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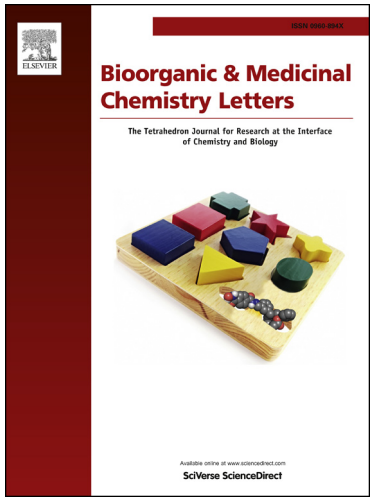

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A Naphthalocyanine Based Near-Infrared Photosensitizer: Synthesis and In Vitro Photodynamic Activities

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Abstract

A hydrophilic near-infrared (NIR) photosensitizer featuring a naphthalocyanine core and peripheral carboxylate acid groups was synthesized and characterized. Its photophysical and photochemical properties were studied and compared with phthalocyanine. Due to the extended π-conjugation, both the Q band and fluorescence emit of this naphthalocyanine bathochromically shift to NIR region. It also exhibits superior NIR photodynamic efficiency to phthalocyanine as evidenced by high efficiency in generating singlet oxygen (Φ_Δ = 0.66) and in vitro phototoxicity toward Hela human cervical cancer cells. Therefore, this novel naphthalocyanine could potentially be a NIR photosensitizer for photodynamic therapy.

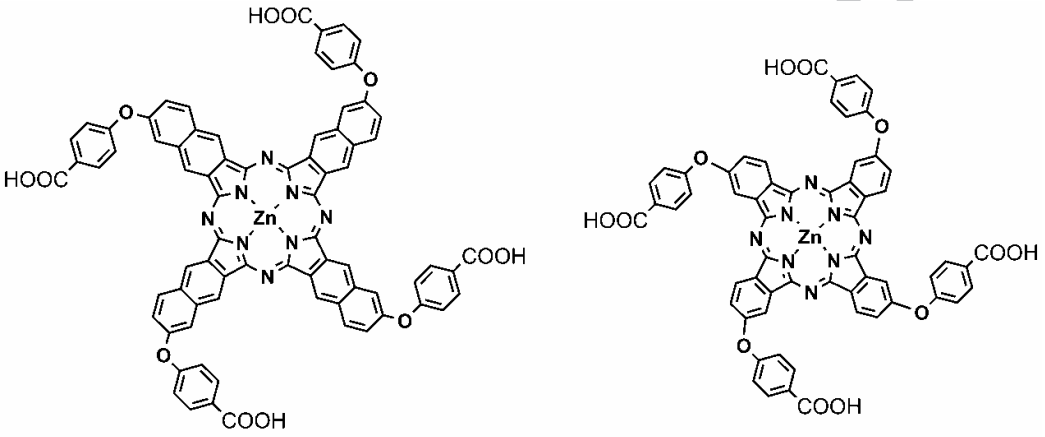
Keywords: Naphthalocyanine, Photodynamic therapy; Singlet Oxygen; Photosensitizer.

Photodynamic therapy (PDT) is an emerging modality for the diagnosis and treatment of various cancers and metastases. It essentially takes advantages of the interaction of light with drug (photosensitizer) and oxygen to initiate apoptosis or necrosis of cancer cells and destroy the tumor , while sparing normal tissue.[1, 2] Porphyrin (Por) is traditional clinical photosensitiser for the treatment of many cancers, [2-5]however , its weak absorption in the red to near-infrared (NIR) region where light penetrates deeper through tissue make it unsuitable for the treatment of deep-seated or large solid tumors. [6] Thus , considerable research efforts have been devoted to the development of new generation photosensitizers that have stronger absorptions in the near-infrared region to match the body's optimal therapeutic window (700-1000nm).[6-10]

