Methyl Pyropheophorbide- a Analogues: Potential Fluorescent Probes for the Peripheral-Type Benzodiazepine Receptor. Effect of Central Metal in Photosensitizing Efficacy

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · JULY 2005

Impact Factor: 5.45 · DOI: 10.1021/jm050039k · Source: PubMed

8 AUTHORS, INCLUDING:

Mahabeer Prasad Dobhal
University of Rajasthan
63 PUBLICATIONS **891** CITATIONS
SEE PROFILE

Janet Morgan
Roswell Park Cancer Institute
58 PUBLICATIONS **2,306** CITATIONS
SEE PROFILE

Thomas J Dougherty
Roswell Park Cancer Institute
158 PUBLICATIONS **8,324** CITATIONS
SEE PROFILE

Ravindra Pandey
Roswell Park Cancer Institute
297 PUBLICATIONS **7,820** CITATIONS
SEE PROFILE

Methyl Pyropheophorbide-*a* Analogues: Potential Fluorescent Probes for the Peripheral-Type Benzodiazepine Receptor. Effect of Central Metal in Photosensitizing Efficacy

Yihui Chen,[†] Xiang Zheng,[†] Mahabeer P. Dobhal,[†,‡] Amy Gryshuk,[†,§] Janet Morgan,[§] Thomas J. Dougherty,[†] Allan Oseroff,[§] and Ravindra K. Pandey*[,†,‡]

PDT Center, Department of Dermatology, and Department of Nuclear Medicine and Radiology, Roswell Park Cancer Institute, Buffalo, New York 14263

Received January 14, 2005

Abstract: Pyropheophorbides and their metal complexes were synthesized to investigate their applications as nonradioactive peripheral benzodiazepine receptor (PBR) binding probes and photosensitizers for use in photodynamic therapy. They were found to be localized in mitochondria and showed significant binding to PBR. In some cases, the PBR binding values were similar to that for **17** (PK11195, 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)isoquinoline-3-carboxamide). However, no direct correlation between **17** displacement ability and photosensitizing efficacy of photosensitizers was observed.

Peripheral benzodiazepine receptor (PBR) is an 18 kDa protein of the outer mitochondrial membrane.[1] It is involved in numerous functions including steroid biosynthesis, mitochondrial respiration, heme biosynthesis, cell proliferation, and calcium channel modulation.[1] In addition, PBR plays an important role in the regulation of stress response, anxiety disorders, mood disorders, and neurodegenerative disorders (Parkinson's disease and Alzheimer's disease). Elevated PBR expression is also related to certain cancers, such as those of breast, colon−rectum, and prostate tissue, and has been proposed as a novel prognostic indicator of an aggressive phenotype for these indications.[2] Therefore, the PBR provides an attractive target molecule for the development of compounds that may be used for the regulation of the above-mentioned PBR-dependent functions.

Another intriguing aspect of the PBR is its association with photodynamic therapy (PDT). PDT is a relatively new modality for cancer treatment; it refers to the photosensitization of tumor-avid photosensitizers by light, resulting in singlet-oxygen-mediated cytotoxicity to tumor cells. Most photosensitizers for PDT are porphyrin-based compounds. Verma et al.[3] were the first to study the binding affinity of various porphyrins to the PBR with prominent physiological functions. A previous report from our laboratory has shown the importance of the PBR as a binding site for a series of chlorin-type photosensitizers such as pyropheophorbide-*a* ethers.[4] It was found that the PDT effect was inhibited by **17** (PK11195, 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)isoquinoline-3-carboxamide) (PBR ligand) for the active derivatives of the alkyl ether derivatives of pyropheophorbide-*a*, but no effect was found for the less active analogues. Kessel et al.[5] studied three very similar analogues (protoporphyrins III, IX, and XIII) to understand the role of the peripheral benzodiazepine receptor in the apoptotic response to PDT. Only protoporphyrin IX had significant affinity for the PBR. These data suggest that the relationship between PDT efficacy and PBR affinity may hold only for sensitizers with PpIX-like configurations. A recent report from Oleinick[6] on the importance of PBR receptor in phthalocyanine photosensitizer Pc-4 concluded that the binding of Pc-4 to PBR is less relevant to the phototoxicity of Pc-4-PDT than other mitochondrial events, such as photodamage to Bcl-2, and that the observed inhibition of Pc-4-PDT induced apoptosis by **17** likely occurs through a mechanism independent of PBR.[7] Therefore, the role of PBR in PDT is still a subject of discussion.

