Süleyman Demirel Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
Cilt 21, Sayı 3, 689-695, 2017

Süleyman Demirel University
Journal of Natural and Applied Sciences
Volume 21, Issue 3, 689-695, 2017

DOI: 10.19113/sdufbed.89035

Effect of New Water-Soluble Dendritic Phthalocyanines on Human Colorectal and Liver Cancer Cell Lines

Ebru YABAŞ*[1], Mustafa SÜLÜ[2], Aykut ÖZGÜR[3], Yusuf TUTAR[4]

[1]Cumhuriyet Üniversitesi, İmranlı Meslek Yüksekokulu, Kimya ve Kimyasal İşleme Teknolojileri Bölümü, 58980, Sivas
[2]İnönü Üniversitesi, Fen Edebiyat Fakültesi, Kimya Bölümü, 44280, Malatya
[3]Gaziosmanpaşa Üniversitesi, Mühendislik ve Doğa Bilimleri Fakültesi, Biyomühendislik Bölümü, 60150, Tokat
[4]Cumhuriyet Üniversitesi, Eczacılık Fakültesi, Temel Eczacılık Bilimleri Bölümü, 58140, Sivas

(Alınış / Received: 27.03.2017, Kabul / Accepted: 13.06.2017, Online Yayınlanma / Published Online: 08.08.2017)

Keywords
Dendrimer,
Anticancer activity,
Liver cancer,
Colorectal cancer

Abstract: Human hepatocellular carcinoma (HepG2) cells and colorectal adenocarcinoma (DLD-1) cells were treated with the synthesized water soluble phthalocyanine derivatives to understand the effect of the compounds both on colorectal and liver cancer cells. The compounds inhibited cell proliferation and displayed cytotoxic effect on these cancer cell lines however; the effect of the compounds on healthy control fibroblast cell line was comparatively lower. The compounds can be employed for cancer treatment as anticancer agents.

Yeni Suda Çözünür Dendritik Ftalosiyaninlerin İnsan Kolorektal ve Karaciğer Kanser Hücre Hatları Üzerine Etkisi

Anahtar Kelimeler
Dendrimer,
Antikanser aktivitesi,
Karaciğer kanseri,
Kolorektal kanser

Özet: İnsan hepatoselüler karsinoma (HepG2) hücreleri ve kolorektal adenokarsinoma (DLD-1) hücreleri, bileşiklerin hem kolorektal hemde karaciğer kanseri hücrelerindeki etkisini anlamak için sentezlenen suda çözünür ftalosiyanin türevleriyle etkileştirildi. Bileşikler hücre proliferasyonunu inhibe etti ve bu kanser hücre dizileri üzerinde sitotoksik etki gösterdi; buna karşın bileşiklerin sağlıklı kontrol fibroblast hücre hattı üzerindeki etkisi nispeten daha düşüktü. Bu bileşikler kanser tedavisinde antikanser ajanlar olarak kullanılabilir.

1. Introduction

Colorectal cancer is one of the most common causes of deaths in the world. The most frequent metastastatic place of the colorectal cancer is the liver. Major cause of the deaths in patients with colorectal cancer is the liver metastasis and almost half of colorectal cancer patients are diagnosed with the liver metastases eventually. Treatment of colorectal cancer liver metastasis includes a combination of surgery, chemotheraphy, and radiotheraphy. 5-fluorouracil, leucovorin, kapesitabin, irinotecan, oxaliplatin, and their combinations are used for treatment of colorectal cancer liver metastasis. These chemotherapeutics inhibit DNA synthesis in cancer cells and prevent DNA replication and transcription, causing cell death. However, their low selectivity causes some limitations and side effects. To compensate selectivity and limitation problems, monoclonal antibodies (cetuximab, bevasizumab, panitumomab) are used in combination with these drugs in colorectal cancer liver metastasis treatment. Therefore, researchers and global pharmaceutical companies focus on the development of efficient colorectal and liver cancer drugs [1,2].

Dendrimers are highly branched macromolecules that radiate from a central core. Because of their three-dimensional structure, surface functionality, versatility, solubility, optic properties, and chirality, dendrimers have been used widely in different areas such as catalysis, sensor technology, liquid crystal materials, and biomedical technology [3-5]. Dendritic macromolecules have been especially used in cancer diagnosis and therapy [6-9] and as drug delivery carriers [10-13] during the last few years. Phthalocyanine and its derivatives are used as functional materials in several technique fields, such as catalyst, data storage, sensors, non-linear optics, photosensitizers in photodynamic therapy in cancer treatment since 1990s [14-19]. Phthalocyanine-cored

*Corresponding author: eyabas@cumhuriyet.edu.tr

dendrimers have the potential to use as photodynamic therapeutic drugs for cancer [20,21]. For example, Pc-cored PAMAM dendrimers synthesized by Sakamato et al [22] are fluorescent and are feasible to use in photodynamic therapeutic drugs for cancer. The phthalocyanine-cored dendrimer was firstly synthesized by Kobayashi in 1997 is poly(ether-amide) dendrimer containing octakis[3,5-(dicarboxy)-phenoxy] ZnPc [23,24].