One of the most promising second-generation photosensitizers for PDT is phthalocyanine (Pc), which is the synthetic analogue of Por with intense red-shifted absorption. Naphthalocyanine (Nc) is a Pc derivative , which possesses four extra benzene rings condensed to the periphery of the Pc macrocycle,[11-13] thus imparting strong absorbance at long wavelengths (750-900 nm) to it. The light penetration through tissues in this region is approximately twice that of porphyrin-mediated PDT.[6] In addition, Ncs are also reported to be much more stable photochemically and photophysically than conventional used porphyrin photosensitizers.[1] These features have led to the impetus for Nc-based photosensitizers for PDT. However, due to their complicated synthetic procedures and poor solubility in common organic solvents, only a few Ncs have been reported in the biomedical area so far despite their great potential in PDT. In particular, hydrophilic Ncs remain extremely rare. As a matter of fact, the hydrophilicity of photosensitizers is very important and crucial for their biological application.[12, 14-19] Herein, we report the design and synthesis of a hydrophilic naphthalocyanine bearing carboxylate functionalities **1**, and its in vitro photodynamic activities. For comparison, the corresponding Pc counterpart **2** was also prepared and studied parallel. It is expected that this hydrophilic NIR photosensitizer **1**, can have strong absorption in the NIR region, high efficiency to generate singlet

oxygen, and efficient in vitro photodynamic activity.

Carboxylate group is a commonly used hydrophilic moiety that can change the water-solubility of the photosensitizers, thereby affecting their photodynamic activity. To impart the hydrophilicity of the molecules, which is believed to be an advantage characteristic in photosensitizers, 4-carboxyphenoxy group was selected to incorporate into Nc **1** via simple aromatic necleophilic substitution reaction. Pc **2** has been prepared before but its biological studies remain unexplored.[20, 21] In this work, a direct comparison of the PDT efficacy between hydrophilic Nc and Pc is reported.



1 **2**

The synthesis of this tetra-substituted Nc **1** is shown in Scheme 1. Treatment of 6-bromo-2,3-dicyanonaphthalene (**3**), which was synthesized following reported procedures,[22, 23] with methyl *p*-hydroxybenzoate (**4**) in the presence of K_2CO_3 in DMF gave the corresponding substituted product **5**. Cyclization of this precursor **5** in the presence of zinc acetate and a catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in n-heptanol under refluxing led to the formation of Nc **6** with concomitant transesterfication of the ester groups to give the heptyl esters as observed by us before.[18, 24] This tetra-substituted Nc could be isolated and purified readily by column chromatography due to its good solubility in common organic solvents, such as THF, pyridine, DMF, $CHCl_3$, benzene, and DMSO. Hydrolysis of the ester groups of **6** using NaOH in a mixed solvent system (THF/MeOH/H_2O), according to the method described by us,[18] gave the naphthalocyanine bearing four sodium carboxylate groups. Upon addition of 1 M HCl until pH 2, a green precipitate of **1** was obtained in

quantitative yield. Although this peripheral tetra- substituted Nc existed as a mixture of structural isomers, the ^{1}H NMR recorded in THF was simpler than expected. Two sets of well-separated multiplets in the region 7.4-9.3 were observed for naphthalocyanine ring protons and carboxyphenoxy moieties, respectively, along with a broad signal at ca. 11.0 for the carboxylic acid protons.

The UV-vis spectra of **1** and **2** in N, N-dimethylformamide (DMF) are displayed in Figure 1. The Q band for the Nc **1** at 763 nm is significantly red-shifted by ca. 90 nm compared with that for the Pc counterpart **2** at 674 nm. The inset shows the fluorescence spectra of **1** and **2,** in which fluorescence of **1** appears with a ca. 80 nm red-shift compared to that of **2**. These large bathochromic shifts could be attributed to the extended -conjugation of Nc. The solubility of this hydrophilic Nc in water was evidenced by two Q bands at 698 nm and 778 nm in slightly basic water, which could be ascribed to aggregated and monomeric absorption, respectively. The pH from acidic to basic does affect the solubility of **1** in water to some extent because of the existence of carboxylate groups. The electronic absorption and basic photophysical data are compiled in Table 1.

The efficiency of these compounds in generating singlet oxygen ( ) was also compared by steady-state method using 1, 3-diphenylisobenzofuran (DPBF) as the scavenger. The absorption of DPBF at 415 nm was monitored along with irradiation time, from which the values of could be determined by method described previously using ZnPc as the reference (= 0.56 in DMF).[25-27] To evaluate their photosensitizing efficiency in the NIR region, two sets of light source consisted of a halogen lamp and a color glass filter (> 610 nm or = 750 10 nm) were used to initiate the singlet oxygen generation. Upon illumination with red light (> 610 nm), both **1** and **2** can efficiently generate singlet oxygen in DMF with the values of 0.66 and 0.45 for **1** and **2**, respectively (Figure 2). This is the best singlet oxygen quantum yield that was reported for Nc so far,[12, 13, 28-30] mainly because

of its good solubility and the coordinating property of DMF to Zn(II) disrupted the (-(interactions of these macrocycles . By contrast, upon illumination with NIR light (= 75 10 nm), the singlet oxygen generation of **2** was greatly reduced because of its weak absorption at NIR region, while that of Nc remains unchanged under the same conditions. These data are also compiled in Table 1. The photobleaching rates of DPBF under different light sources were compared in Figure 3. As a result, Nc **1** is a superior NIR singlet oxygen generator to Pc **2**.