It has been shown that mitochondria are important intracellular targets for PDT and play a critical role in energy production and involvement in apoptosis.[8,9] For porphyrins and porphyrin-based structures, accumulation of photosensitizers can occur in the membranes of organelles such as the mitochondria or lysosomes. In our studies with a series of photosensitizers related to chlorins and bacteriochlorins, we observed that most of the effective photosensitizers are localized in mitochondria.[10] Interestingly, in a series of alkyl ether analogues of pyropheophorbide-*a* analogues, the overall lipophilicity was found to play an important role in the site of intracellular localization. For example, the lower alkyl ether analogues (C1−C8) that are localized in mitochondria were found to be more effective in vivo than higher alkyl analogues (C9−C12) with a preferrential localization in lysosomes. Among the alkyl ether analogues of pyropheophorbide-*a*, the corresponding hexyl ether derivative (HPPH), which produced the best in vivo photosensitizing activity, also showed affinity toward the PBR. However, the PBR binding data obtained from HPPH was significantly lower than the data observed for **17**.

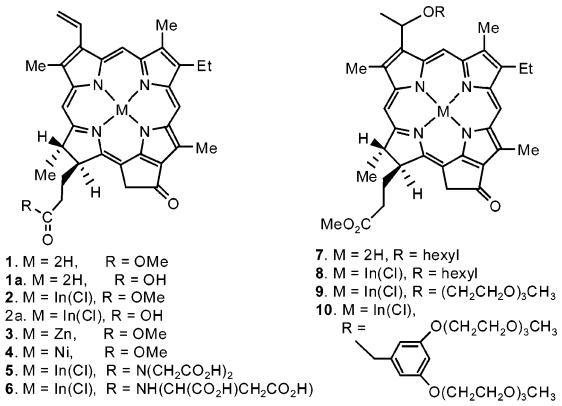
The present work describes the synthesis of a series of pyropheophorbides (free-base and the corresponding metalated analogues), their PBR binding affinity, intracellular localization pattern, and photosensitizing efficacy. The main objectives of this study were (i) to develop nonradioactive, efficient fluorescent probes for PBR binding and (ii) to investigate any correlation between the PBR binding affinity of these compounds and photosensitizing efficacy.

The compounds synthesized for our study are shown in Schemes 1 and 2. For our initial study, to determine the effect of the central metal, pyropheophorbide-*a* **1** was converted into the corresponding Zn(II), In(III), and Ni(II) complexes. Among these analogues the In(III) complex showed the best PDT efficacy and significant PBR binding affinity. The Ni(II) complexes, because of its inability to produce singlet oxygen, did not show any PDT efficacy.[11] However, a strong PBR binding affinity with this compound was observed. Because our objective

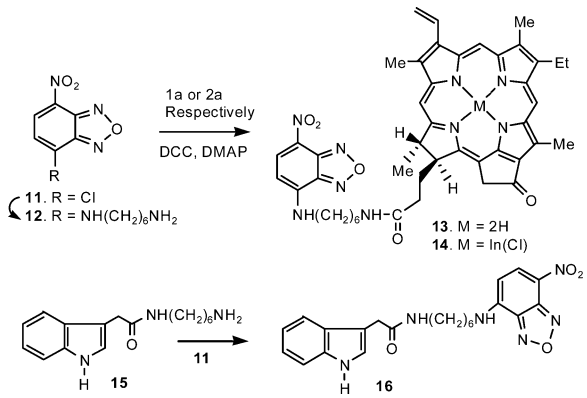
* To whom correspondence should be addressed. Phone: 716-845-3203. Fax: 716-845-8920. E-mail: ravindra.pandey@roswellpark.org.
[†] PDT Center.
[‡] Department of Nuclear Medicine and Radiology.
[§] Department of Dermatology.