On the other hand, it is important to synthesis water soluble phthalocyanine compounds for biomedical applications [25,26]. In the literature, different water soluble dendritic phthalocyanine compounds have been synthesized and their photophysical [27,28,29], spectroscopic [30,31], electron transfer processes [32,33], electrical and CO_2 sensing properties [34] have been investigated. Therefore, synthesis of new water soluble dendritic phthalocyanine compounds and investigation of their anticancer activities could be worthwhile.

In this study, water soluble dendritic metal-free **3a** and zinc phthalocyanine **3b** compounds were synthesized and characterized. The potential of anticancer activity of **3a** and **3b** was evaluated in human colorectal and liver cancer cell lines. Furthermore, the cytotoxicity of compounds was determined in healthy fibroblast cell line. Experimental data indicated that these compounds displayed minimal activity against healthy fibroblast cells but effectively toxicated the cancer cell lines. The study also aimed to offer new potential therapeutic agents for the treatment of human colorectal cancer liver metastasis.

2. Material and Method

2.1. Chemistry

The solvents used in the reaction were dried by molecular sieves or suitable methods [35]. Argon gas was used for inert media in all reactions. Tetrakis[(2-trimethylaminoethylsulfanyl)phthalocyanine]tetraiodide and tetrakis[(2-trimethylaminoethylsulfanyl)phthalocyaninatozinc(II)]tetraiodide were synthesized according to the literature [36]. UV-vis and IR spectra were obtained on Shimadzu UV-vis spectrometer and Perkin Elmer Spectrum 100 spectrometer equipped with ATR, respectively. ^{1}H-NMR spectra was recorded by a Bruker 300 MHz spectrometer. Melting points of the compounds were determined on an Electrothermal 9100 digital melting point instrument. DLD-1, HepG2, L929 cell lines and Dulbecco's modified eagle's medium (DMEM), fetal bovine serum, sterile phosphate buffer saline (PBS) were obtained from ATCC (American Type Culture Collection, USA) and from PAA Ltd. 0.25% Trypsin-EDTA solution was from Biological Industries Ltd, respectively. XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) cell proliferation kit was supplied from Roche Diagnostic.

2.2. Synthesis of compounds

2.2.1. Compounds 1a and 1b

The solution of triethyl methanetricarboxylate sodium salt ($NaC(CO_2C_2H_5)_3$) was prepared with reaction of metallic Na (12.30 mg, 0.54 mmol) and triethyl methanetricarboxylate (0.23 mL, 1.07 mmol) in dry ethanol (6 mL) at room temperature. Tetrakis [(2-trimethylaminoethylsulfanyl) phthalocyanine] tetraiodide (200.0mg, 0.13mmol) or tetrakis[(2-trimethylaminoethylsulfanyl) phthalocyaninatozinc (II)]tetraiodide (203.0 mg, 0.13 mmol) was added to the prepared solution of $NaC(CO_2C_2H_5)_3$. The mixture was refluxed for 3h. After cooled down to room temperature, 2.0 mL of 40% NaOH solution was added to reaction medium, stirred for 10 min, 2.0 mL H_2O was added to it and continued to reflux for 4h. The reaction mixture was concentrated by evaporation of ethanol and the dark green aqueous solution was extracted with ether. The product was precipitated by acidify of the aqueous phase with diluted hydrochloric acid. The green solid was filtered off, washed with H_2O (3x 20 mL) and acetone (2x 3 mL) and was dried in vacuum. The green solids **1a** and **1b** were soluble in DMSO and DMF.

(**1a**) Yield 100.0 mg (46%). Mp: >300^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 ^{o}C): δ= -4.70 (br s, 2H, Pc-N**H**, disappeared on D_2O addition); 7.85-6.73 (br m, 12H, Pc Ar-**H**); 3.31 (s, 16H, S-C**H$_2$-CH$_2$**); 3.31 (s, 24 H, O-C**H$_2$**); 2.38 (s, 36H, CH_2-C**H$_3$**). UV-Vis (DMSO) λ_{max}/nm 710, 682, 646, 407, 342. IR (ATR) υ (cm^{-1}) 2960; 1755; 1582; 1446; 742.

(1b) Yield 110.0 mg (49%). Mp: >300^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 ^{o}C): δ= 7.80-6.65 (br m, 12H, Pc Ar-**H**); 3.34 (s, 16H, S-C**H$_2$-CH$_2$**); 3.34 (s, 24 H, O-C**H$_2$**); 2.38 (s, 36H, CH_2-C**H$_3$**). UV-Vis (DMSO) λ_{max}/nm 688, 610, 365. IR (ATR) υ (cm^{-1}) 2960; 1759; 1585; 1446; 748.

2.2.2. Compounds 2a and 2b

Mixtures of **1a** (100.0 mg, 0.06 mmol) and $H_2NC(CH_2OH)_3$ (103.0 mg, 0.84 mmol) in DMSO (3 mL) were heated at the presence of K_2CO_3 (332.0 mg, 2.4 mmol) at 180^{o}C for 24h. After cooled down to room temperature, the product was precipitated in ether, filtered off and dried. The crude residue was washed with H_2O (3x 5 mL), ethanol (2x 3 mL) and acetone (3x 3 mL), respectively.