The in vitro photodynamic activities of compounds **1** and **2** formulated with Cremophor EL were investigated against Hela cells. In the absence of light, all these compounds were not cytotoxic. As shown in figure 4, upon irradiation with red light (> 610 nm), both naphthalocyanine **1** and phthalocyanine **2** are highly photocytotoxic with IC_{50} of 3.7 and 3.5 μM, respectively, showing they have similar in vitro PDT efficiency under these conditions. By contrast, Pc **2** becomes less effective on the phototoxicity when irradiated by NIR light, which can again be attributed to its negligible absorption in NIR region.

As reveled by fluorescence microscopy, both Nc **1** and Pc **2** could enter the Hela cells causing intracellular fluorescence. While the fluorescence was rather uniform in the cytoplasm of the cells for both of them, **2** appeared as brighter than **1** (Figure 5). To account for the different cellullar activities of these two compounds, their absorptions in DMEM cell culture medium was examined. As shown in figure 6, **2** gives a typical absorption spectrum of nonaggregated phthalocyanine, but two Q bands are observed for **1** ascribed to the aggregated and monomeric bands, respectively. The results suggest that Nc is more aggregated than Pc in the culture medium due to the strong intermolecular interactions, which is undesirable for photodynamic action. Since the lower cellular fluorescence intensity of **1** maybe caused by either higher aggregation tendency or lower cellular uptake**,** the photosensitizer concentrations inside the cells were quantified by an extraction method. DMF was used to extract the photosensitizers inside the cells after incubation with **1** and **2** for 2 h. The cellular uptake of **1** was about 2-fold lower than

that of **2** (figure 7). To take into account that these two compounds had similar in vitro PDT efficiency (figure 4), the photocytotoxicity of **1** was in accord with its higher singlet oxygen generation.

In summary, we have synthesized and characterized a novel water-soluble naphthalocyanine, which shifts the Q-band absorption from red to the NIR region comparing to its phthalocyanine counterpart. Our in vitro evaluation results indicate that this compound has efficient photodynamic activities in NIR region, and is therefore a highly promising NIR photosensitizer for PDT. Further studies of this system are in progress.

Acknowledgments

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References and notes

1. Ali, H.; van Lier, J. E. *Chem. Rev.* **1999,** *99*, 2379.
2. Dougherty, T. J.; Gomer, C. J.; Jori, G.; Kessel, D.; Korbelik, M.; Moan, J.; Peng, Q. *J. Natl. Cancer Inst.* **1998,** *90*, 889.
3. Hu, Z. Y.; Pan, Y. F.; Wang, J. W.; Chen, J. J.; Li, J.; Ren, L. F. *Biomed. Phar.* **2009,** *63*, 155.
4. Anand, S.; Ortel, B. J.; Pereira, S. P.; Hasan, T.; Maytin, E. V. *Cancer Lett.* **2012,** *326*, 8.
5. Dolmans, D. E..; Fukumura, D.; Jain, R. K. *Nat. Rev. Cancer* **2003,** *3*, 380.
6. Sternberg, E. D.; Dolphin, D.; Bruckner, C. *Tetrahedron* **1998,** *54*, 4151.
7. Lu, T.; Shao, P.; Mathew, I.; Sand, A.; Sun, W. *J. Am. Chem. Soc.* **2008,** *130*, 15782.
8. Muranaka, A.; Yonehara, M.; Uchiyama, M. *J. Am. Chem. Soc.* **2010**,*132*, 7844.
9. Brasseur, N.; Nguyen, T.-L.; Langlois, R.; Ouellet, R.; Marengo, S.; Houde, D.; van Lier, J. E. *J. Med. Chem.* **1994,** *37*, 415.
10. Camerin, M.; Rello, S.; Villanueva, A.; Ping, X.; Kenney, M. E.; Rodgers, M. A. J.; Jori, G. *Eur. J. Cancer* **2005,** *41*, 1203.
11. Bucking, M.; Dickson, E. F. G.; Farahani, M.; Fischer, F.; Holmes, D.; Jori, G.; Kennedy, J. C.; Kenney, M. E.; Peng, X.; Pottier, R. H.; Weagle, G. *J.Photochem. Photobiol., B* **2000,** *58*, 87.
12. Wohrle, D.; Shopova, M.; Muller, S.; Milev, A. D.; Mantareva, V. N.; Krastev, K. K. *J. Photochem. Photobiol., B* **1993,** *21*, 155.