10.1021/jm050039k CCC: $30.25
Published on Web 05/10/2005

Scheme 1. Structures of **1**−**10**



Scheme 2. Structures of **11**−**16**[a]



[a] Prepared by reacting **15** with NBD-chloride **11**, following the literature procedure.[1]

was to investigate the PBR binding affinity with photosensitizing ability, this study was extended to a series of In(III) analogues with variable lipophilicity in which the vinyl group at position 3 was replaced with hexyl ether **8** and mono- and di-PEG substituents **9** and **10**. Kozikowski[1] showed that incorporation of NBD chloride **11** to PBR binding indole **15** enhances the PBR binding affinity. Therefore, to investigate the effect of such introduction in pyropheophorbide-*a*, a corresponding NBD conjugate **13** and the related In(III) complex **14** were synthesized (Scheme 2). For a comparative study, **16** (Kozikowski's compound) was prepared by reacting **15** with NBD chloride **11,** following the literature procedure.[1]

Porphyrin-based compounds are reported to be ligands for the mitochondrial PBR. The first report on correlation between the photodynamic activities of several porphyrin photosensitizers and their binding affinities for the PBR was from Verma et al.[3] Therefore, before evaluation of the methylpheophorbide-*a* analogues for PBR binding, their intracellular localization characteristic was determined. Interestingly, they all were found to be localized in mitochondria. A representative localization pattern of the most effective photosensitizers **7** and the corresponding In(III) complex **8** is shown in Figure 1.

Among the methyl pyropheophorbide-*a* **1** and the related metal complexes **2**−**4**, the In(III) and Ni(II) analogues **2** and **4**, respectively, produced a PBR binding affinity similar to that of **17**. Among the compounds investigated, the binding affinities was in the order of

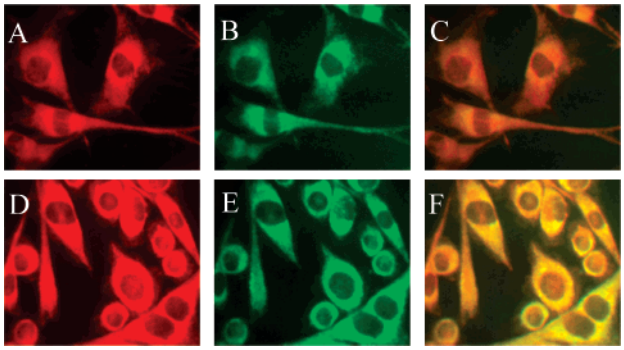


Figure 1. Comparative intracellular localization of **7** and **8** with MitoTracker Green: (A) **7**; (B) MitoTracker Green; (C) overlap of **7** with MitoTracker Green; (D) **8**; (E) MitoTracker Green; (F) overlap of **8** with MitoTracker Green.

4 > **2** > **1** > **3**, i.e., nickel complex > indium complex > free base > zinc complex. Because of the limited singlet-oxygen-producing ability of the Ni(II) complex **4**, we focused our attention on In(III) analogues, and pyropheophorbide-*a* derivatives **7**−**10** and **14** with variable lipophilicity were used for our studies.

For investigation of the effect of lipophilicity in PBR binding, compounds with hydrophilic substituents containing carboxylic groups **5** and **6** or mono-/di-PEG substituents **9** and **10** were synthesized. Despite a similar lipophilicity between **5** and **6**, **6** showed higher PBR binding affinity than **5**. These results suggest that lipophilicity alone may not be an indicator of PBR binding affinity.

In recent years, several indole analogues have also been investigated for PBR binding. Among these analogues, an indole−NBD conjugate **16** has been reported as an excellent PBR binding agent. The use of the NBD moiety in the conjugate was based on its fluorescence properties, but its presence also enhanced the PBR binding affinity. However, in contrast to **16**, the presence of the NBD moiety in **13** and **14** diminished the PBR binding affinity (Figure 2C). The IC_{50} values for these pyropheophorbide-*a* analogues are summarized in Table 1 and show the following order of PBR binding affinities: **4** > **2** > **1** > **6** > **5** ∼ **3** > **10** > **9** > **14** > **8** > **7** > **13**. Because of their strong fluorescence in the range 650−720 nm, these compounds could be promising candidates as fluorescent probes and may have a significant advantage (cost effective, easy to handle) over the radioactive probes (e.g., tritiated **17**) currently being used.

The in vitro PDT efficacy of photosensitizers was investigated in RIF tumor cells by following the MTT assay.[12] After careful comparison, the in vitro PDT efficacy (LD_{50} light dose either at 0.06 or 1 micromolar concentration) was in the order of **2** = **9** > **8** > **7** > **10** > **14** > **6** ∼ **1** > **3** > **5** > **13** (Table 2).

Compared to the free-base analogues, the corresponding indium complexes generally produced higher PBR binding affinities and also were found to have more effective in vitro photosensitizing ability. For example, **2** at 0.06 μM and a light dose of 0.625 J/cm^2 gave 100% cell kill, whereas **1** at the same concentration required a light dose of 2 J/cm^2 and only 60% tumor cell-kill was observed (Figure 3A). At 1 μM, although the zinc complex **3** showed some PDT efficacy, it was less effective than the corresponding free-base analogue **1**.

