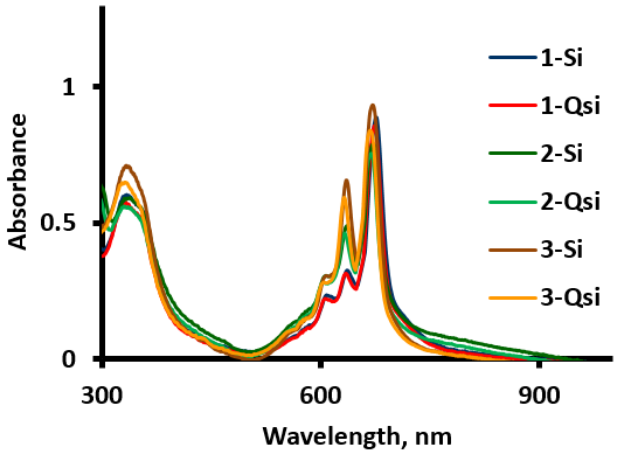


Fig. 1 The UV-vis spectra of compounds (**1-3-Si** and **1-3-QSi**) in DMSO at room temperature (Concentration: 6 µM).

summarized in Table 1. The planar rings of the target phthalocyanines did not include substituents which could result in high solubility and less aggregation. Additionally, DMSO could not strongly coordinate axially to the metal ion and in turn, could not decrease π-π interactions between the rings.[46] Therefore, the observation of a higher shoulder in the UV-vis spectra arising from H-aggregates is possible. According to the obtained results, the target compounds exhibited high anticancer features despite the probable aggregation.

Singlet oxygen quantum yield

Singlet oxygen quantum yield (ϕ_Δ) is an essential measure to evaluate the potential of photosensitizing agents for the production of singlet oxygen.[25] In this study, the singlet oxygen quantum yields of compounds (**1-3-QSi**) were calculated using the phosphorescence signal of singlet oxygen at 1229 nm.[47] The results are given in the ''Supporting Information'' and listed in Table 1. The highest value was obtained for compound **3-QSi**. Although the singlet oxygen quantum yields of compounds (**1-3-QSi**) were obtained lower than the standard (ZnPc; ϕ_Δ= 0.67 in DMSO), they were sufficient for photodynamic therapeutic applications and were higher than those obtained for water-soluble axially di-substituted silicon phthalocyanines.[48,49]

Table 1. UV-vis spectroscopic properties of compounds (**1-3-Si** and **1-3-QSi**).

Compound	B-band (log ε)	Vibronic band (log ε)	Q-band (log ε)	ϕ_Δ
1-Si	334 (5.00)	636 (4.73)	676 (5.17)	-
2-Si	332 (4.99)	636 (4.88)	672 (5.09)	-
3-Si	329 (5.06)	635 (5.03)	671 (5.19)	-
1-QSi	331 (4.97)	635 (4.72)	674 (5.15)	0.33
2-QSi	330 (4.96)	633 (4.88)	669 (5.09)	0.37
3-QSi	326 (5.03)	632 (4.99)	667 (4.14)	0.44

DNA cleavage assay

The mutation of DNA is well-known as a threat to all living organisms. Due to the excellent biological characteristics of metal complexes, the possibility and advantage of their interaction with DNA have been studied in recent decades.[50] The investigation of the DNA cleavage activity of different metal complexes such as metal phthalocyanines can help design novel advanced drugs, especially for the fight against cancers and bacteria.[51,52] In this study, stock samples of compounds (**1-3-QSi**) were prepared in DMSO and their pBR322 DNA cleavage activities were examined by performing agarose gel electrophoresis. The obtained results were shown in Fig. 2.

Although compound **1-QSi** led to the conversion of pBR322DNA from Form I to Form II at all the studied concentrations (lanes 1-3), compound **2-QSi** exhibited weak interaction with DNA (lanes 4-6). However, 200 µg/mL of compound **3-QSi** displayed strong interaction with DNA and made DNA transformed from Form I to Form II (lane 7). There are some similar studies in the literature. Çoban *et al*. prepared zinc phthalocyanine containing ferrocene and investigated its DNA cleavage features. The phthalocyanine exhibited DNA cleavage activity.[53] Amitha *et al*. examined the DNA cleavage activity of quaternized tetra-substituted copper and zinc phthalocyanines bearing Betti base (BB) on peripheral positions. Both of the compounds cleaved the pBR322 DNA molecule.[54] Barut *et al*. explored the DNA cleavage studies of new axially di-substituted silicon phthalocyanines containing two types of morpholine substituents. The studied macromolecules displayed acceptable pBR322 DNA cleavage activities under irradiation.[55] Compared to the literature, compounds **1-QSi** (50-200 µg/mL) and **3-QSi** (200 µg/mL) can be used as anticancer drugs after further investigations.

CT-DNA binding using gel electrophoresis

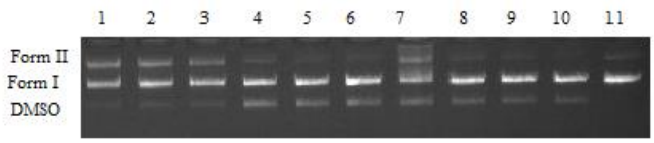


Fig. 2 DNA Cleavage activity of compounds (**1-3-QSi**). Lane (1) pBR 322DNA + 200 µg/mL of **1-QSi**. Lane (2) pBR 322DNA + 100 µg/mL of **1-QSi**. Lane (3) pBR 322DNA + 50 µg/mL of **1-QSi**. Lane (4) pBR 322DNA + 200 µg/mL of **2-QSi**. Lane (5) pBR 322DNA + 100 µg/mL of **2-QSi**. Lane (6) pBR 322DNA + 50 µg/mL of **2-QSi**. Lane (7) pBR 322DNA + 200 µg/mL of **3-QSi**. Lane (8) pBR 322DNA + 100 µg/mL of **3-QSi**. Lane (9) pBR 322DNA + 50 µg/mL of **3-QSi**. Lane (10) pBR 322DNA + 10µL DMSO, (11) Pbr322DNA + 10 µL distillated water.

