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Synthesis of Multifunctional Organic Nanoparticles Combining Photodynamic Therapy and Chemotherapeutic Drug Release

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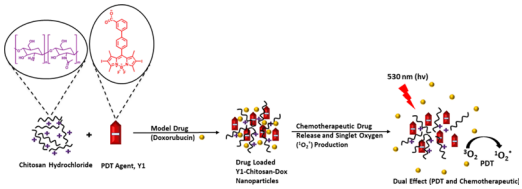
Abstract: Cancer is a group of diseases that are caused by uncontrolled proliferation of cells in various parts of the body and it is one of the most studied diseases worldwide. Photodynamic therapy (PDT) is a treatment that uses photosensitizers called photosensitizing agents in addition to light to kill cancer cells. Photosensitizers work only after they have been activated by certain types of light. PDT contains three basic components, these are a photosensitizer, visible light and molecular oxygen ($^3O_2$). The efficiency of the therapeutic action is directly related to the property of the photosensitizer. In this study, chitosan and BODIPY based nanoparticles that were capable of carrying out drug delivery and producing singlet oxygen ($^1O_2$) were synthesized for the first time. For this purpose, organic nanoparticles showed PDT feature were synthesized via the formation of ionic complexes formed with opposite charged ionic interactions between the synthesized BODIPY derivative (PDT agent) and chitosan hydrochloride at appropriate pH (pH=6). Later, during the formation of this ionic complex, a chemotherapeutic model drug (Doxorubicin) was added to the medium and chemotherapeutic drug-loaded chitosan and BODIPY-based nanoparticles were synthesized. Finally, while drug-free (**Y1**-Chitosan nanoparticles) and drug-loaded organic nanoparticles (**Y1**-Chitosan-Dox nanoparticles) showed very good PDT properties, they were found to be effective on MCF7 cancer cells and less toxic to L929 cells.



Keywords: photodynamic therapy, chitosan, BODIPY, doxorubicin, drug release.

1. Introduction

Cancer, which is one of the diseases that cause the most deaths in the world, is one of the diseases on which most research is conducted today. In many cases diagnosed at advanced stage clinical chemotherapy treatment is almost indispensable. In available cancer treatment, a combination of chemotherapy, radiotherapy, and surgical intervention is generally used. In many cases, the preferred treatment method varies according to the type and location of the cancer, but there are many cases where these treatment methods are insufficient.[1-4]

Chitosan nanoparticles have attracted considerable attention in drug delivery. The main reasons for this, their drug protection capabilities from degradation, their high drug loading capabilities and their slow and permanent drug delivery capacities can be considered.[5-7] In addition, the cationic property of chitosan allows the ionic cross binding of chitosan with the anions and causes easily approaching to the cell membranes. Furthermore, the mucosa adhesion feature prolongs the retention on the target surfaces. Chitosan nanoparticles are synthesized from a natural chitosan polymer showing biocompatible and biodegradable and containing acetylated and deacetylated units. Chitosan nanoparticles have shown promising results for the treatment of diabetes and cancer.[8-13]

Doxorubicin, selected as a model chemotherapeutic drug in this study, is a chemotherapy drug and is used to treat many types of cancer. However, its clinical use is limited with its severe adverse effects such as congestive heart failure and low solubility. Doxil is the first clinically approved nanoparticle-based drug delivery system. Moreover, clinical trials of broad classes of nanomaterials including dendrimers, polymers and metallic nanoparticles are still ongoing. Doxorubicin can also be used in combination with other chemotherapy drugs.[14-16]

BODIPY compounds are important candidates for chemosensor, laser dye, photodynamic agent, and solar cell applications depending on the substituted groups.[17-19] Furthermore, the easy manufacturing process, low cost and good stability make BODIPY derivatives ideal agents for PDT. In addition, BODIPY derivatives are widely used in the synthesis of various systems that transfer energy and harvest light due to their known good spectral properties. One of the important reasons for the widespread use of BODIPY compounds is that the BODIPY framework can be easily functionalized from various positions, depending on the properties of the reagents and reaction conditions.[20-22]

In the current study, chitosan and BODIPY based nanoparti-

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cles, which were capable of carrying out drug delivery and producing singlet oxygen were synthesized for the first time. For this purpose, organic nanoparticles showed PDT feature were synthesized via the formation of ionic complexes formed with opposite charged ionic interactions between the synthesized BODIPY derivative **Y1** (PDT agent) and chitosan hydrochloride at appropriate pH (pH=6). Finally, the synthesis of chitosan and BODIPY based and chemotherapeutic drug loaded dual effect organic nanoparticles with the addition of chemotherapeutic model drug (doxorubicin) during the formation of these ionic complexes was aimed. Therefore, organic nanoparticles that had synergistic effect of the PDT and controlled drug delivery and contain chitosan as a polymer that can approach to the cells and show adhesive property to the mucosa were synthesized for the first time. Additionally, the effects of organic nanoparticles on the MCF-7 breast cancer cells and L929 normal cells were investigated.

2. Experimental

2.1. Synthesis of compound 3

To a deoxygenated solution of aldehyde compound, (0.88 mmol, 200 mg) and 2,4-dimethylpyrrole (1.94 mmol, 184 mg) in CH_2Cl_2 (500 mL) and one drop of TFA was added and the mixture was stirred overnight. The red solution was treated with DDQ (0.88 mmol, 199.76 mg), stirred for 30 min, then 5 mL of Et_3N and 3 mL of $BF_3{\cdot}Et_2O$ were added, and the mixture was stirred at r.t. for further 40 min. The organic phase was separated, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography using etylacetate/hexanes (5:1, v/v) (28%) (see Supplementary Figure S1).