13. Ishii, K.; Shiine, M.; Shimizu, Y.; Hoshino, S.-i.; Abe, H.; Sogawa, K.; Kobayashi, N. *J. Phys. Chem.B* **2008,** *112*, 3138.

14. Yates, N. C.; Moan, J.; Western, A. *J. Photochem. Photobiol., B* **1990**, *4*, 379.

15. Xu, H.; Ng, D. K. P. *Chem. Asian J.* **2009**, *4*, 104.

16. Saydan, N.; Durmus, M.; Dizge, M. G.; Yaman, H.; Gurek, A. G.; Antunes, E.; Nyokong, T.; Ahsen, V. *J. Porphyrins Phthalocyanines* **2009**, *13*, 681.

17. Soares, A. R. M.; Tome, J. P. C.; Neves, M.; Tome, A. C.; Cavaleiro, J. A. S.; Torres, T. *Carbohydr. Res.* **2009,** *344*, 507.

18. Liu, W.; Jensen, T. J.; Fronczek, F. R.; Hammer, R. P.; Smith, K. M.; Vicente, M. G. H. *J. Med. Chem.* **2005,** *48*, 1033.

19. Medina, W. S. G.; dos Santos, N. A. G.; Curti, C.; Tedesco, A. C.; dos Santos, A. C. *Chem. Biol. Interact.* **2009,** *179*, 402.

20. a) Verdree, V.; Pakhomov, S.; Su, G.; Allen, M.; Countryman, A.; Hammer, R.; Soper, S. *J. Fluoresc.* **2007,** *17*, 547; b) Leng, X.; Choi, C.; Luo, H.; Cheng, Y.; Ng, D. K. P. *Org. Lett.* **2007**, *9*, 2497.

21. Yan, Y.; Lu, S.; Li, B.; Zhu, R.; Zhou, J.; Wei, S.; Qian, S. *J. Phys. Chem. A* **2006**, *110*, 10757.

22. Kitahara, K.; Asano, T.; Hamano, K.-i.; Tokita, S.; Nishi, H. *J. Heterocycl. Chem.* **1990**, *27*, 2219.

23. Kovshev, E. I.; Puchnova, V. A.; Luk'yanets, E. A. *Zh. Org. Khim.***1971,** *7*, 369.

24. Luan, L.; Chen, J.; Ding, L.; Wang, J.; Cheng, X.; Ren, Q.; Liu, W. *Chem. Lett* .**2012**, *41*, 1012.

25. Shen, X.; Jiang, X.; Huang, C.; Zhang, H.; Huang, J. *Tetrahedron* **2010**, *66*, 9041.

26. Spiller, W.; Kliesch, H.; Wöhrle, D.; Hackbarth, S.; Röder, B.; Schnurpfeil, G. *J. Porphyrins Phthalocyanines* **1998,** *2*, 145.

27. Maree, M. D.; Kuznetsova, N.; Nyokong, T. *J. Photochem. Photobiol., A* **2001,** *140*, 117.

28. Spikes, J. D.; van Lier, J. E.; Bommer, J. C. *J. Photochem. Photobiol., A* **1995,** *91*, 193.

29. Mantareva, V.; Shopova, M.; Spassova, G.; Wohrle, D.; Muller, S.; Jori, G.; Ricchelli, F. *J. Photochem. Photobiol. B* **1997,** *40*, 258.

30. Muller, S.; Mantareva, V.; Stoichkova, N.; Kliesch, H.; Sobbi, A.; Wohrle, D.; Shopova, M. *J. Photochem. Photobiol., B* **1996,** *35*, 167.

Figure captions:

Scheme 1. Synthetic route for naphthalocyanine **1.**

Table 1. Electronic absorption and photophysical data for **1** and **2** in DMF.