The binding of compounds (**1-3-QSi**) with CT-DNA was studied by applying an agarose gel electrophoresis. All the studied samples contained 50 µM of the quaternized phthalocyanine and different concentrations of CT-DNA ranging from 0.25 mg/mL to 2 mg/mL. The compounds bounded to the CT-DNA through groove binding or intercalation. The results are portrayed in Fig. 3. The decrease in the CT-DNA intensity did not proportionally follow the reduction in the CT-DNA concentration since there was a partial, weak interaction

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between compound **1-QSi** and the CT-DNA (lanes 3-6). Compounds **2-QSi** interacted strongly with CT-DNA (lanes 7-10) whereas there was a moderate interaction between **3-QSi** and the CT-DNA (lanes 11-14). Compound **2-QSi** exhibited strong binding activity with CT-DNA as six positive nitrogen atoms made the CT-DNA neutral through the electrostatic interactions with the negative charge of phosphate groups of 0.5 mg/mL CT-DNA (lanes 9-10).

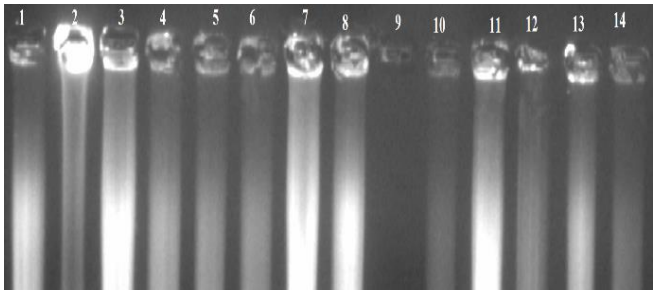


Fig. 3 Agarose gel electrophoresis of compounds (**1-3-QSi**). Ingredients of lanes: lane1: CT-DNA (2 mg/mL) in ddw, lane2: CT-DNA (2 mg/mL) in buffer (pH=7), lanes 3-6: CT-DNA (2-0.25 mg/mL)+buffer+ compound **1-QSi** (50 µM), lanes 7-10: CT-DNA (2-0.25 mg/mL)+ buffer+ compound **2-QSi** (50 µM), lanes 11-14: CT-DNA (2-0.25 mg/mL)+ buffer+ compound **3-QSi** (50 µM).

Some literature has reported similar studies. Koçan *et al*. studied the DNA binding activity of morpholinoethoxy-substituted metal-free, zinc(II), and indium(III) phthalocyanines using CT-DNA molecule. These water-soluble phthalocyanines displayed acceptable CT-DNA binding activities.[56] Zeballos et al. studied the CT-DNA binding activities of 2,9(10),16(17),23(24)-tetrakis [(N-butyl-N-methylammonium)ethylsulfanyl] phthalocyaninatozinc (II) tetraiodide, 2,9(10),16(17),23(24)-tetrakis [(N-dibutyl-N-methylammonium)ethoxy] phthalocyaninato zinc (II) tetraiodide, and 2,9(10),16(17),23(24)-tetrakis [(2-trimethylammonium)ethylsulfanyl] phthalocyaninato zinc (II) tetraiodide. 2,9(10),16(17),23(24)-tetrakis [(N-butyl-N-methylammonium)ethylsulfanyl] phthalocyaninato zinc (II) tetraiodide exhibited the highest activity owing to the linkage of to the phthalocyanine ring.[57] Al-Raqa et al. prepared new quaternized axially di-substituted silicon (IV) phthalocyanine bearing quaternized 4-(4-pyridinyl)phenyl groups and investigated its CT-DNA binding properties. The studied compound bounded to the CT-DNA molecule.[58]

Determination of binding of 1-3QSi compounds to DNA using UV-vis measurements

UV-vis spectroscopy is well-known as an appropriate method to analyze the binding mode of compounds with DNA molecules (intercalative or non-intercalative mode). Intercalative interactions result in a bathochromic shift of the UV-vis spectra and a decrease in the absorbance intensity by increasing the DNA concentration. Non-intercalative interactions lead to a hypsochromic shift along with an increase in absorbance intensity by increasing DNA concentration.[57,59] In this study, the UV-vis spectra of compounds (**1-3-QSi**) were examined in the absence and presence of different concentrations of CT-DNA after incubation for 1 hour at 37°C. The changes in the respective UV-vis spectra of compounds (**1-3-QSi**) were studied at 674, 669, and 667 nm. As the DNA concentration increased, the Q-band intensity of the UV-vis spectra of compounds (**1-3-QSi**) decreased and slightly shifted to the red region. The UV-vis spectra of compound **1-QSi** in the absence and presence of different concentrations of CT-DNA are given in Fig. 4 as an example. The obtained results confirmed that there are intercalative interactions between CT-DNA and compounds (**1-3-QSi**). All the data are listed in Table 2. Accordingly, the CT-DNA binding activities of compounds (**1-3-QSi**) increased in the following order: **1-QSi** < **3-QSi** < **2-QSi**.