2.2. Synthesis of compound Y1

In a 250 mL round-bottom flask, compound **3** (0.122 mmol, 54 mg) and I_2 (0.244 mmol, 69.25 mg) were dissolved in ethanol (100 mL). Iodic acid, HIO_3 (0.244 mmol, 42.92 mg) was dissolved in a few drops of water and was added into the previous solution. The reaction mixture was stirred at r.t. When the reaction was completed, saturated sodium thiosulfate solution was added (50 mL) and extracted with dichloromethane. The organic phase was then extracted twice more with water. Combined organic phases were dried with Na_2SO_4 and evaporated under reduced pressure. The final product was purified by silica gel column chromatography using etylacetate/hexanes (5:1, v/v) (45%) (see Supplementary Figure S4).

2.3. Synthesis of Y1-chitosan nanoparticles with PDT effect

First, 2.9 mg of chitosan hydrochloride was dissolved in 9 mL of water (0.32 mg/mL), and 1 mL (3.1 mg/mL) of **Y1**'s solution in THF was added. The pH of the mixture was determined to be 4-5. Then the pH of the mixture was adjusted to 6 with NaOH solution. The formation of very fine particles was observed (Figure 1). The mixture was stirred at r.t. for a further 2 hours. After the mixture was centrifuged at 10,000 rpm for 1 minute, the upper part was removed by decantation. Ultra-pure water was added to the remaining precipitate and kept in a sonic bath for 5 min. It was centrifuged again. This process was repeated two more times. The pink colored precipitate was dried in vacuum and stored in the refrigerator.

2.4. Synthesis of drug-loaded Y1-chitosan-dox nanoparticles

For the synthesis of drug-loaded **Y1**-Chitosan-Dox nanoparticles, 2.9 mg of chitosan hydrochloride was dissolved in 9 mL of water (0.32 mg/mL), and 2 mg of doxorubicin hydrochloride solution in 300 μL methanol (6.67 mg/mL) was added dropwise. Then, 1 mL of compound **Y1** solution in THF (0.40 mg/mL) was added to this mixture. The pH of the mixture was determined to be 4-5. Then the pH of the mixture was adjusted to 6 with NaOH solution. The mixture turned from pink to purple (see Supplementary Figure S9). Very fine precipitate formation was observed. The mixture was then continued to stir for another two hours at room temperature. After the mixture was centrifuged at 10000 rpm for 1 minute, the upper part was removed by decantation. Ultra-pure water was added to the remaining precipitate and kept in a sonic bath for 5 minutes. It was centrifuged again. This process was repeated two more times. The purple colored precipitate was dried in vacuum and stored in the refrigerator.

2.5. *In-vitro* drug release of Y1-chitosan-dox nanoparticles

For drug release experiments *in-vitro*, 10 mL of Doxorubicin loaded **Y1**-Chitosan-Dox Nanoparticles solution was placed into dialysis membrane (MW cutoff 3500) and dialyzed against 250 mL of ultrapure water at 37 ℃. Aliquots of 3.0 mL were withdrawn from the solution periodically. The volume of solution was maintained constant by adding 3.0 mL of ultrapure water after each sampling. The amount of Doxorubicin released from **Y1**-Chitosan-Dox Nanoparticles was measured using UV absorbance at 480 nm (Figure 8).

2.6. Determination of PDT property of Y1-chitosan nanoparticles

For the determination of PDT property of **Y1**-Chitosan nanoparticles, 1,3-Diphenylisobenzofuran (DPBF) was used as a singlet oxygen trap molecule. In a typical procedure used 530 nm LED lamp as a light source, **Y1**-Chitosan nanoparticles and trap molecule were mixed in THF. Before LED irradiation, initially, several dark measurements were taken. Subsequently, the THF solution was exposed to 530 nm LED light at various times. Absorbance decrease of trap molecule was monitored suggesting singlet oxygen generation in the presence of light.

2.7. Determination of PDT property of drug loaded Y1-chitosan-dox nanoparticles

For the determination of PDT property of **Y1**-Chitosan-Dox nanoparticles, 1,3-Diphenylisobenzofuran (DPBF) was used as a singlet oxygen trap molecule. In a typical procedure used 530

nm LED lamp as a light source, **Y1**-Chitosan-Dox nanoparticles and trap molecule were mixed in THF. Before LED irradiation, initially, several dark measurements were taken. Subsequently, the THF solution was exposed to 530 nm LED light at various times. Absorbance decrease of trap molecule was monitored suggesting singlet oxygen generation in the presence of light.

2.8. Cell lines and cell culture

Human breast cancer cells MCF-7 (HTB-22) and mouse fibroblast cells L929 (CRL-6364) were obtained from American Type Culture Collection (ATCC, USA). MCF-7 and L929 cells were grown in DMEM (Gibco) and it was supplemented with 10% Fetal Bovine Serum (FBS) (Sigma- Aldrich) and 1% penicillin/streptomycin (Gibco, Thermo Fisher Scientific) antibiotic mixtures. Cells were cultured at 37 ℃ within 5% CO_2 humidified atmosphere. Doxorubicin (Sigma-Aldrich), **Y1**, **Y1**-Chitosan Nanoparticles and **Y1**-Chitosan-Dox nanoparticles were suspended in ultrapure water (pH=6) and it was further diluted with DMEM prior to treatment with a final percentage of DMSO less than 0.1%. The untreated cells were also exposed to DMEM containing 0.1% DMSO.

2.9. Cytotoxicity assay

The effect of different concentrations of Doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles on MCF-7 and L929 cell viability (with or without LED application) was determined the XTT (2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) test (Roche). The cells were seeded in 96-well plates at a density of 1×10^4 cells per well in 100-μL DMEM culture media and incubated overnight before treatment. The cells were then treated with Doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles at different concentrations (20, 10, 5, 2.5, 1 μg/mL) for 24 h. After incubation, the media containing different concentrations of Doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles, and **Y1**-Chitosan-Dox nanoparticles were removed, the wells were washed twice with PBS and 100 μL of fresh DMEM was added to the wells. After LED application ((530 nm LED (3 min.)), the cells were incubated for another 24 h. The media containing different concentrations of Doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles, and **Y1**-Chitosan-Dox nanoparticles was then removed, the wells were washed twice with PBS and 100 μL of colorless DMEM plus 50 μL of XTT mixture solution was added to each well and incubated for 4 h. Finally, the absorbance was determined using an ELISA microplate reader (Thermo) at 450 nm and the cell viability was evaluated as a viable cell amount percentage compared to control, as untreated cells.