Figure 1.UV-vis Absorption spectra of **1** (solid) and **2** (dash) in DMF (both at 4 μM). The inset shows the normalized florescence spectra of **1** (solid) and **2** (dash) in DMF excited at 680 and 610 nm, respectively.

Figure 2. Typical spectra for the determination of singlet oxygen quantum yield of **1** (A, C) and **2** (B, D) in DMF; A, B: exposed to light > 610nm; C, D: exposed to light = 750 10 nm.

Figure 3. Comparison of the photobleaching rate of DBPF (K_{DPBF}) by the photosensitizers.

Figure 4. Cytotoxic effects of **1** (squre, solid line) and **2** (circle, dash line) against Hela cells in the absence (solid symbols) and presence (hollow symbols) of light (> 610 nm, ~1 J cm^{-2}).

Figure 5. Visualization of intracellular fluorescence of **1** (A) and **2** (B) in Hela cells after being incubated (both at 10μM) for 2 h (acquired with an Olympus IX71 inverted microscope equipped with a U-MWU2 fluorescence unit, excited at 330 - 380 nm and monitored at > 420nm).

Figure 6 Electronic absorption spectra of **1** (solid) and **2** (dash), formulated with cremophor EL, in DMEM (both at 10 μM).

Figure 7 Cellular uptake of **1** and **2** determined by an extraction method using DMF

as solvent.

Scheme 1.

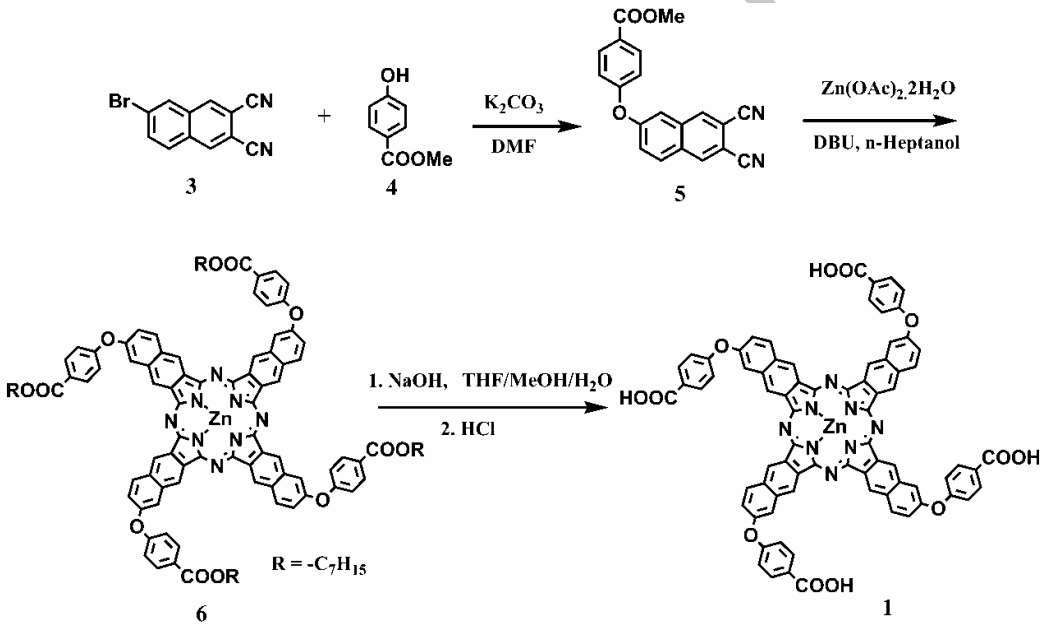


Table 1.

Electronic absorption and photophysical data for naphthalocyalnine **1** and phthalocyanine **2** in DMF.

Compound	$_{max}$/nm	$_{em}$/nm[a]	[b]	K_{DPBF}[c,d] (>610nm)	K_{DPBF}[c,e] (750 10nm)
1	341, 390, 680, 726, 763	764	0.66	2.8×10^{-2}	2.7×10^{-2}
2	355, 610, 643, 674	680	0.45	1.8×10^{-2}	7.1×10^{-3}

[a] Excited at 680 nm for **1** or 610 nm for **2**.

[b] singlet oxyen quantum yield measured in DMF relative to ZnPc (= 0.56).

[c] K_{DPBF} represents the photobleaching rate of DPBF by the photosensitizer, taken as the slope of the central linear region of the decay curve.