2b was synthesized using a similar procedure to **2a.** The dark green solids **2a** and **2b** were soluble in DMSO and DMF.

(2a) Yield 90.0 mg (58%). Mp: >300^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 ^{o}C): δ= -4.58 (br s, 2H, Pc-N**H**,

disappeared on D_2O addition); 7.92-7.07 (br m, 12H, Pc Ar-**H**); 3.40 (s, 16H, S-C**H₂-CH₂**); 3.27 (s, 72 H, C-C**H₂**); 8.71 (br s, 12H, amide-N**H**); 5.92 (br s, 36H, -O**H**). UV-Vis (DMSO) λ_{max}/nm 712, 685, 614, 401, 351. IR (ATR) υ (cm^{-1}) 3440-3381; 2967; 1761; 1602; 1486; 773. Anal. Calc. for $C_{104}H_{150}N_{20}S_4O_{48}$: C 48.48; H 5.87; N 10.87%, found: C 48.27; H 5.79; N 10.80%.

(2b) Yield 95.0 mg (60%). Mp: >300°C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ= 7.87-7.12 (br m, 12H, Pc Ar-**H**); 3.42 (s, 16H, S-C**H₂-CH₂**); 3.25 (s, 72 H, C-C**H₂**); 8.63 (br s, 12H, amide-N**H**); 5.64 (br s, 36H, -O**H**). UV-Vis (DMSO) λ_{max}/nm 690, 618, 363. IR (ATR) υ (cm^{-1}) 3448-3380; 2969; 1764; 1604; 1486; 773. Anal. Calc. for $C_{104}H_{148}N_{20}S_4O_{48}Zn$: C 47.32; H 5.65; N 10.61%, found: C 47.19; H 5.58; N 10.51%.

2.2.3. Compounds 3a and 3b

Phthalocyanine **2a** (100.0 mg, 0.039 mmol) or **2b** (100.0 mg, 0.038 mmol) were added to NaOH solution (30%, 5 mL). This mixture was refluxed until a clear solution was formed. At the end of the reaction, the dark green reaction solution was precipitated in methanol (100 mL) and filtered, respectively. The resulting solid was dissolved in H_2O and the solution was again precipitated with methanol until neutral. The product washed with methanol, acetone and dried in vacuum etuv at 80°C.

The green solids **3a** and **3b** were soluble in H_2O and DMSO at room temperature.

(3a) Yield 70.0 mg (53%). Mp: >300°C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ= -4.52 (br s, 2H, Pc-N**H**, disappeared on D_2O addition); 8.04-7.12 (br m, 12H, Pc Ar-**H**); 3.44 (s, 16H, S-C**H₂-CH₂**); 3.30 (s, 72 H, C-C**H₂**); 8.66 (br s, 12H, amide-N**H**). UV-Vis (DMSO) λ_{max}/nm 714, 688, 621, 406, 350. IR (ATR) υ (cm^{-1}) 3397-3380; 2968; 1765; 1608; 1486; 773. Anal. Calc. for $C_{104}H_{114}N_{20}S_4O_{48}Na_{36}$: C 37.09; H 3.41; N 8.32%, found: C 36.98; H 3.29; N 8.21%.

(3b) Yield 70.0 mg (54%). Mp: >300°C. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ= 7.99-7.10 (br m, 12H, Pc Ar-**H**); 3.45 (s, 16H, S-C**H₂-CH₂**); 3.33 (s, 72 H, C-C**H₂**); 8.57 (br s, 12H, amide-N**H**). UV-Vis (DMSO) λ_{max}/nm 692, 624, 365. IR (ATR) υ (cm^{-1}) 3396-3380; 2970; 1767; 1609; 1486; 774. Anal. Calc. for $C_{104}H_{112}N_{20}S_4O_{48}Na_{36}Zn$: C 36.40; H 3.29; N 8.16%, found: C 36.26; H 3.18; N 8.09%.

2.3. In vitro studies

2.3.1. Cell culture

In this study, the following cell lines were used: DLD-1 (human colorectal adenocarcinoma), HepG2 (human hepatocellular carcinoma) and L929 (mouse fibroblast). Cells were cultured in 25 cm^2 and 75 cm^2 flasks containing DMEM (low glucose) with 10% fetal bovine serum, 1% l-glutamine, 100 IU/mL penicillin and 10 mg/mL streptomycin.

2.3.2. Cytotoxicity and anticancer activity tests

Cytotoxicity and anticancer activity assays were performed using colorimetric XTT cell proliferation test [37,38]. The cells was cultivated in a flat 96-well culture plate ($10x10^4$ cells/well was seeded in the 100 µL culture medium). Each plate included a blank containing complete medium without cells. Compounds **3a** and **3b** were diluted to obtain five different concentrations ranging from 10^{-4} to 10^{-8} M. The plates were incubated in a humidified CO_2 incubator at 37°C throughout 24 h and 48 h. At the end of these incubation periods, **3a** and **3b** compounds were removed and the wells were washed with steryl PBS three times. 50 µl of the XTT solution was added to each well and the plate was incubated in an incubator for four hours. Wells were gently shaked to evenly distribute the dye and absorbance of the samples against a background control as a blank with ELISA reader at a wavelength of 450 nm was measured.