2.10. Immunofluorescent assay

For caspase 3 immunofluorescence staining, cells were grown on sterile coverslips in 6-well culture dishes. After 24 hours, Doxorubicin, **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles were administered to the cells (with 530 nm LED (3 min.) or without LED application). Cells were purified from the medium and washed with phosphate-buffered saline (PBS) (Sigma Aldrich, Germany). Washed cells were fixed in paraformaldehyde (pH 7.4) (Merk, Germany) for 10 min. at room temperature. After fixation, washed it 3 times with cold PBS. Subsequently, the sodium citrate buffer (pH 6.5) heated at 90 ℃ was treated for 10 min. (Sigma Aldrich, Germany) to reveal the tissue antigen. Cells were washed 2 times with PBS for 5 min. For permeability, cells were incubated in 0.1% Triton™ X-100 (Sigma Aldrich, Germany) solution for 10 min. The PBS was then washed for 5 min. After washing, Ultra V Block (Thermo Scientific, PBQ180830, USA) was dropped and left at room temperature for 30 min. to avoid non-specific binding. Cells were then incubated with Rabbit Polyclonal Caspase 3 CPP32 (Thermo Scientific, USA) primary antibody at 36 ℃ in the dark. It was kept at + 4 ℃ overnight. At the end of the period, it was washed twice with PBS for 5 min. Secondary antibodies to Goat Anti-Mouse IgG H & L antibody (Alexa Fluor® 488) (ab150113) (Abcam, USA) were used for the rabbit Polyclonal Caspase 3 primary antibody. Secondary antibodies were diluted to 1:200 with antibody diluent reagent (Invitrogen, USA) and applied to cells for 1 h. in a humidity chamber. It was then stained with 0.1 μg/mL 4'6-Diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma Aldrich, Germany), and after washing with PBS, the coverslips from the culture dishes were removed and inverted on the slide. Fluorescence microscopy (Olympus BX51, Japan) was studied using filters suitable for fluorescence examination followed by a recording.

3. Results and discussion

3.1. Synthesis and characterization of PDT agent Y1, Y1-chitosan nanoparticles and drug loaded Y1-chitosan-dox nanoparticles

Before the synthesis of **Y1**-chitosan nanoparticles, the synthesis of PDT agent **Y1** was performed. For this purpose, a BODIPY compound (**3**) was synthesized from the synthesis of 4′-Formylbiphenyl-3-carboxylic acid and 2,4-dimethylpyrolle, and then this compound was iodinated to obtain PDT agent **Y1** (see Supplementary Figure S1 and S4). After this stage, the synthesis of chitosan nanoparticles was started. Chitosan carries cationic amino groups in its structure at low pH (pH < 6.5), on the other hand **Y1** compound is a carboxylic acid derivate and it should be deprotonated at this pH value, because of pK_a of benzoic acid (pK_a=4.20).[23] So, **Y1**-Chitosan nanoparticles were synthesized from BODIPY derivative **Y1** (PDT agent) and chitosan hydrochloride at the appropriate pH (pH=6) by the formation of an ionic complex due to the opposite charged ionic interaction between them. Thus, during the synthesis of **Y1**-Chitosan nanoparticles, the formation of these nanoparticles was immediately observed by adjusting the pH to 6 (Figure 1 and Figure 2).

The size distribution of **Y1**-Chitosan nanoparticles was measured from dynamic light scattering (DLS) analysis and SEM analysis with average diameters of 375.8 nm. Additionally, their zeta potentials have been found as 10.7 mV (pH=6.0) (Figure 3 and see Supplementary Figure S7).

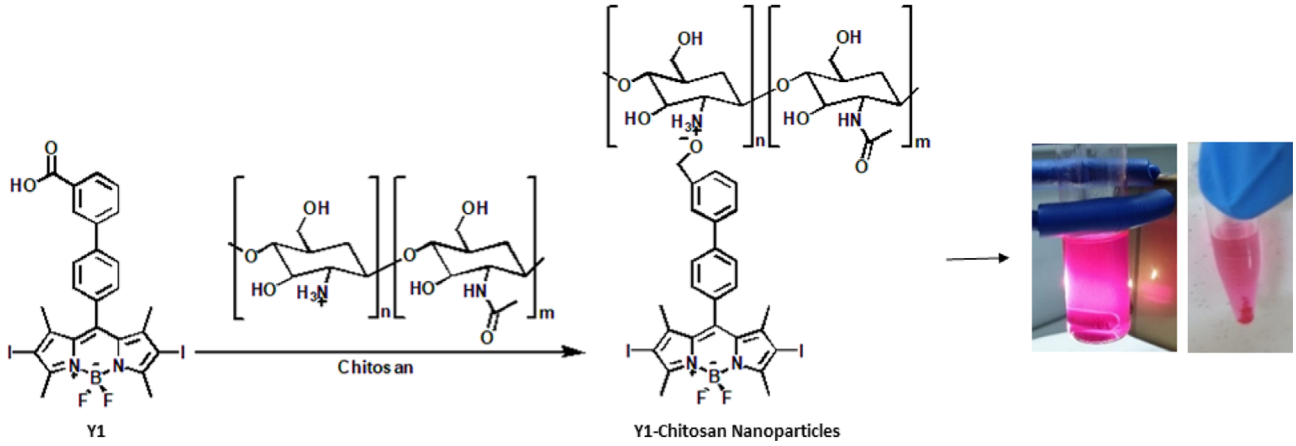

Figure 1. Synthesis of **Y1**-Chitosan Nanoparticles.