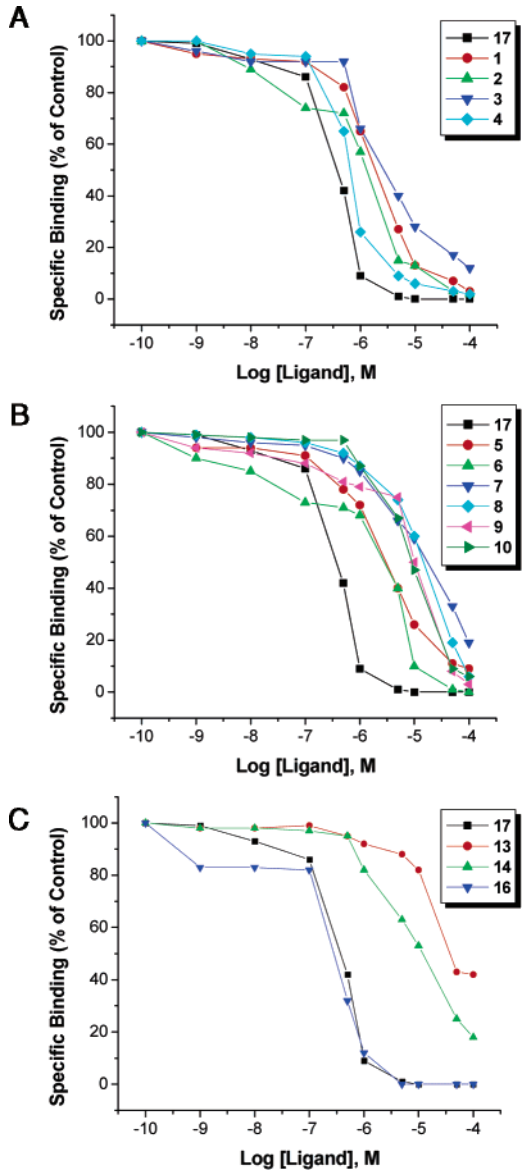


Figure 2. Displacement of titrated **17** (PBR probe) with pyropheophorbide-*a* analogues.

Table 1. IC_{50} Displacement of Titrated **17** from the PBR (See Figure 2)

compd:	**17**	**1**	**2**	**3**	**4**
IC_{50} ($\times 10^{-7}$ M):	3.9	18.2	11.2	28.9	6.6
compd:	**5**	**6**	**7**	**8**	**9**
IC_{50} ($\times 10^{-7}$ M):	28.8	20.4	169.8	147.9	104.7
compd:	**10**	**13**	**14**	**16**	
IC_{50} ($\times 10^{-7}$ M):	89.1	371.5	107.1	3.2	

In summary, a correlation between the in vitro PDT efficacy and the PBR binding affinity of these compounds was observed and it was in the order of **2** > **1** > **3** (Figure 2A and 3A).

The Ni(II) complex **4**, which exhibited limited $^1O_2$ efficiency,[11] showed insignificant photosensitizing ability in RIF tumor cells (MTT assay) (see Figure 3A). Since the PBR binding affinity of the Ni(II) complex **4** is similar to that of **17**, this compound has the potential to be an effective fluorescent PBR binding probe and

Table 2. Light Dose (J/cm^2) for LD_{50} (See Figure 3)

compd:	**1**	**2**	**3**	**4**	**5**	**6**
light dose at 0.06 μM	*a*	0.02	*a*	*a*	*a*	*a*
light dose at 1.00 μM	*b*		0.70	*a*	0.87	0.10
compd:	**7**	**8**	**9**	**10**	**13**	**14**
light dose at 0.06 μM	0.43	0.05	0.02	0.96	*a*	1.64
light dose 1.00 μM					*a*	0.01

[a] Greater than 2.00 J/cm^2 light dose. [b] Dark toxicity.