2.3.3. Statistical analysis

Differences in the mean values of the measured activities were statistically evaluated using the SPSS 17.0 program. (Univariate Variance Analyses and Pearson Correlation). Probability values of $p < 0.05$ were considered to be significant.

3. Results

3.1. Synthesis and characterization

The dendritic phthalocyanines were synthesized on the basis of Newkome's divergent-growth approach [23]. Synthesis of **1**-**3** compounds is summarized in Figure 1. The dendritic phthalocyanines **1a** and **1b** were synthesized by the reaction of tetrakis[(2-trimethylaminoethylsulfanyl)phthalocyanine]tetraiodide [36] or tetrakis[(2-trimethylaminoethylsulfanyl)phthalocyaninatozinc(II)]tetraiodide [36] with triethyl methanetricarboxylate sodium salt solution prepared by the reaction of Na metal and triethyl methanetricarboxylate in dry ethanol at room temperature. The dendritic phthalocyanines **2a** and **2b** were prepared by the reaction of $H_2NC(CH_2OH)_3$ with compound **1a** or **1b** in DMSO at the presence of K_2CO_3, respectively. The reaction of compound **2a** or **2b** with aqueous NaOH solution yielded water soluble dendritic metal-free and zinc(II) phthalocyanines **3a** and **3b**, respectively.

UV-vis, ^{1}H-NMR and IR spectra confirmed the structure of all compounds. Compounds **1a**, **1b**, **2a** and **2b** were soluble in DMF and DMSO while **3a** and **3b** were also water soluble.

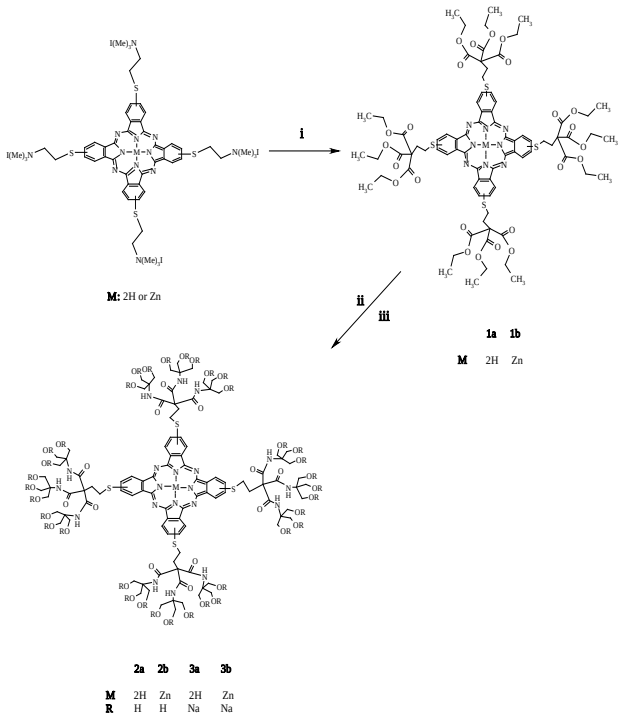
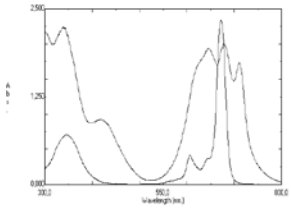


Figure 1. Synthesis of dendritic phthalocyanines **1**-**3**. Reagents; i) $NaC(CO_2C_2H_5)_3$, dry ethanol, 40% NaOH aqueous solution; ii) $H_2NC(CH_2OH)_3$, K_2CO_3, DMSO; iii) 30% NaOH aqueous solution.

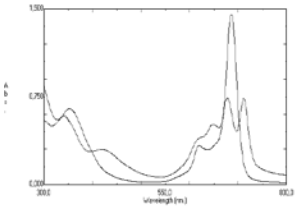
IR spectra of dendritic phthalocyanine compounds **1a**, **1b**, **2a**, **2b**, **3a** and **3b** are shown the peaks for C=O groups in ester substituents at 1755 cm^{-1}, 1759 cm^{-1}, 1761 cm^{-1}, 1764 cm^{-1}, 1765 cm^{-1} and 1767 cm^{-1}, respectively [34,39-42]. The vibration bands of –NH and –OH groups in tris substituents in compounds **2a** and **2b** appear as broad peaks between 3440-3381 cm^{-1} and 3448-3380 cm^{-1} [34,43,44]. The vibration band of the -OH group in the IR spectra of **2a** and **2b**, it disappeared after conversion to water soluble dendritic phthalocyanines **3a** and **3b**. The IR spectra of all compounds **1**-**3** showed peaks between at 1582-1609 cm^{-1} for aromatic C=C stretching vibration and at 1446-1486 cm^{-1} for the stretching vibration of C=N. A peak for the substituted benzene ring observed at 741-774 cm^{-1} for all compounds **1**-**3** [39,45].