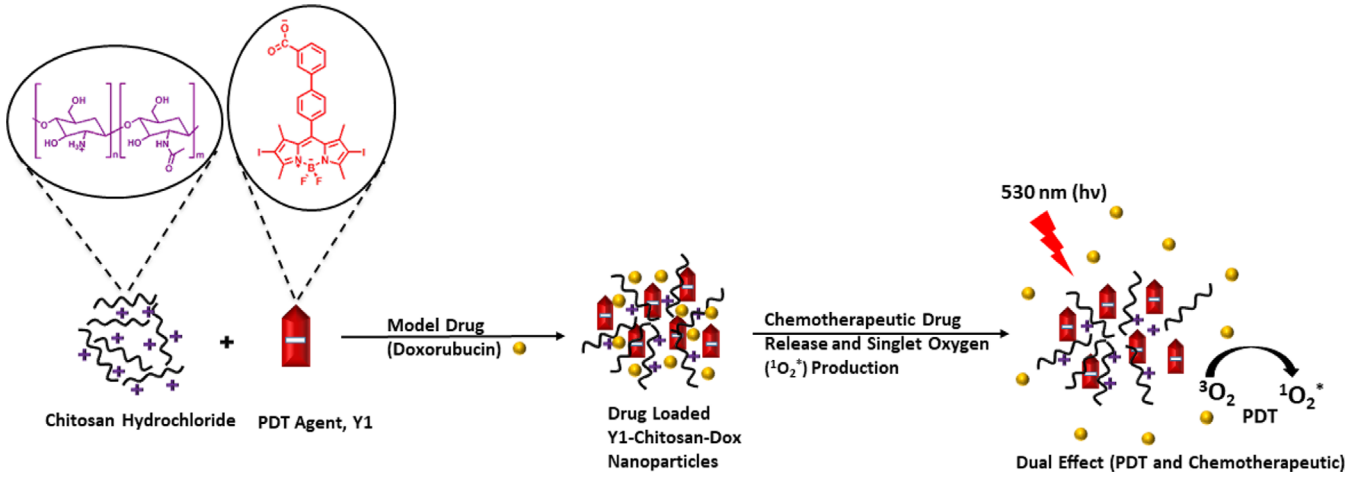

Figure 2. Synthesis and working principle of **Y1**-Chitosan-Dox nanoparticles.

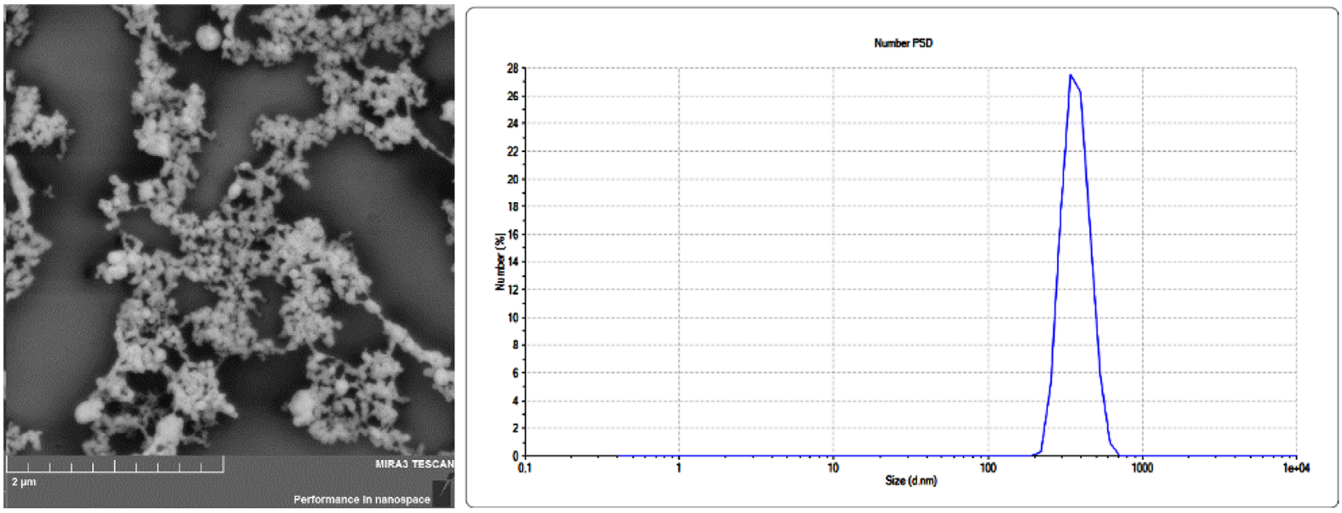

Figure 3. The SEM image of **Y1**-Chitosan nanoparticles and the size distribution of **Y1**-Chitosan nanoparticles.

Later, as a model drug, chitosan nanoparticles loaded with Doxorubicin were synthesized. During this synthesis, when the pH of the medium was adjusted to 6, the color of the solution changed from pink to purple (see Supplementary Figure S9). The size distribution of drug loaded **Y1**-Chitosan-Dox nanoparticles was measured from dynamic light scattering (DLS) analysis and SEM analysis with average diameters of 375.4 nm. In addition, their zeta potentials have been found as 25.7 mV (pH=6.0)