Table 2 CT-DNA binding data for compounds (**1-3-QSi**).

Compound	Wavelength (nm)	Change in Absorbance	Red shift (nm)	K_b ($\times 10^4 M^{-1}$)
1-QSi	674	Hypochromism	2	2.4
2-QSi	669	Hypochromism	3	11.5
3-QSi	667	Hypochromism	3	6.7

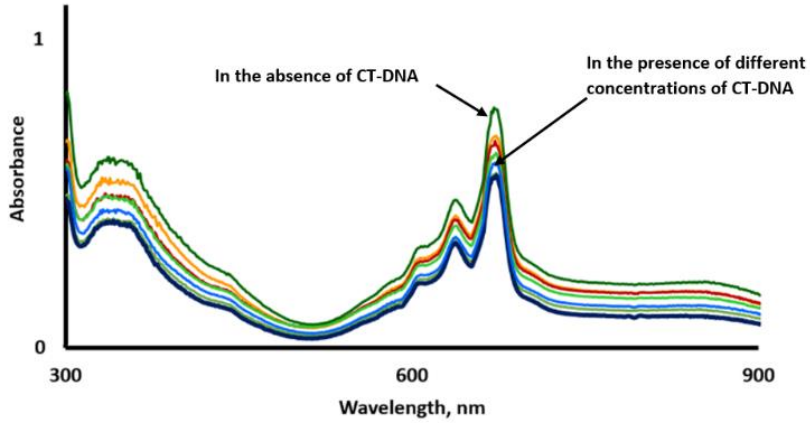


Fig. 4 The UV-vis spectra of compound **1-QSi** in the absence and presence of different concentrations of CT-DNA (0.25-2 mg/mL).

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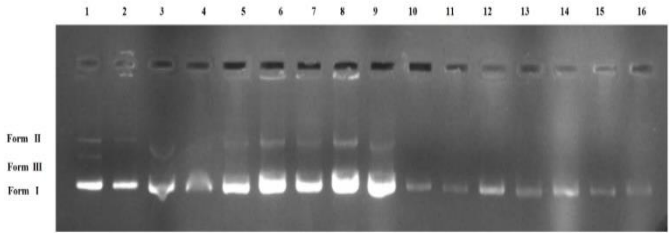


Fig. 5 The inhibitory effect of compounds (**1-3-Si** and **1-3-QSi**) against human topoisomerase I. Lane 1: pBR322 in ddwater; lane 2: pBR322 in buffer (TGS) and water; lane 3: pBR322 + 1 Unit Topo I; lane 4: pBR322 + 1 Unit Topo I + buffer (TGS)+ ddwater; lane 5-7: pBR322 + 1 Unit Topo I + buffer (TGS)+ compound **1-QSi** (200, 100, 50 µM)+ ddwater; lane 8-10: pBR322 + 1 Unit Topo I + buffer (TGS)+ compound **2-QSi** (200, 100, 50 µM)+ ddwater; lane 11-13: pBR322 + 1 Unit Topo I + buffer (TGS)+ compound **3-QSi** (200, 100, 50 µM)+ ddwater; lane 14-16: pBR322 + 1 Unit Topo I + buffer (TGS)+ irrinotecan (200, 100, 50 µM)+ ddwater.

Human topoisomerase-I inhibition

DNA relaxation assay as an efficient method is applied to measure the topoisomerase inhibition activities of materials. An agarose gel electrophoresis is used in this approach. In this study, the inhibition activities of different concentrations of compounds (**1-3-QSi**) against the human topoisomerase-I enzyme were examined by pBR322 DNA and irinotecan was used as a positive control.[60-62] The method that has been described in Ref. 62 was applied with some modifications. The images of the related agarose gel electrophoresis studies are shown in Fig. 5. Lane 3 was utilized as a negative control. As demonstrated, Topo I affected pBR322DNA strand segregation in the absence of the buffer and compounds (**1-3-QSi**). However, it did not exhibit the same behaviour in the presence of the buffer (lane 4). As seen in lanes 5-7, compound **1-QSi** did not display inhibition activity against the topo I enzyme at the studied concentrations. Additionally, only 50 µM of compound **2-QSi** inhibited the topo I enzyme (lanes 8-10). However, compound **3-QSi** displayed high inhibition activities in all the studied concentrations (lanes 11-13). The inhibition behaviour of the positive control irinotecan is shown in lanes 14-16. There are similar studies in the literature. Özel et al. studied the topoisomerase I inhibition features of 2(3),9(10),16(17),23(24)-Tetrakis-{2-[3-(dimethylamino)phenoxy]ethoxy} phthalocyaninato oxotitanium (IV) iodide, 2(3),9(10),16(17),23(24)-tetrakis-{2[3(diethylamino)phenoxy]ethoxy} phthalocyaninato oxotitanium (IV) iodide, and 2(3),9(10),16(17),23(24)-tetrakis-(2-{2-[3-(dimethylamino)phenoxy]ethoxy}ethoxy)-phthalocyaninato oxotitanium (IV) iodide. The water-soluble titanium(IV) phthalocyanine bearing (2-{2-[3-(dimethylamino)phenoxy]ethoxy}ethoxy) groups displayed the highest inhibition activity.[63] Baş et al. prepared the quaternized axially di-substituted silicon (IV) phthalocyanine and naphthalocyanine bearing 1-acetylpiperazines. They exhibited moderate activity against topoisomerase I.[61] In comparison to the literature, all the studied concentrations of compound **3-QSi** and 50 µM of compound **2-QSi** exhibited high Topoisomerase-I inhibition activities and can be utilized as cancer drugs after further investigation.