[d] measured with >610nm cut-off glass filter. [e] measured with 750 10nm band-pass glass filter.

Figure 1.

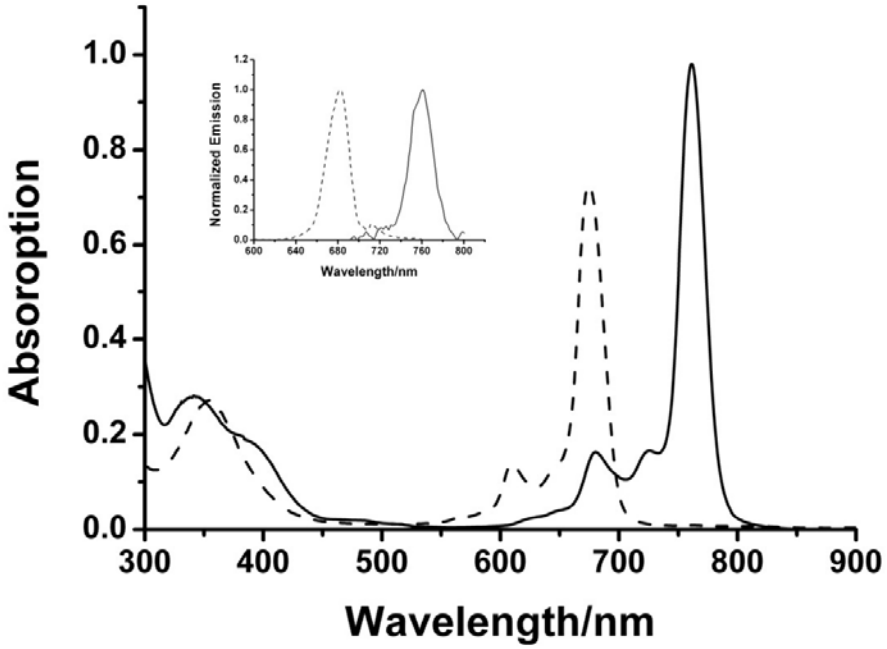


Figure 2.

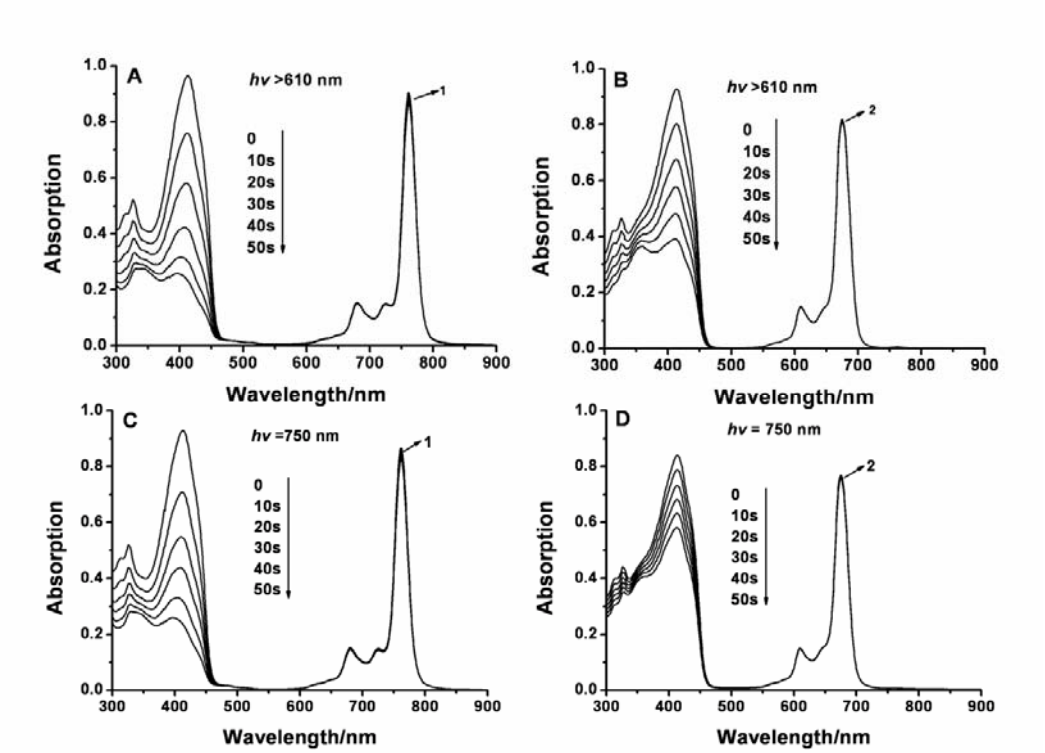


Figure 3.