^{1}H-NMR spectra of the all compounds **1-3** showed aromatic protons at 8.04-6.65 ppm as a broad multiplet and aliphatic protons at 3.45-2.38 ppm [40,42]. -NH protons of phthalocyanine ring cavity at metal free phthalocyanine compounds **1a**, **2a** and **3a** appeared at -4.70 ppm, -4.58 and -4.52 ppm broad singlet, respectively [45,46]. In addition, ^{1}H-NMR spectra of dendritic phthalocyanine compounds **2a**, **2b**, **3a** and **3b**, -NH groups in tris substituents were given at 8.71 ppm, 8.63 ppm, 8.66 ppm and 8.57 ppm broad singlet, respectively [40,43]. The -OH protons in ^{1}H-NMR spectra of dendritic phthalocyanine compounds **2a** and **2b** were observed at 5.92 ppm and 5.64 ppm as a broad singlet, respectively [44,47]. In the ^{1}H-NMR spectra of water soluble dendritic phthalocyanine compounds **3a** and **3b** were not observed to -OH proton peak, this result shows that all of the -OH groups in the compounds **2a** and **2b** have been hydrolyzed.

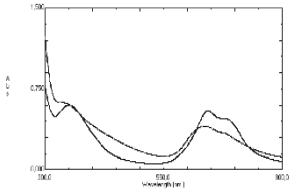
The UV-vis spectra of metal-free dendritic phthalocyanine compounds **1a**, **2a** and **3a** in DMSO showed two intense absorptions between 710 nm and 682 nm. The UV-vis spectra of dendritic zinc phthalocyanine compounds **1b**, **2b** and **3b** in DMSO give a peak at around 688 nm, 690 nm and 692 nm, respectively [48]. In addition, B-band of componds **1**-**3** appeared at between 342-365 nm (Figure 2).



(a) UV-vis spectra of compounds **1a** (--) and **1b** (—) in DMSO.



(b) UV-vis spectra of compounds **2a** (--) and **2b** (—) in DMSO.



(c) UV-vis spectra of compounds **3a** (--) and **3b** (—) in water.

Figure 2. UV-vis spectra of compounds **1**-**3**.

3.2. Cytotoxicity and anticancer activity of 3a and 3b

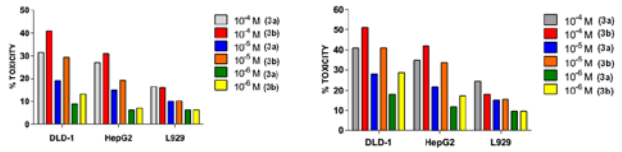
Cytotoxic effects of compounds were analysed in L929 fibroblasts by XTT assay performed in cultures exposed for 24h and 48h. Fibroblast cells were selected because they are widely distributed in many types of tissues. Especially, L929 cell line is recommended in many biological experiments such as material biocompatibility testing, drug cytotoxicity testing and cell biology studies. Moreover, the cells can be cultured easily and have a proper doubling time of 24 h. According to the results, water soluble dendritic phthalocyanines **3a** and **3b** showed low cytotoxicity at the three extreme concentrations (10^{-4}, 10^{-5} and 10^{-6} M). Furthermore, increasing incubation time did not induce further cytotoxic effects on L929 cell line dramatically.

Antitumor properties of **3a** and **3b** were tested in vitro against two human cancer cell lines (DLD-1 and HepG2) derived from colorectal and liver. According to the results, **3a** and **3b** showed great anticancer potency and were able to kill both DLD-1 and HepG2 cells at 10^{-4} M and 10^{-5} M. Furthermore, cell viability was found to decrease with incubation time.

Table 1. Anticancer activity of **3a** and **3b** as % cell viability after 24h and 48h.

	24 HOURS			48 HOURS		
	L929	**DLD-1**	**HepG2**	**L929**	**DLD-1**	**HepG2**
10^{-4} M 3a	83.48	68.59	73.00	75.53	59.05	65.00
10^{-4} M 3b	84.00	49.00	63.05	82.14	41.71	55.00
10^{-5} M 3a	90.00	71.00	84.97	85.00	72.00	78.22
10^{-5} M 3b	89.79	63.55	80.84	84.59	59.00	66.41
10^{-6} M 3a	93.75	91.12	93.79	90.38	82.12	88.24
10^{-6} M 3b	92.75	86.81	93.00	88.38	71.28	82.88

Especially, the strongest anticancer activity was found for **3b**, and also addition of 10^{-4} M **3b** led to 19,59% and 9,95% higher toxicity than 10^{-4} M **3a** for colorectal and liver cancer cells respectively after 24 h, and these ratios were determined as 17,34% and 10% after 48 h. Furthermore, 10^{-5} M **3b** caused 7,45% and 4,13%, 13% and 11,81% higher toxicity than 10^{-5} M **3a** for colorectal and liver cancer cells after 24 h and 48 h, respectively (Table 1) (Figure 3).