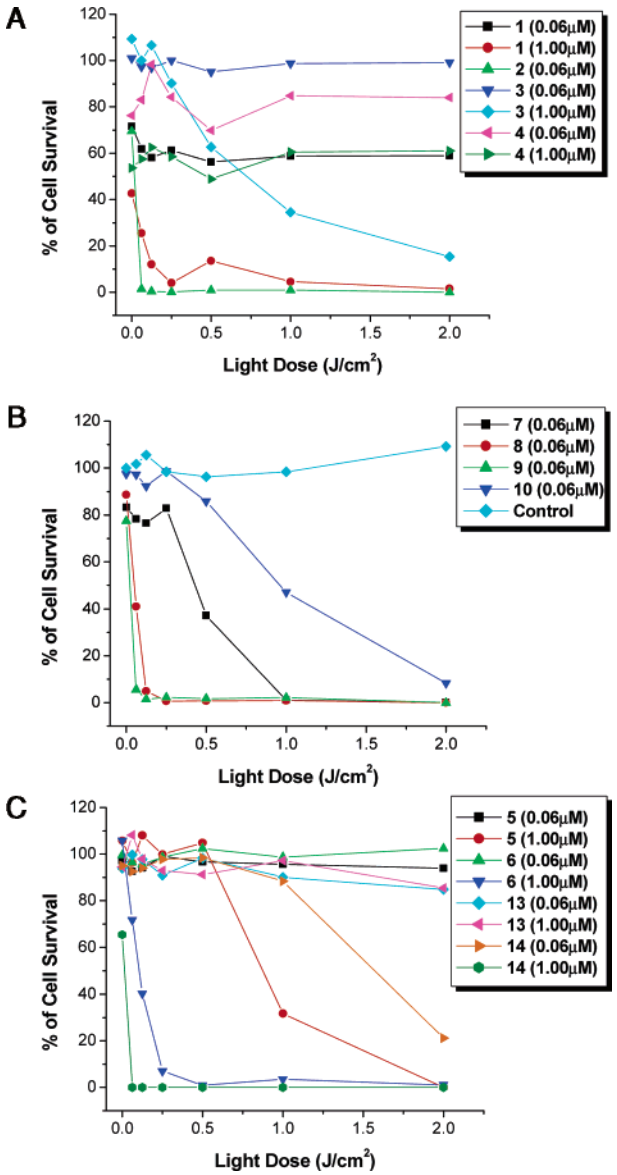


Figure 3. In vivo PDT efficacy studies of pyropheophorbide-*a* analogues (RIF tumor).

possesses an ability to replace the radioactive probes currently being used. Interestingly, as can be seen in Figures 2 and 3, compared to free-base analogues, the related indium complexes were found to have higher PDT efficacy with enhanced PBR binding ability (**8** > **7**, **14** > **13**). Although the overall lipophilicity of **5** and **6** was similar, a significant difference in their in vitro photosensitizing efficacy was observed (Figure 3C), with **6** being more effective than **5**, which correlates with the PBR binding values (Figure 2B). On the other hand, **1** has higher PBR binding affinity than **7** but was less

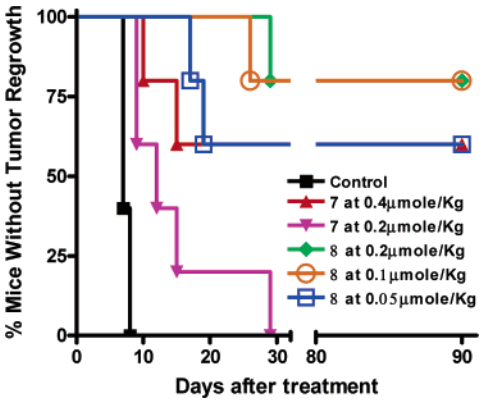


Figure 4. In vivo photosensitizing efficacy of **7** and **8** in C3H mice bearing RIF tumors (10 mice/group). The mice were treated with laser light (135 J/cm^2, 75 mW/cm^2, 665 nm for **7** and 655 nm for **8**) at 24 h postinjection of the drug. The control has no photosensitizer and no light exposure.

efficacious. In summary, in the pyropheophorbide-*a* series, no direct correlation between PBR binding affinity and in vitro PDT efficacy was observed.

On the basis of in vitro screening, the indium complexes in general were found to be more effective than the corresponding free-base counterparts. Among the free-base and metalated analogues investigated so far, **7** and the related In(III) analogue **8** produced the best photosensitizing activity. Therefore, these compounds were tested for in vivo PDT efficacy in C3H mice bearing RIF tumors at variable drug doses. The results summarized in Figure 4 show that at 0.2 μmol/kg, compared to **7**, the corresponding In(III) complex **8** was more effective with 8/10 mice tumor-free on day 90. The therapeutic response of **7** at 0.4 μmol/kg was similar to that of the In(III) analogue at 0.05 μmol/kg, which was 8-fold more than the parent compound **7**.