Cell culture

The normal physiology and biochemistry of cells, the effect of drugs and toxic compounds on the cells, and mutagenesis and carcinogenesis are mostly explored by performing cell culture. Drug delivery and penetration of drugs are affected by some

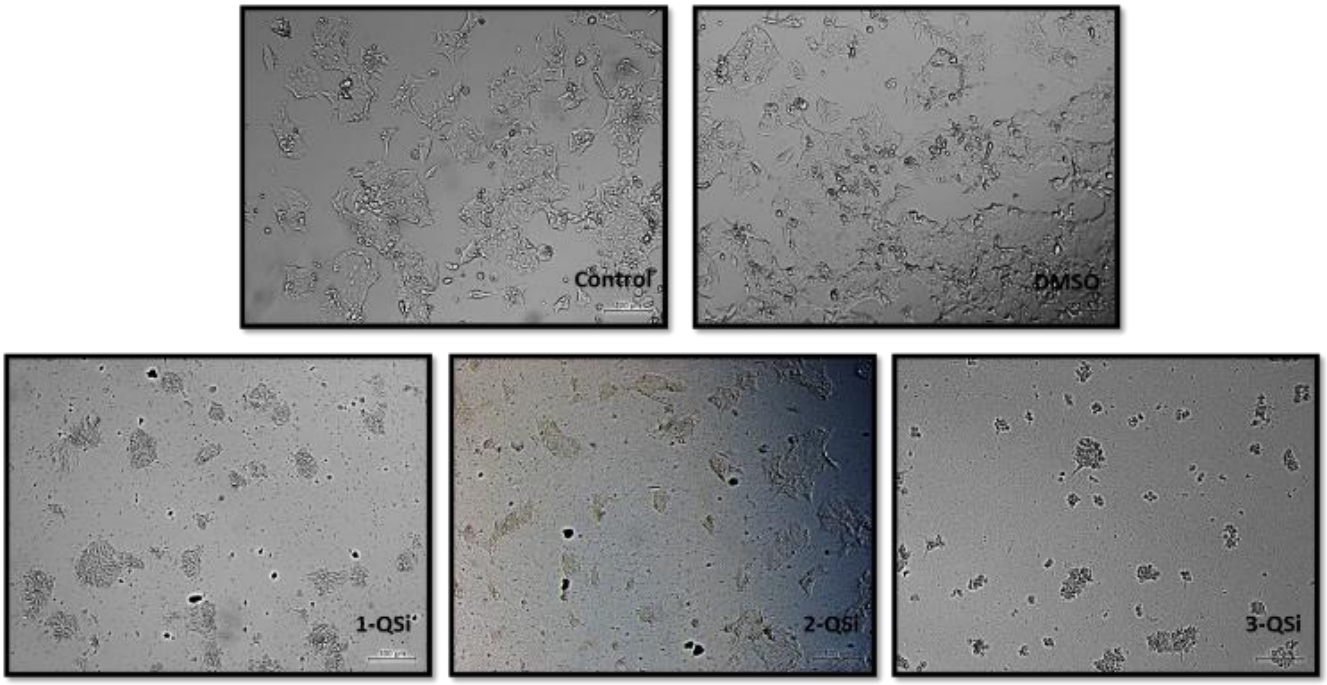


Fig. 6 The microphotographs of the DLD-1 cells. Cells morphologic character with 100 µM of each compound and control groups, for 24 hours, under inverted microscopy x10.

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parameters including the shape, size, and surface of the anticancer agents.[64] Generally, cationic amine-containing compounds have been utilized for cancer treatment since the positively charged amines collect on the surface of lesions much more than neutral or negatively charged materials.[65,66] It is also well-known as a consistent and reproducible application for a batch of clonal cells. In this study, the DLD-1 cell line was cultured under normal culture conditions. Control and DMSO group cells maintained the original morphology during the culture. However, the morphology of the cells that were incubated with compounds (**1-3-QSi**) was changed. Their shrunk structures are given in Fig. 6.

MTT assay

Cellular metabolic activities that consist of cytotoxicity, proliferation, and cell viability are measured by performing the MTT assay as an indicator.[67,68] Generally, the phototoxicity of phthalocyanines relies on the extent of their uptake into cells, intracellular distribution, and the ability for generating reactive oxygen species (ROS).[69,70] Indeed, the nature of the axial ligands on Si(IV) phthalocyanines plays a vital role in their aggregation, photophysical, and phototoxic properties. Therefore, the phototoxicity of phthalocyanines is a combination of these parameters.[71] In this study, an *in vitro* cytotoxicity test was performed and the phototoxic influence of compounds (**1-3-QSi**) on the DLD-1 cell line was examined using an MTT cell proliferation assay.[72,73] The IC_{50} values are summarized in Table 3. The cytotoxic and phototoxic results are shown in Fig. 7. An increase in the concentration of compounds (**1-3-QSi**) was followed by a decrease in cell proliferation whereas a statistically remarked alteration did not appear for the photodynamic therapy group ($P<0.05$). Some literature has presented similar studies. Akin et al. investigated anticancer properties of a series of water-soluble sulfonated metal phthalocyanines (M= GaCl, InCl, Co, Cu, Mn, Ni). They exhibited low dark cytotoxic effects.[74] Lo et al. synthesized a number of new symmetric and unsymmetric axially di-substituted silicon (IV) phthalocyanines bearing 1,3-bis(dimethylami-no)-2-propoxy and/or alkoxy groups. They displayed acceptable photocytotoxicity against cell lines namely human hepatocellular carcinoma HepG2 and murine macrophage J774.[75] Zhu et al. prepared bis (2-morpholine) ethoxy phthalocyaninatosilicon and bis (2-(N-methyl-morpholine)) ethoxy phthalocyaninatosilicon diiodide and examined their anticancer activities against B16 melanoma tumour cells. A high IC_{50} value was obtained for bis (2-(N-methyl-morpholine)) ethoxy phthalocyaninatosilicon diiodide.[76] The acceptable IC_{50} values proved that compounds (**1-3-QSi**) can prevent the proliferation of DLD-1 cells. All the studied compounds exhibited similar cytotoxicity against the DLD-1 cell line and the results were compatible with the literature.[55,60,62,72,73]