(a) **(b)**

Figure 3. % Toxicity observed in different cell lines of compounds **3a** and **3b (a)** after 24 hours of incubation **(b)** after 48 hours of incubation.

In current studies, anticancer activities of anionic metal-free **3a** and Zn(II) **3b** phthalocyanine dendrimers were evaluated. It has been observed that these compounds have potential for development as cancer drugs.

In our study, we observed antitumor activity of anionic phthalocyanine-based dendrimers in colorectal and liver cancer cells. The presence of phthalocyanine increased antitumor activity as well as participation of Zn in phthalocyanine core triggers this activity. Especially zinc phthalocyanine compounds are observed to be more effective in biomedical applications in the literature [49,50]. It is known that, dendrimers provide better opportunity for drug delivery due to their high functionality, low polydispersity, and three-dimensional architecture. Apart from these, dendrimers protect drugs from biological degradation and increase their stability [51,52]. Our novel metal-free **3a** and zinc(II) **3b** phthalocyanine dendrimers may offer new compounds for using site specific drug delivery.

4. Discussion and Conclusion

The water soluble dendritic metal-free and zinc(II) phthalocyanines were designed for highly soluble and stable agents for colorectal liver metastases treatment. We studied anticancer activity of water soluble dendritic phthalocyanines **3a** and **3b** in colorectal and liver cancer cell lines and also the cytotoxicity of the compounds in healthy fibroblast cell line. The designed compounds may use as anticancer agent to destroy cancer cells.

The presented results confirm that new water soluble dendritic metal-free **3a** and zinc phthalocyanines **3b** may be utilized for colorectal and liver metastases treatment. Moreover, these new compounds have great potential for further investigations and applications.

Acknowledgment

This work was funded through a seed grant from the Turkish National Academy of Sciences (TUBA GEBIP 2008-29 for YT).

References

[1] Ece, E., Kilickap, S., Özgür, A., Tutar, Y. 2015. Treatment of Colorectal Cancer Liver Metastases: Clinical and Molecular Aspects. OMICS Group, USA.

[2] Misiakos, E. P., Karidis, N. P., Kouraklis,G. 2011. Current treatment for colorectal liver metastases. World Journal of Gastroenterology, 17(2011), 4067-4075.

[3] Newkome, G. R., Moorefield, C. N., Vogtle, F. 2002. Dendrimers and Dendrons. Witey-VCH Verlag GmbH.

[4] Vögtle, F., Richardt, G., Werner N. 2009. Dendrimer Chemistry. Witey-VCH Verlag GmbH.

[5] Scheirs, J. 2001. Dendrimers and Other Dendritic Polymers. John Wiley & Sons.

[6] Zhang, Y., Thomas, T. P., Lee, K. H., Li, Zong, M. H., Desai, A. M., Kotlyar, A., Huang, B., Holl, M. M. B., Baker Jr., J. R. 2011. Polyvalent saccharide-functionalized generation 3 poly(amidoamine)dendrimer-methotrexate conjugate as a potential anticancer agent. Bioorganic& Medicinal Chemistry, 19(2011), 2557-2564.

[7] Johansson, E. M. V., Dubois, J., Darbre, T., Reymond, J. L. 2010. Glycopeptide dendrimer colchicine conjugates targeting cancer cells. Bioorganic&Medicinal Chemistry, 18(2010), 6589-6597.

[8] Abdel-Rahman, M. A., Al-Abd, A. M. 2013. Thermoresponsive dendrimers based on oligoethylene glycols: Design, synthesis and cytotoxic activity against MCF-7 breast cancer

cells. European Journal of Medicinal Chemistry, 69(2013), 848-854.

[9] Govender, P., Edafe, F., Makhubela, B. C. E., Dyson, P. J., Therrien, B., Smith, G. S. 2014. Neutral and cationic osmium(II)-arene metallodendrimers: Synthesis, characterisation and anticancer activity. Inorganica Chimica Acta, 409(2014), 112-120.

[10] Murugan, E., Geetha Rani, D. P., Yogaraj, V. 2014. Drug delivery investigations of quaternised poly(propylene imine) dendrimer using nimesulide as a model drug. Colloids and Surfaces B, 114(2014), 121-129.

[11] Cortez-Maya, S., Hernández-Ortega, S., Ramírez-Apan, T., Lijanova, I. V., Martínez-García, M. 2012. Synthesis of 5-aryl-1,4-benzodiazepine derivatives attached in resorcinaren-PAMAM dendrimers and their anti-cancer activity. Bioorganic& Medicinal Chemistry, 20(2012), 415-421.

[12] Sharma, A., Gautam, S. P., Gupta, A. K. 2011. Surface modified dendrimers: synthesis and characterization for cancer targeted drug delivery. Bioorganic& Medicinal Chemistry, 19(2011), 3341-3346.

[13] Kesharwani, P., Jain, K., Jain, N. K. 2014. Dendrimer as nanocarrier for drug delivery. Progress in Polymer Science, 39(2014), 268-307.

[14] Leznoff, C. C., Lever, A. B. P. 1989-1996. Phthalocyanines Properties and Applications. Vol.1-4, VCH Publisher.