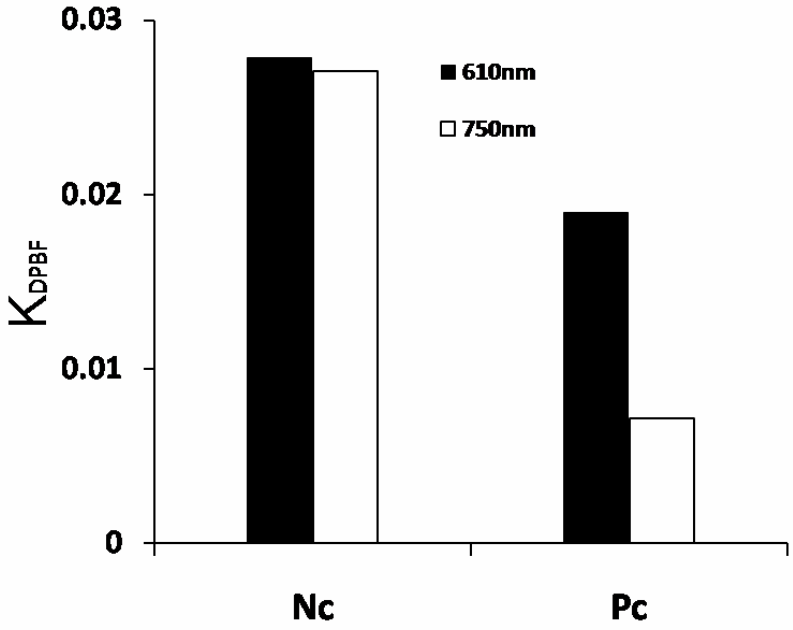


Figure 4.

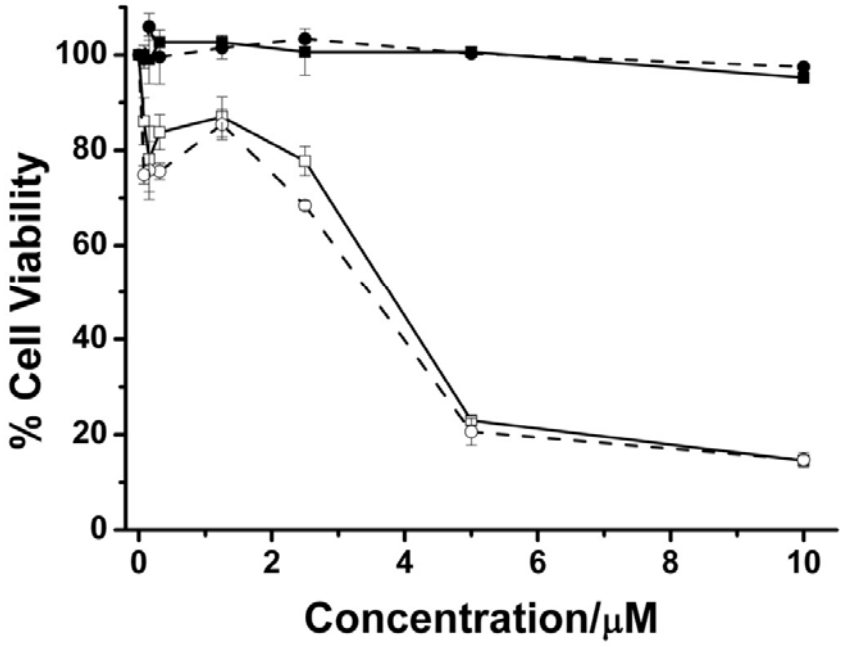
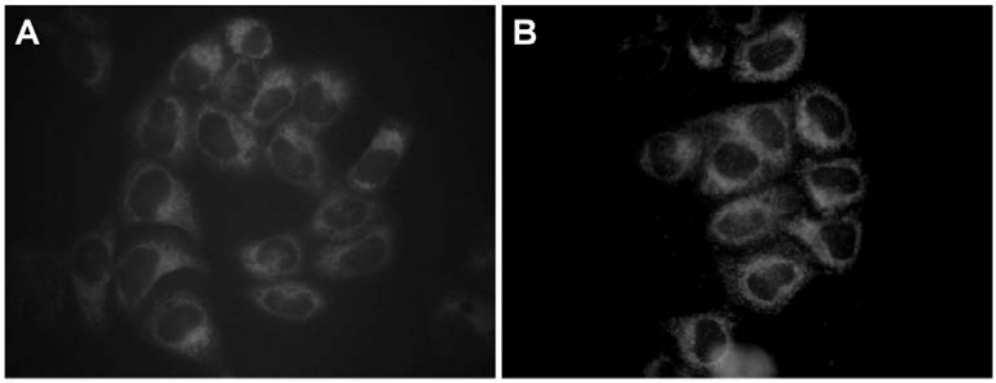