Table 3. IC_{50} values of compounds (**1-3-QSi**) on DLD-1 cell line.

Compound	IC_{50} Values (μM)	
	Darkness Group (Without irradiation)	Photodynamic therapy group (White lamp irradiation)
1-QSi	11.35	9.31
2-QSi	13.76	11.92
3-QSi	15.38	11.92
Cisplatin	80.6	80.5

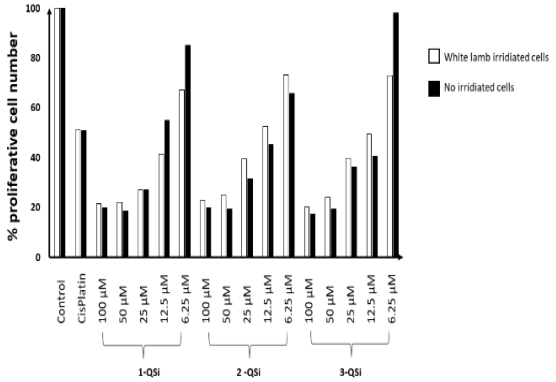


Fig. 7 MTT cell proliferation assay. **1-3-QSi** with/without white lamp irradiation.

Apoptosis assay

Apoptosis is a programmed cell death that is well-known as one of the essential parameters for aging, development, and disease.[77,78] An *in vitro* apoptosis assay was applied in this study. The analysis of the live, early and late apoptosis, and cell death (total apoptotic) on the DLD-1 cells was carried out utilizing the Annexin V assay kit. The results are portrayed in Fig. 8. Due to the insignificant effect of the white lamb irradiation on the MTT test, white lamb irradiation was not performed for the apoptosis studies. The IC_{50} values were calculated to study the apoptotic effect of compounds (**1-3-QSi**).[74,75] Albeit compounds **2-QSi** and **3-QSi** displayed statistically intensive total apoptosis activities in comparison to living cells ($P<0.001$), the total apoptosis activity of compound **1-QSi** was not higher than the live cells. However, compounds (**1-3-QSi**) induced apoptosis to a statistically significant extent ($P=0.05$) compared to the control group. There is some literature reporting similar studies. Schmidt *et al.* prepared new tetra-triethyleneoxysulfonyl substituted zinc phthalocyanine and dihydroxy -2,9(10), 16(17), 23(24) – tetrakis (4,7,10- trioxaundecan-1-sulfonyl) silicon phthalocyanine. The zinc phthalocyanine displayed acceptable apoptotic behaviours.[79] Lai et al. studied anticancer properties of the unsymmetrically substituted bis amino phthalocyanine. The studied compound led to apoptosis which was confirmed by losing membrane asymmetry and forming a DNA ladder.[80] As a

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result, macromolecules (**1-3-QSi**) exhibited high apoptotic activities that were compatible with the literature.

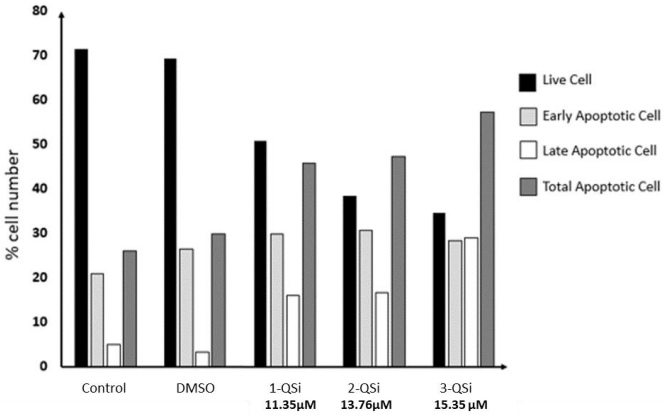


Fig. 8 Percentage of the DLD-1 cells apoptosis and live cell in the absence/presence of compounds (**1-3-QSi**).

Experimental

Equipment

IR spectra were recorded on a PerkinElmer Spectrum One spectrometer in a 4000-400 cm^{-1} range at room temperature. ^1H NMR spectra were recorded on an Agilent VNMRS 500 MHz spectrometer and chemical shifts (δ) are given in ppm. Electronic absorption spectra were obtained using a Scinco Lab Pro Plus UV/Vis spectrophotometer. Mass spectra were obtained using a Bruker Microflex MALDI–TOF mass spectrometer.