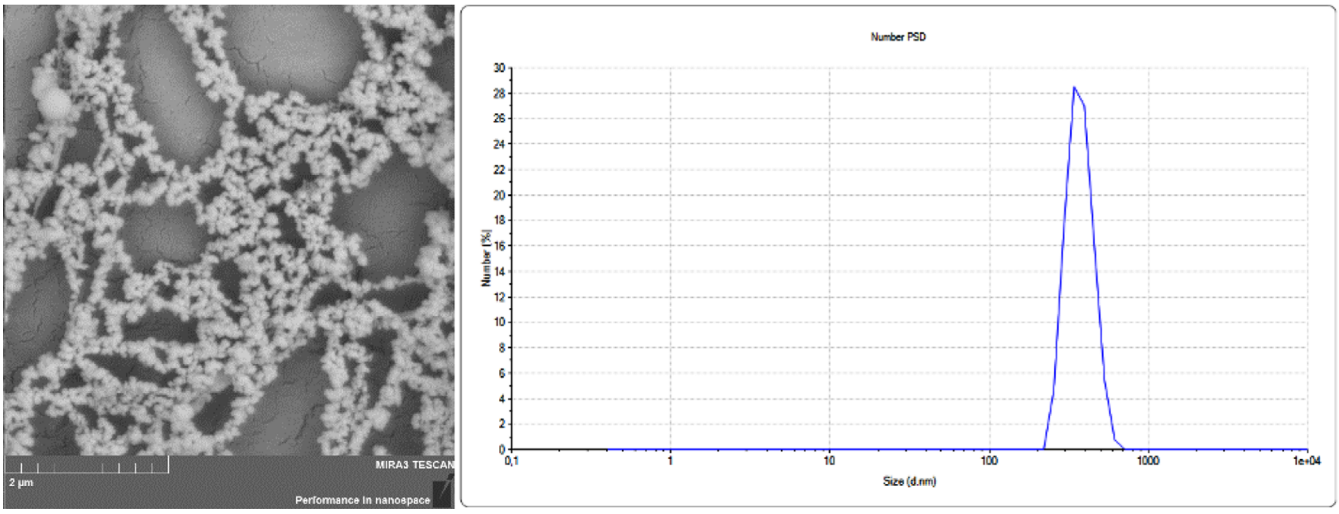

Figure 4. The SEM image of **Y1**-Chitosan-Dox nanoparticles and the size distribution of **Y1**-Chitosan-Dox nanoparticles.

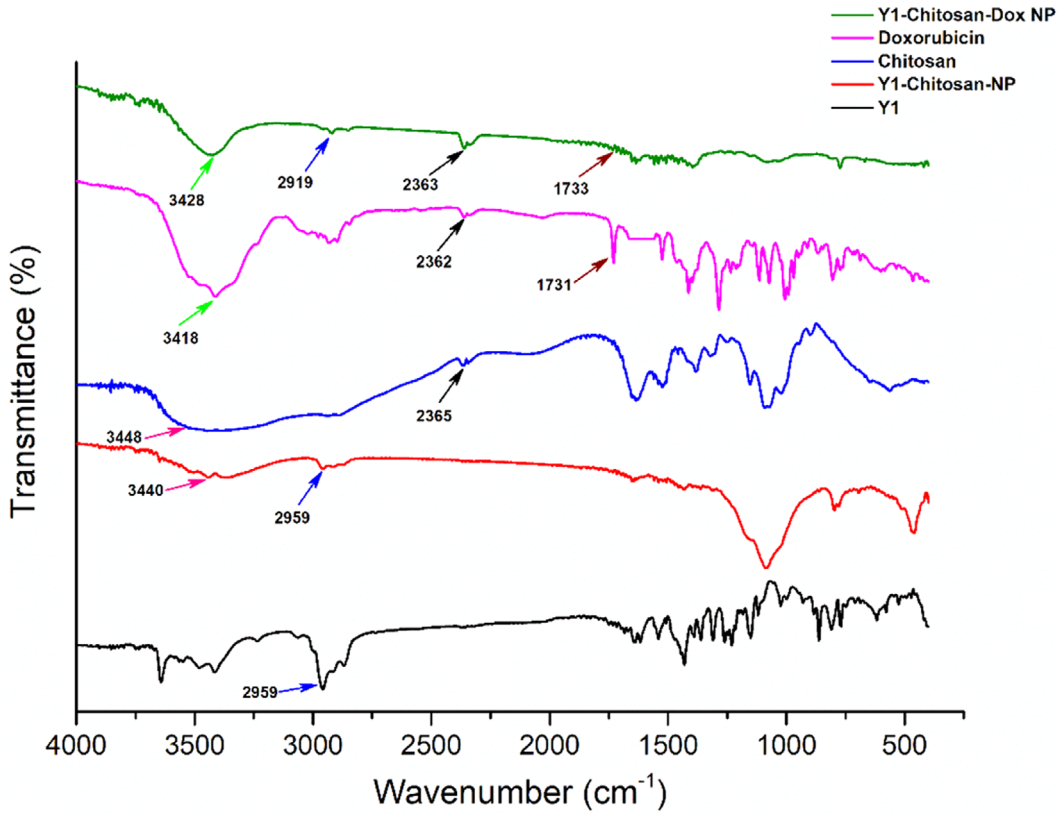

Figure 5. FTIR spectra of **Y1**, **Y1**-Chitosan nanoparticles, Chitosan, Doxorubicin and **Y1**-Chitosan-Dox nanoparticles.

(Figure 4 and see Supplementary Figure S10).

The FTIR spectra of Chitosan, Doxorubicin, **Y1**, **Y1**-Chitosan Nanoparticles and Drug Loaded **Y1**-Chitosan-Dox Nanoparticles are shown in Figure 5. Accordingly, at 2959 cm^{-1} there is a –CH stretching vibration of **Y1** compound. It is understood that **Y1**-Chitosan nanoparticles also have -CH stretching vibration at the same place, i.e. at 2959 cm^{-1}. The peak of -CH stretching vibration of **Y1**-Chitosan-Dox nanoparticles shifted slightly to 2919 cm^{-1}. It is also seen that the -OH stretching peak of the pure Doxorubicin compound is at 3418 cm^{-1}, this peak shifted to 3428 cm^{-1} in **Y1**-Chitosan-Dox nanoparticles. Moreover, the peaks of $C=NH^{+}$ charged amines of Doxorubicin and **Y1**-Chitosan-Dox nanoparticles are at 2362 cm^{-1} and 2363 cm^{-1}, respectively. Also, the peaks of carbonyl group (-C=O) stretching vibration of pure Doxorubicin and **Y1**-Chitosan-Dox nanoparticles are at 1731 cm^{-1} and 1733 cm^{-1}, respectively.