Figure 5.



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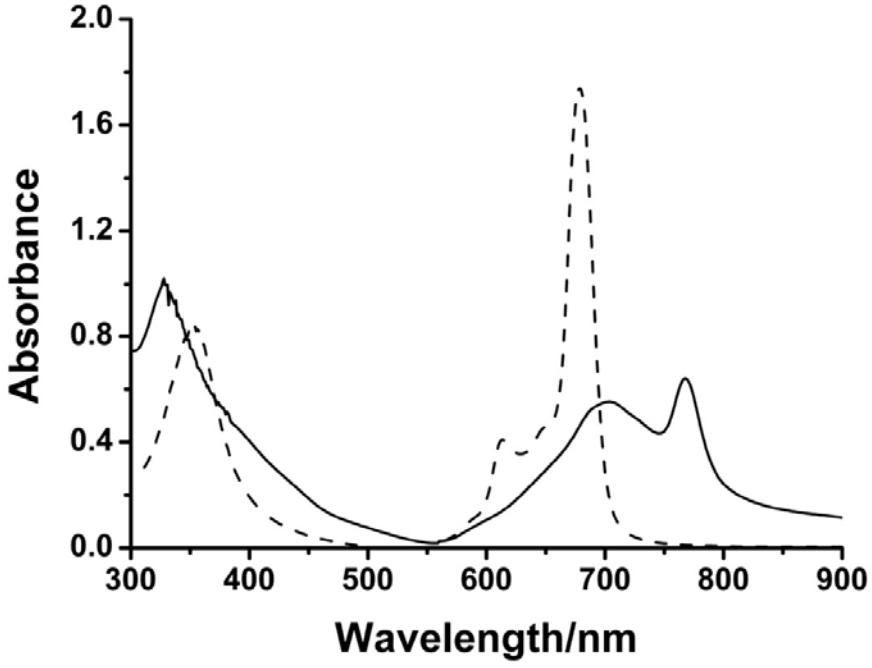
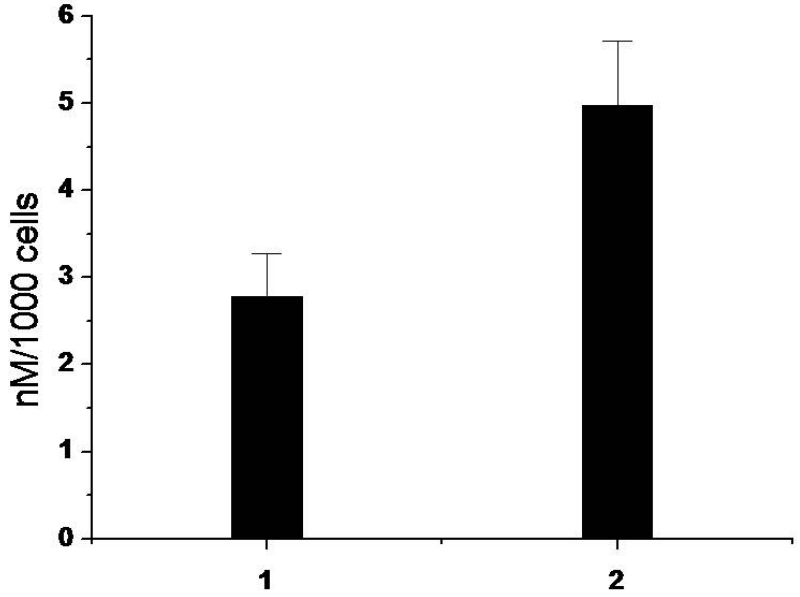


Figure 7.



Graphic Abstract