Synthesis and characterization

Silicon phthalocyanine dichloride was purchased from Merck Company. All the consumed biological materials and chemicals were purchased from commercial suppliers. Compounds (1-Si and 1-QSi) were synthesized according to the procedures that have been described in Ref. 81 with some modifications.

General procedure for the synthesis of compounds 2 and 3

2,4,6-tris((dimethylamino)methyl)phenol (**1**) (500 mg, 1.88 mmol) and 2-chloroethanol (152 mg, 1.88 mmol) or 2-(2-chloroethoxy)ethanol (235 mg, 1.88 mmol) were dissolved in ethanol (10 mL). Then sodium hydroxide (1.50 g, 37.68 mmol) was added to the solution. The reaction content was stirred at refluxed temperature for 48 hours under a nitrogen atmosphere. After cooling to room temperature, the crude product was filtered off and washed several times with ethanol. The resulting solid was purified by applying column chromatographic technique on silica gel as a stationary phase and methanol as a mobile phase.

2-(2,4,6-tris((dimethylamino)methyl)phenoxy)ethan-1-ol (2)

Yield 420 mg (72%). FT-IR υ (cm^{-1}): 3396 (OH), 2980-2861 (C-H aliphatic), 1147 (C-N), 1095 (C-O-C). ^1H NMR (500 MHz; DMSO-d_6): δ (ppm) 8.52 (s, Ar-H, 2H), 4.81 (bs, OH, 1H), 3.83-3.79 (m, Ar-CH_2N, CH_2-OH, 4H), 3.66-3.58 (m, Ar-O-CH_2, 2H), 3.46-3.41 (m, Ar-CH_2N, 4H), 1.76-1.71 (s, CH_3, 12H), 1.61-1.54 (s, CH_3, 6H).

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2-(2-(2,4,6-tris((dimethylamino)methyl)phenoxy)ethoxy)ethan-1-ol (3)

Yield 465 mg (70%). FT-IR υ (cm^{-1}): 3384 (OH), 2948-2924 (C-H aliphatic), 1126 (C-N), 1076 (C-O-C). ^1H NMR (500 MHz; DMSO-d_6): δ (ppm) 8.5 (s, Ar-H, 2H), 5.75 (bs, OH, 1H), 3.90-3.87 (m, Ar-O-CH_2, 2H), 3.59-3.56 (m, Ar-CH_2N, CH_2-OCH_2-CH_2, 12H), 1.77-1.73 (m, CH_3, 12H), 1.58 (bs, CH_3, 6H).

General procedure for the synthesis of compounds 2-Si and 3-Si

Silicon (IV) phthalocyanine dichloride (100 mg, 0.163 mmol), the related ligand {compound **2** (101 mg, 0.327 mmol) or compound **3** (0.116 mg, 0.327 mmol)}, and sodium hydride (31.5 g, 1.31 mmol) were stirred in toluene (3 mL) at 130 °C for 48 hours under an inert atmosphere. The content was cooled to room temperature, filtered off, washed several times with hot n-hexane and water. The pure product was obtained using a column chromatography on silica gel as a stationary phase and *n*-hexane-THF (2:1) as the mobile phase.

Compound 2-Si

Yield 110.50 mg (56%), mp > 250 °C. FT-IR υ (cm^{-1}): 3055 (C-H aromatic), 2965-2853 (C-H aliphatic), 1377-1163 (C-N), 1078 (C-O-C). ^1H NMR (500 MHz; DMSO-d_6): δ (ppm) 8.94-8.92 (m, Ar-H, 8H), 8.43-8.40 (m, Ar-H, 8H), 8.20-8.15 (m, Ar-H, 4H), 3.99-3.97 (m, Ar-CH_2N, 4H), 3.93-3.90 (m, Ar-CH_2N, 8H), 1.32-1.30 (m, CH_3, 12H), 1.28-1.26 (m, CH_3, 24H), 0.95-0.92 (m, Ar-O-CH_2, 4H), 0.64 (bs, CH_2-O-Si, 4H). UV–vis (DMSO): λ_{max} nm (log ε) 332 (4.99), 636 (4.88), 672 (5.09). MS m/z calcd. for $[M]^+$ 1157.49 found 1097.23 $[M\text{-}4CH_3]^+$, 1113.32 $[M\text{-}3CH_3]^+$, 1184.74 $[M\text{-}CH_3+Na+H_2O+H]^+$.

Compound 3-Si

Yield 130 mg (64%), mp > 250 °C. FT-IR υ (cm^{-1}): 3047 (C-H aromatic), 2956-2884 (C-H aliphatic), 1367-1119 (C-N), 1080 (C-O-C). ^1H NMR (500 MHz; DMSO-d_6): δ (ppm) 9.66 (bs, Ar-H, 8H), 8.53-8.47 (m, Ar-H, 8H), 8.20-8.10 (m, Ar-H, 4H), 3.81-3.77 (m, Ar-CH_2N, 4H), 3.67-3.62 (m, Ar-CH_2N, 8H), 1.67-1.60 (m, CH_3, 12H), 1.53-1.46 (m, CH_3, 24H), 0.11 (bs, Ar-O-CH_2, 4H), -1.61-(-1.71) (m, CH_2-O-CH_2CH_2, 12H). UV–vis (DMSO): λ_{max} nm (log ε) 329 (5.06), 635 (5.03), 671 (5.19). MS m/z calcd. for $[M]^+$ 1245.59 found 1248.77 $[M+3H]^+$, 1612.551 $[M+2DHB+Na+2H_2O]^+$, 1653.83 $[M+2DHB+2Na+3H_2O]^+$.