3.2. PDT Property of Y1-Chitosan Nanoparticles

UV-vis spectra of **Y1** and **Y1**-Chitosan nanoparticles are given in the supporting information (see Supplementary Figure S8). To assess the PDT properties of **Y1**-Chitosan nanoparticles,

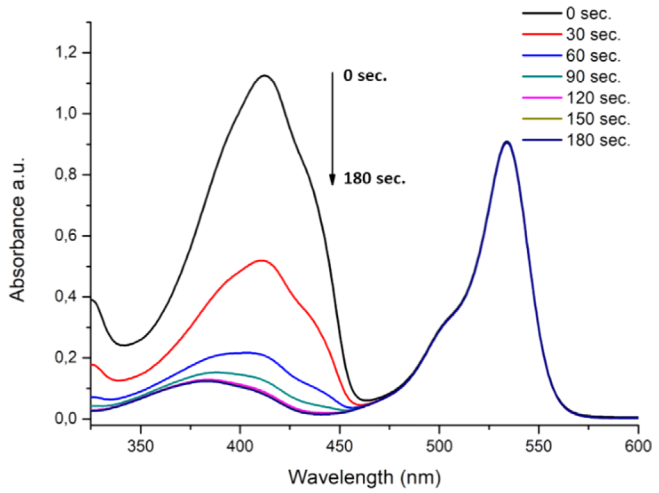

Figure 6. Decrease in the absorbance of DPBF in the presence of 0.13 mg/mL, **Y1**-Chitosan nanoparticles (total volume 3000 μL) at 416 nm in THF after irradiated with 530 nm LED.

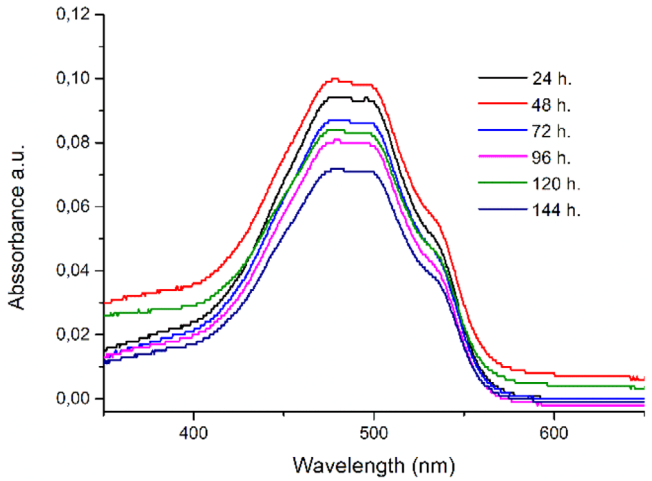

Figure 8. The absorbance spectrum for Doxorubicin release from **Y1**-Chitosan-Dox Nanoparticles.

1,3-diphenylisobenzofuran (DPBF) was used as a singlet oxygen trap and **Y1**-Chitosan nanoparticles were exposed to 530 nm LED light in the presence of trap molecule in THF. During the irradiation with the LED lamp, the decrease in the absorbance of the trap molecule was monitored (Figure 6). Accordingly, **Y1**-Chitosan nanoparticles produced extremely good singlet oxygen.

3.3. PDT property of drug loaded Y1-chitosan-dox nanoparticles

PDT properties of drug-loaded **Y1**-Chitosan-Dox nanoparticles were also studied in the same way. Again, the decrease in the absorbance of the trap molecule was observed using a 530 nm LED lamp in THF (Figure 7). However, the decrease in the absorbance of the trap molecule was slightly less than **Y1**-Chitosan Nanoparticles. The main reason for this may be the loading of less PDT agent in the drug loaded nanoparticles.

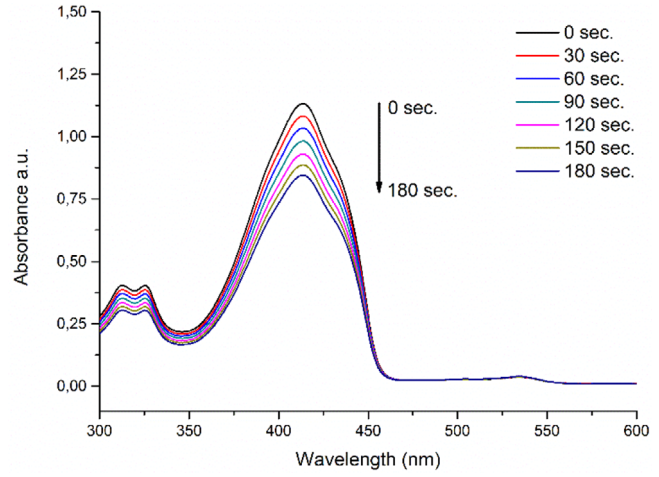

Figure 7. Decrease in the absorbance of DPBF in the presence of 0.13 mg/mL, **Y1**-Chitosan-Dox nanoparticles (total volume 3000 μL) at 416 nm in THF after irradiated with 530 nm LED.

3.4. *In-vitro* drug release property of Y1-chitosan-dox nanoparticles with the PDT effect

UV-vis measurements were performed to determine whether the Doxorubicin-loaded **Y1**-Chitosan-Dox nanoparticles were dialyzed against ultrapure water or not (Figure 8). According to the result, it was understood that the nanoparticles released Doxorubicin slowly into the medium. Accordingly, Doxorubicin reaches saturation after 48 h. After that, reverse diffusion occurs and the amount of Doxorubicin in the medium decreases. This decrease will continue until the system reaches equilibrium.

3.5. Cytotoxicity assay

In this work, Doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles (with or without LED) has been evaluated for its cytotoxicity in MCF-7 and L929 cells. The cells were treated with various concentrations of Doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles solutions and cytotoxicity was assessed using the XTT assay. To evaluate whether Doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles show selective cytotoxicity between non-cancerous and cancer cells, L929 cells treated with Doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles at 1-20 μg/mL concentrations for 24 h. According to the XTT results compared to the MCF-7 cells, Doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles showed less toxic effects to the L929 cells in groups with and without LED (Figure 9(B)). On the other hand, if we compare the effects of **Y1**-Chitosan-Dox Nanoparticles and Doxorubicin on L929 cells (with or without LED), it is understood that **Y1**-Chitosan-Dox nanoparticles have less toxic effect due to the slow release of Doxorubicin. This result supports that Doxorubicin is loaded into **Y1**-Chitosan-Dox nanoparticles.