[15] Bekaroğlu, Ö. 1996. Phthalocyanines Containing Macrocycles. Applied Organometalic Chemistry, 10(1996), 605-613.

[16] Jiang, Z., Shao, J., Yang, T., Wang, J., Jia, L. 2014. Pharmaceutical development, composition and quantitative analysis of phthalocyanine as the photosensitizer for cancer photodynamic therapy. Journal of Pharmaceutical and Biomedical Analysis, 87(2014), 98-104.

[17] Nyokong, T., Antunes, E. 2013. Influence of nanoparticle materials on the photophysical behavior of phthalocyanines. Coordination Chemistry Reviews, 257(2013), 2401-2418.

[18] Bonnet, R. 1995. Photosensitizers of the porphyrin and phthalocyanine series for photodynamic therapy. Chemical Society Reviews, 24(1995), 19-33.

[19] Miller, J. D., Baron, E. D., Scull, H., Hsia, A., Berlin, J. C., McCormick, T., Colussi, V., Kenney, M. E., Cooper, K. D., Oleinick, N. L. 2007. Photodynamic therapy with the phthalocyanine photosensitizer Pc4: the case experience with preclinical mechanistic and early clinical-translational studies. Toxicology Applied Pharmacology, 224(2007), 290-299.

[20] Li, W. S., Aida, T. 2009. Dendrimer porphyrins and phthalocyanines. Chemical Reviews, 109(2009), 6047- 6076.

[21] Figueira, F., Pereira, P. M. R., Silva, S., Cavaleiro, J. A. S., Tomé, J. P. C. 2014. Porphyrins and Phthalocyanines decorated with dendrimers: Synthesis and biomedical applications. Current Organic Synthesis, 11(2014), 110-126.

[22] Sakamoto, K., Kanazawa, S. 2011. U.S. Patent 8, 030, 342.

[23] Kimura, M., Nakada, K., Yamaguchi, Y., Hanabusa, K., Shirai, H., Kobayashi, N. 1997. Dendritic metallophthalocyanines: Synthesis and Characterization of a zinc(II) phthalocyanine[8]^3-arborol. Chemical Communications, 13(1997), 1215-1216.

[24] Kobayashi, N. 1999. Phthalocyanines. Current Opinion in Solid State and Materials Science, 4(1999), 345-353.

[25] Dumoulin, F., Durmuş, M., Ahsen, V., Nyokong, T. 2010. Synthetic pathways to water-soluble phthalocyanines and close analogs. Coordination Chemical Reviews, 254(2010), 2792-2847.

[26] Ongarora, B. G. 2012. Syntheses and characterization of Water-Soluble Phthalocyanines For Diagnosis and treatment of cancer, Moi University, PhD, Uasin Gishu County, Kenya.

[27] Nishida, M., Momotake, A., Shinohara, Y., Nishimura, Y., Arai, T. 2007. Synthesis and photophysical properties of water-soluble dendrimers bearing a phthalocyanine core. Journal of Porphyrins and Phthalocyanines, 11(2007), 448-454.

[28] Peng, Y., Zhang, H., Wu, H., Huang, B., Gan, L., Chen, Z. 2010. The synthesis and photophysical properties of zinc (II) phthalocyanine bearing poly(aryl benzyl ether) dendritic substituents. Dyes and Pigments, 87(2010), 10-16.

[29] Ng, D. K. P. 2003. Dendritic phthalocyanines: synthesis, photophysical properties, and aggregation behavior. Chimie, 6(2003), 903-910.

[30] Li, Xi-you, He, X., Ng, A. C. H., Wu, C., Ng, D.K.P. 2000. Influence of Surfactants on the Aggregation Behavior of Water-Soluble Dendritic Phthalocyanines. Macromolecules, 33(2000), 2119-2123.

[31] Hahn, U., Torres, T., Amphiphilic zinc phthalocyanine dendrimers by the Click Chemistry approach. Journal of Porphyrins and Phthalocyanines, 15(2011), 364-372.

[32] Hahn, U., Engmann, S., Oelsner, C., Ehli, C., Guldi, D. M., Torres, T. 2010. Immobilizing Water-Soluble Dendritic Electron Donors and Electron Acceptors-Phthalocyanines and

Perylenediimides-onto Single Wall Carbon Nanotubes. Journal of American Chemical Society, 132(2010), 6392-6401.

[33] He, D., Peng, Y., Yang, H., Ma, D., Wang, Y., Chen, K., Chen, P., Shi, J. 2013. Single-wall carbon nanotubes covalently linked with zinc (II) phthalocyanine bearing poly (aryl benzyl ether) dendritic substituents: Synthesis, characterization and photoinduced electron transfer. Dyes and Pigments, 99(2013), 395-401.

[34] Sülü, M., Altındal, A., Bekaroğlu, Ö. 2005. Synthesis, characterization and electrical and CO_2 sensing properties of triazine containing three dendritic phthalocyanine. Synthetic Metals, 155(2005), 211-221.

[35] Armarego, W. L. F., Chai, C. L. L. 2003. Purificaiton of Laboratory Chemicals, 5 third ed., Butterworth/Heinemann, Tokyo.