General procedure for the synthesis of compounds 2-QSi and 3-QSi

The newly synthesized macromolecule compound **2-QSi** (30 mg, 0.026 mmol) or compound **3-QSi** (30 mg, 0.024 mmol)) and iodomethane [for compound **2-QSi** (22 mg, 0.156 mmol), for compound **3-QSi** (20.5 mg, 0.144 mmol)] were stirred in the mixture (3 mL) of chloroform:dichloromethane(100:1) at room

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temperature for 72 hours. The resulting product was filtered off, washed several times with chloroform, and dried in a vacuum.

Compound 2-QSi

Yield 32 mg (61.5%), mp > 250 °C. FT-IR υ (cm^{-1}): 3077 (C-H aromatic), 2969-2948 (C-H aliphatic), 1378-1127 (C-N), 1088 (C-O-C). ^{1}H NMR (500 MHz; DMSO-d_6): δ (ppm) 8.30 (bs, Ar-H, 8H), 7.57-7.53 (m, Ar-H, 8H), 7.14-7.10 (m, Ar-H, 4H), 3.93-3.86 (m, Ar-CH_2N, 4H), 3.49 (m, Ar-CH_2N, 8H), 1.43-1.38 (m, CH_3, 18H), 1.33-1.30 (m, CH_3, 36H), 0.65-0.54 (m, CH_2-CH_2-O, 8H). UV–vis (DMSO): λ_{max} nm (log ε) 330 (4.96), 633 (4.88), 669 (5.09). MS m/z calcd. for $[M]^+$ 2009.15 found 543.83 $[M-3I+3H]^{3+}$, 1114.27 $[M+DHB+2Na+H_2O]^{2+}$, 1669.81 $[M-3I+Na+H_2O+H]^+$.

Compound 3-QSi

Yield 31 mg (61%), mp > 250 °C. FT-IR υ (cm^{-1}): 3073 (C-H aromatic), 2982-2948 (C-H aliphatic), 1365-1122 (C-N), 1086 (C-O-C). ^{1}H NMR (500 MHz; DMSO-d_6): δ (ppm) 8.29 (bs, Ar-H, 8H), 7.61-7.53 (m, Ar-H, 8H), 7.11 (m, Ar-H, 4H), 4.12 (bs, Ar-CH_2N, 4H), 3.49 (m, Ar-CH_2N, 8H), 1.44-1.34 (m, CH_3, 18H), 1.21 (m, CH_3, 36H), 0.92-0.86 (m, O-CH_2, 4H), 0.82-0.76 (m, O-CH_2-O-CH_2, 8H), 0.63 (bs, CH_2-O-Si, 4H). UV–vis (DMSO): λ_{max} nm (log ε) 326 (5.03), 632 (4.99), 667 (4.14). MS m/z calcd. for $[M]^+$ 2097.25 found 1097.01 $[M-I+CHCA+2H_2O]^{2+}$, 1651.604 $[M-4I+2Na+OH]^+$.

Biological Studies

Supercoiled pBR322 plasmid DNA, CT-DNA, and the kits of human Topoisomerase were obtained from Thermo Scientific, Sigma, and Topogen, respectively.

Singlet oxygen quantum Yield

The capacity of compounds (**1-3-QSi**) for singlet oxygen production was examined through the study of the phosphorescence of $^1O_2$, using a Horiba–Jobin–Yvon Fluorimeter with a Hamamatsu NIR PMT 5509.[47] The singlet oxygen quantum yields were calculated by applying below equation:

$$\phi_\Delta = \phi_\Delta(std)\ (I_\Delta \times A\ (std) / I_\Delta(std) \times A)$$

where ϕ_Δ(std) is the singlet oxygen quantum yield of unsubstituted zinc (II) phthalocyanine (ZnPc; ϕ_Δ = 0.67 in DMSO). IΔ and A are the area under the curve of the peak of singlet oxygen at 1229 nm and the absorbance, respectively.

Pharmacological/biological assays

DNA cleavage experiments

An agarose gel electrophoresis was applied to examine the DNA cleavage activity of the quaternized phthalocyanines (**1-3-QSi**). Supercoiled pBR322 plasmid DNA (100 µg) was used without irradiation. Supercoiled pBR322 plasmid DNA was treated with different concentrations of compounds (**1-3-QSi**) ranging from 50 to 200 µM in DMSO and DDW. All samples were incubated in PCR tubes at 37°C for 24 hours. Then, the mixtures were loaded on 1% agarose gel with ethidium bromide staining in TAE buffer (Tris acetic acid-EDTA). Electrophoresis was performed at 90 V for 70 minutes. The results were visualized using the BioRad Gel Doc XR system and analysed by applying the Image Lab Version 4.0.1 Software program.