As presented in Figure 10(A), Doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles, and **Y1**-Chitosan-Dox nanoparticles only groups exhibited cytotoxic activity in a concentration-dependent manner. As expected, after LED irradiation cytotoxicity of **Y1**, **Y1**-

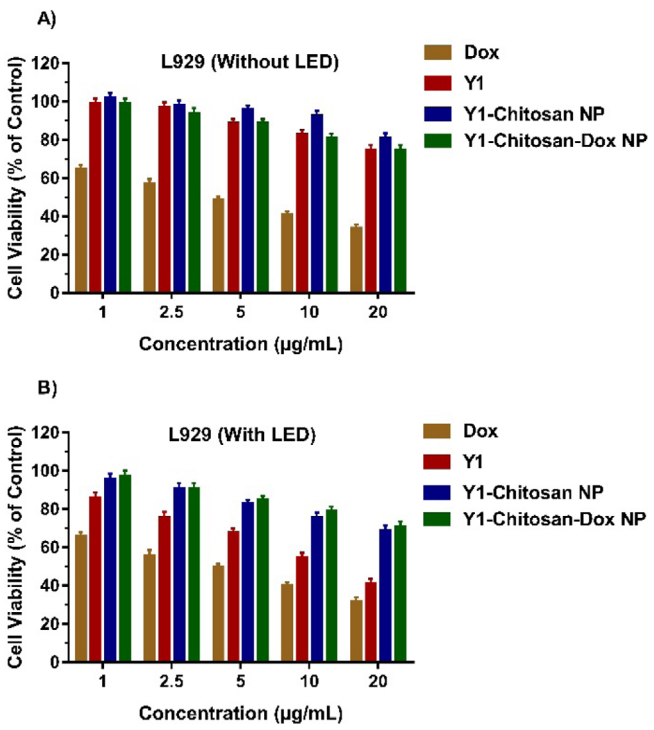

Figure 9. Concentration-dependent growth inhibition of L929 cells by doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles without (A) or with (B) LED. Cell viability was determined using the XTT assay and results are expressed as mean ± SD in triplicate.

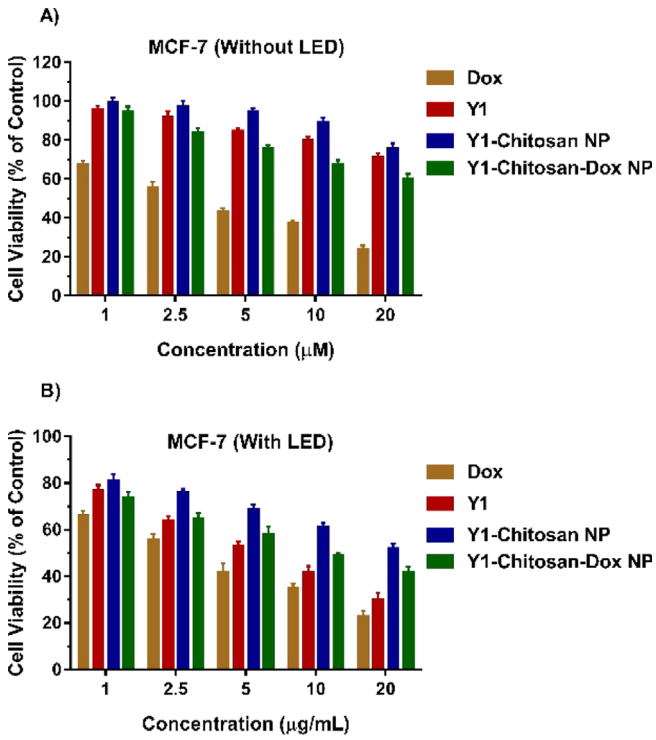

Figure 10. Concentration-dependent growth inhibition of MCF-7 cells by doxorubicin, **Y1**, **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles without (A) or with (B) LED. Cell viability was determined using the XTT assay and results are expressed as mean ± SD in triplicate.

Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles increased; however, it has been observed that LED application has no effect on the toxicity of Doxorubicin. **Y1**-Chitosan-Dox nanoparticles showed cytotoxic activity on MCF-7 cells, but this cytotoxicity is less than Doxorubicin and **Y1** (with LED). This is because both compounds are loaded in Chitosan. The important point here is that while **Y1**-Chitosan and **Y1**-Chitosan-Dox nanoparticles show toxic effects on MCF-7 (with LED), they are less toxic on L929 cells. This result shows that **Y1**-Chitosan-Dox nanoparticles work well and are less toxic.

3.6. Fluorescence microscopy

Commonly used chemotherapeutics induce apoptosis in the targeted cancer cell. Caspases are cysteine proteases that are crucial for the morphological and biochemical changes that occur during apoptosis. Caspases-3 and -7 are the central driving caspases in the cell. Caspase-3 targets structural substrates that lead to cell death and DNA fragmentation.[24] Although Caspase-3 and -7 share substrate and balance each other during apoptosis, Caspase-3 is said to affect a wider range of substrates and have more specific functions than Caspase-7.[25] The anticancer activity can be detected by caspase-3 activation of the apoptotic pathway in cancer cells. As such, Caspase-3 activation is used as a marker for the efficacy of drug therapy. We evaluated the effect of the compounds developed in this study on MCF-7 cells in Caspase-3 localization. With DAPI/CASPASE-3 double staining, apoptotic cells appear with green fluorescent staining, and cell nuclei appear in blue fluorescence with DAPI staining. As shown in Figure 11, the most apoptotic cells appear with Doxorubicin both with LED (3 min.) and without LED. This is an expected result according to the cytotoxicity assay results. It is seen that the efficiency of **Y1**-chitosan nanoparticles increases with LED application. This increase is due to the presence of **Y1** compound in these nanoparticles. Finally, it is understood that apoptosis also increases with LED application in **Y1**-Chitosan-Dox nanoparticles. The extremely good apoptotic properties of both **Y1**-chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles as well as being less toxic to L929 cells make these nanoparticles ideal for clinical application and the design of new nanoparticles.