[36] Dabak, S., Gümüş, G., Gül, A., Bekaroğlu, Ö. 1996. Synthesis and properties of new phthalocyanines with tertiary or quaternarized aminoethylsulfanyl substituents. Journal of Coordination Chemistry, 38(1996), 287-293.

[37] Koca, İ., Özgür, A., Coşkun, K.A., Tutar, Y. 2013. Synthesis and anticancer activity of acyl thioureas bearing pyrazole moiety. Bioorganic&Medicinal Chemistry, 21(2013), 3859-3865.

[38] Özgür, A., Yenidunya, E., Koca, İ., Tutar, Y. 2015. Acyl Thiourea Derivatives Containing Pyrazole Ring Selective Targeting of Human Aurora Kinases in Breast and Bone Cancer. Letters Drug Design Discovery, 12(2015), 180-189.

[39] Ağırtaş, M. S., Çelebi, M., Gümüş, S., Özdemir, S., Okumuş, V. 2013. New water soluble phenoxy phenyl diazenyl benzoic acid substituted phthalocyanine derivatives: Synthesis, antioxidant activities, atypical aggregation behavior and electronic properties. Dyes and Pigments, 99(2013), 423-431.

[40] Kimura, M., Sugihara, Y., Muto, T., Hanabusa, K., Shirai, H., Kobayashi, N. 1999. Dendritic Metallophthalocyanines-Synthesis, Electrochemical Properties, and Catalytic Activities. Chemistry-A European Journal, 5(1999), 3495-3500.

[41] Newkome, G. R., Yao, Z., Baker, G. R., Gupta, V. K., Russo, P. S., Saunders, M. J. 1986. Chemistry of micelles series. Part 2. Cascade molecules. Synthesis and characterization of a benzene [9] 3-arborol. Journal of American Chemical Society, 108(1986), 849-850.

[42] Jang, W. D., Nakagishi, Y., Nishiyama, N., Kawauchi, S., Morimoto, Y., Kikuchi, M., Kataoka, K. 2006. Polyion complex micelles for photodynamic therapy: Incorporation of dendritic photosensitizer excitable at long wavelength relevant to improved tissue-penetrating property. Journal of Controlled Release, 113(2006), 73-79.

[43] Cardona, C. M., McCarley, T. D., Kaifer, A. E. 2000. Synthesis, electrochemistry, and interactions with beta-cydodextrin of dendrimers containing a single ferrocene subunit located "off-center. Journal of Organic Chemistry, 65(2000), 1857-1864.

[44] Ihre, H., Omayra L De Jesus, P., Frechet, J. M. J. 2001. Fast and convenient divergent synthesis of aliphatic ester dendrimers by anhydride coupling. Journal of American Chemical Society, 123(2001), 5908-5917.

[45] Yabaş, E., Sülü, M., Saydam, S., Dumludağ, F., Salih, B., Bekaroğlu, Ö. 2011. Synthesis, characterization and investigation of electrical and electrochemical properties of imidazole substituted phthalocyanines. Inorganica Chimica Acta, 365(2011), 340-348.

[46] Youngblood, W. J. 2006. Synthesis of a new trans-A2B2 phthalocyanine motif as a building block for rodlike phthalocyanine polymers. Journal of Organic Chemistry, 71(2006), 3345-3356.

[47] Joralemon, M. J., O'Reilly, R. K., Matson, J. B., Nugent, A. K., Hawker, C. J., Wooley, K. L. 2005. Dendrimers Clicked Together Divergently. Macromolecules, 38(2005), 5436-5443.

[48] Nyokong, T. 2010. Electronic spectral and electrochemical behavior of near infrared absorbing metallophthalocyanines. Structure and Bonding, 135(2010), 45-88.

[49] Portilho, F. A., Cláudio, C. E. O., Miranda-Vilela, A. L., Estevanato, L. L. C., Longo, J. P. F., Santos, M. F. M. A., Boca, A. L., Martins, O. P., Simioni, A. R., Morais, P. C., Azevedo, R. B., Tedesco, A. C., Lacava, Z. G. M. 2013. Antitumor activity of photodynamic therapy performed with nanospheres containing zinc-phthalocyanine, Journal of Nanobiotechnology, 11(2013), 41-55.

[50] Setaro, F., Ruiz-González, R., Nonell, S., Hahn, U., Torres, T. 2014. Synthesis, photophysical studies and $^1O_2$ generation of carboxylate-terminated zinc phthalocyanine dendrimers. Journal of Inorganic Biochemistry, 136(2014), 170-176.

[51] Spataro, G., Malecaze, F., Turrin, C. O., Soler, V., Duhayon, C., Elena, P. P., Majoral, J. P., Caminade, A. M. 2010. Designing dendrimers for ocular drug delivery, European Journal of Medicinal Chemistry, 48(2010), 326-334.

[52] Cheng, Y., Wang, J., Rao, T., He, X., Xu, T. 2008. Phermacautial applications of dendrimers: promising nanocarriers for drug delivery. Frontiers in Bioscience, 13(2008), 1447-1471.