DNA binding experiments using gel electrophoresis

The interaction of the studied compounds with CT-DNA was studied by performing an agarose gel electrophoresis method. 200 µM solutions of quaternized phthalocyanines (**1-3-QSi**) were prepared in DMSO. Then, a series of samples containing 50 µM of the newly synthesized phthalocyanine and different concentrations of CT-DNA (0.25-2 mg/mL in ddw) were prepared and the total volume was adjusted to a final volume of 25µl with ddw in PCR tubes. The samples were incubated at 37°C for 1 hour. Then, the mixtures were loaded with the buffer on 1% agarose gel with ethidium bromide staining in TAE. The electrophoresis was performed at 90 V for 1 hour. The results were visualized by applying the BioRad Gel Doc XR system.[60]

Topoisomerases inhibition

Irinotecan (2 mg/mL) was used as a positive control in the topoisomerases inhibition studies. The human DNA topoisomerase I and E.coli topoisomerase assays were applied according to the method that has been extensively explained in Ref. 60 with minor modifications. The relaxation of supercoiled pBR322 plasmid DNA (100 µg) in the absence/ presence of the studied compounds (50 µM, 200 µM) was studied in buffer (10 × TGS) and dilution buffer in the kit to measure the human topoisomerase I (Topo I) inhibition activity of quaternized phthalocyanines (**1-3-QSi**). The samples were incubated at 37°C for 1 hour and 4 µL of 5 × loading buffer solution including 4.5% SDS, 0.25% bromophenol blue, and 45% glycerol was added. Electrophoresis was performed in 1% agarose gel in 1 × TAE at 90 V for 90 minutes.[82]

Cell culture

The human colorectal cell line DLD-1 was obtained from American Type Culture Collection (CCL-221™, ATCC, USA). The studied cells were cultured in high glucose DMEM supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin-streptomycin. The samples were incubated at 37°C under 5% CO_2 using a humidified incubator and cultured in 75 cm^2 flasks.[83,84]

MTT assay

The cells were seeded as $1x10^4$ cells per well in 96 well plates and left for 24 hours for MTT test. For MTT test the study was carried out in two groups: Darkness (the normal culture conditions) and photodynamic therapy group (white lamp irradiation). The samples were prepared by adding different concentrations of the studied compounds to the cells to obtain 3.125, 6.25, 12.5, 25, 50, and 100 µM. DMSO (30%) and non-treated cells were utilized as a negative control and the control group, respectively. Cisplatin was used as a positive control. For

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the darkness group, 96 well plate was incubated at 37°C for 24 hours under 5% CO_2 using a humidified incubator. For the photodynamic therapy group, 96 well plates were incubated for 1 hour at normal conditions and were exposed to the 3000-lx white light irradiation for 1 hour. Then the plates were kept under the normal incubation conditions. The distance between the plate surface and the light source was 4 cm. After incubation, MTT solution (with a final concentration of 0.5 mg/mL) was added to the wells and the formazan crystals were observed after 3 hours. The well contents aspired and 100 µL DMSO was added to solve formazan crystals. The data were recorded between 560 and 750 nm using an ELISA reader (Glomax, Promega, USA). The half-maximal inhibitory concentration (IC_{50}) was calculated using the obtained optical density (OD) values.[77,85]

Apoptosis assay

1 x 10^5 cells were seeded at 6 well culture plates for 24 hours. Compounds (**1-3-QSi**) were added to the cells. DMSO was used as a negative control. Non-treated cells were utilized as the control group. The photodynamic therapy group was not tested for this assay. Only the darkness group (normal incubated plates) was analysed. After incubation for 24 hours with compounds, the cells were harvested with Trypsin-EDTA and centrifuged. The cell pellet was resuspended with 100 µL dPBS. Then, 100 µL MUSE Annexin V kit reagent was added. The samples were left for 20 minutes at dark room temperature. The cells were analysed by applying a MUSE Cell Analyser (Millipore, USA).[86]

Conclusions

This study reports the synthesis and characterization of new silicon phthalocyanines and their water-soluble analogs. The DNA interactions of the synthesized water-soluble silicon phthalocyanines were studied using pBR322 plasmid DNA and CT-DNA molecules. Compound **1-QSi** exhibited effective plasmid DNA cleavage at all the tested concentrations. Compounds **2-QSi** and **3-QSi** intercalatively or groovely bounded to CT-DNA. Additionally, the human topoisomerase I inhibition activities of compounds (**1-3-QSi**) were studied. Compound **3-QSi** exhibited high strong DNA cleavage and enzyme inhibition activities at all the studied concentrations. Also, the highest singlet oxygen quantum yield was obtained for compound **3-QSi**. Therefore, compound **3-QSi** can be considered as suitable anticancer agent. The obtained result Besides, the anticancer activities of compounds (**1-3-QSi**) against the DLD-1 cell line were examined by applying *in vitro* MTT and Annexin V assays. All the compounds had cytotoxic and apoptotic effects on the DLD-1 colorectal cell line. However, there was no statistically significant difference between the dark group and white lamp irradiation group of the studied phthalocyanines under the applied conditions. The compounds displayed acceptable cytotoxicity activities which are compatible with the literature. The IC_{50} values were obtained between 11.35 and 15.38 µM which are much lower than Cisplatin (80.30). Therefore, the studied compounds strongly affected DLD-1 cell line. All the compounds also increased the early and total apoptosis and led to the maintenance of cell membrane integrity. To conclude, statistically significant values were obtained for compounds **2-QSi** and **3-QSi** on late apoptosis (P**2-QSi**=0.0001 and P**3-QSi**=0.00005). As late apoptosis depends on the permeable cell membrane, damaged cells, and DNA damage, the performed CT-DNA binding gel electrophoresis confirmed the apoptosis results. Therefore, these compounds can exhibit high performance for irradiation-containing applications (e.g. photodynamic therapy) and non-irradiation-containing therapies (e.g. chemotherapeutic therapy).

Author Contributions

Dr. N. Farajzadeh, Dr. N. G. Kuşçulu, Dr. H. Y. Yenilmez, Dr. D. Bahar, and Prof. Dr. Z. A. Bayır have given approval to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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