4. Conclusions

In conclusion, we have successfully synthesized **Y1**-Chitosan nanoparticles from a BODIPY derivative **Y1** (PDT agent) and chitosan hydrochloride at the appropriate pH (pH=6) by the formation of an ionic complex due to the opposite charged ionic interaction between them. Also, **Y1**-Chitosan-Dox nanoparticles loaded with Doxorubicin as a model drug were successfully prepared. They have good PDT properties in both drug-free (**Y1**-Chitosan nanoparticles) and drug-loaded (**Y1**-Chitosan-Dox nanoparticles) organic nanoparticles. However, drug-free nanoparticles have slightly better PDT properties. The release of Doxorubicin as a model drug from drug-loaded nanoparticles was also studied. Accordingly, the drug level in the medium reached

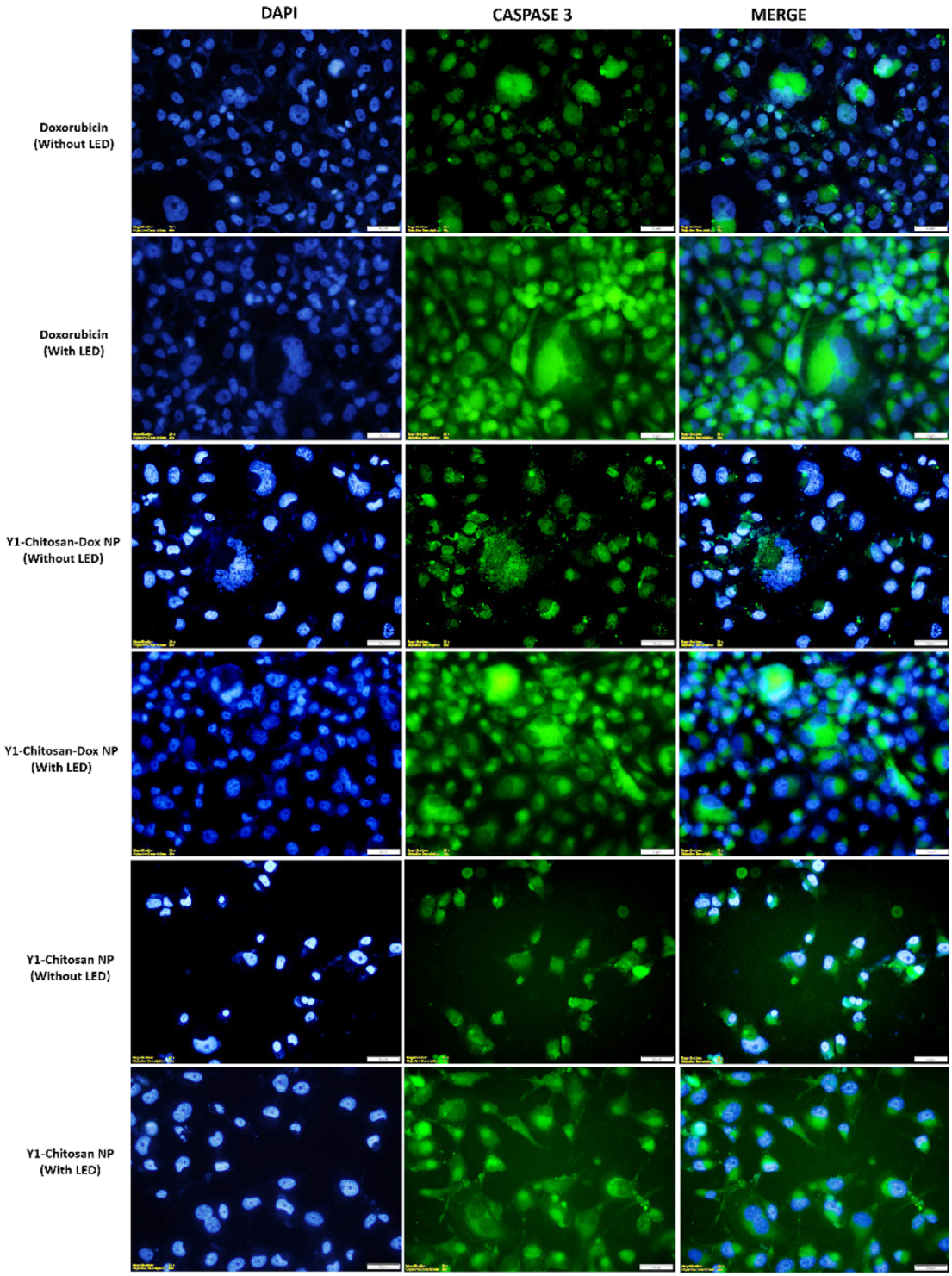

Figure 11. DAPI/CASPASE-3 double staining was performed to determine apoptosis in MCF-7 breast cancer cell lines. 24 hours after the administration of Doxorubicin, **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles (with 530 nm LED (3 minutes) or without LED application) to MCF-7 breast cancer cell lines, apoptotic cells were used as green fluorescence areas with anti-Caspase-3 staining and cell nuclei are monitored by DAPI staining with blue fluorescence.

its peak after 48 h. As a result, according to both cytotoxic and apoptosis studies, it was understood that **Y1**-Chitosan nanoparticles and **Y1**-Chitosan-Dox nanoparticles were effective on MCF7 cancer cells and were less toxic to L929 cells. This result makes these nanoparticles ideal for both clinical applications and the design of new nanoparticles.

Supporting information: Information is available regarding the ^{1}H-NMR and mass spectra of compounds 3 and Y1. The zeta potentials of Y1-Chitosan nanoparticles and drug loaded Y1-Chitosan-Dox nanoparticles are included in the supporting information. The materials are available *via* the Internet at http://www.springer.com/13233.

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