In summary, our results with a series of pyropheophorbide-*a* analogues suggest that the presence of a core metal makes a significant difference in PBR binding and photosensitizing efficacy. Because of their strong fluorescence, these compounds have great potential as fluorophores to replace radioactive PBR probes (e.g., tritiated **17**). The presence of metals also had a significant impact on photosensitizing efficacy, with the In-(III) complexes proving to be the most efficacious, which could be a result of their higher singlet oxygen production. The detailed photophysical characterization of these compounds is currently underway. Replacing cold indium with radioactive indium (In-III, half-life 72 h) may also provide an opportunity to develop bifunctional agents for tumor-imaging (nuclear imaging) and photodynamic therapy. Recently, the In(III) complex of pyropheophorbide was reported as a potential candidate for the treatment of age-related macular degeneration (AMD),[13,14] a leading cause of blindness that is most prevalent in people over the age of 60. Therefore, the compounds discussed herein with variable lipophilicity could be ideal candidates for investigating structure−activity relationships within a particular series of compounds for such indication, and these studies are currently in progress.

Acknowledgment. Financial support from NIH Grant CA 55792 and the shared resources of the RPCI Support Grant P30CA16056 is highly appreciated. We also thank the National Science Foundation under the Integrative Graduate Education and Research Traineeship (Grant DGE0114330) for providing a graduate fellowship to A.G. The help rendered by Adam Sumlin in in vivo experiments is highly appreciated.

Supporting Information Available: Synthesis, NMR spectra, and HRMS analyses of new compounds, detailed PBR binding, and in vitro PDT efficacy data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kozikowski, A. P.; Kotoula, M.; Ma, D.; Boujrad, N.; Tuckmantel, W.; Papadopoulos, V. Synthesis and Biology of a 7-Nitro-2,1,3-benzoxadiazol-4-yl Derivative of 2-Phenylindole-3-acetamide: A Fluorescent Probe for the Peripheral-Type Benzodiazepine Receptor. *J. Med. Chem*, **1997**, *40* (16), 2435−2439 and references therein.

(2) Han, Z.; Slack, R. S.; Li, W. P.; Papadopoulos, V. Expression of Peripheral Benzodiazepine Receptor (PBR) in Human Tumors: Relationship to Breast, Colorectal, and Prostate Tumor Progression. *J. Recept. Signal Transduction Res.* **2003**, *23*, 225−238.

(3) Verma, A.; Facchina, S. L.; Hirsch, D. J.; Song, S. Y.; Dillahey, L. F.; Williams, J. R.; Snyder, S. H. Photodynamic Tumor Therapy: Mitochondrial Benzodiazepine Receptors as a Therapeutic Target. *Mol. Med.* **1998**, *4*, 40−50.

(4) Dougherty, T. J.; Sumlin, A. B.; Greco, W. R.; Weishaupt, K. R.; Vaughan, L. A.; Pandey, R. K. The Role of the Peripheral Benzodiazepine Receptor in Photodynamic Activity of Certain Pyropheophorbide Ether Photosensitizers: Albumin Site II as a Surrogate Marker for Activity. *Photochem. Photobiol*. **2002**, *76* (1), 91−97.

(5) Kessel, D.; Antolovich, M.; Smith, K. M. The Role of the Peripheral Benzodiazepine Receptor in the Apoptotic Response to Photodynamic Therapy. *Photochem. Photobiol.* **2001**, *74* (2), 346−349.

(6) Morris, R. L.; Varnes, M. E.; Kenney, M. E.; Li, Y. S.; Azizuddin, K.; McEnery, M. W.; Oleinick N. L. The Peripheral Benzodiazepine Receptor in Photodynamic Therapy with the Phthalocyanine Photosensitizer Pc 4. *Photochem. Photobiol*. **2002**, *75* (6), 652−661.

(7) Oleinick, N. L.; Morris, R. L.; Belichenko, T. The Role of Apoptosis in Response to Photodynamic Therapy: What, Where, Why, and How. *Photochem. Photobiol. Sci.* **2002**, *1* (1), 1−21.

(8) Kessel, D.; Luo, Y. Intracellular Sites of Photodamage as a Factor in aPoptotic Cell Death. *J. Porphyrins Phthalocyanines* **2001**, *5* (2), 181−184.

(9) Zhang, Z.; Blesington, D.; Hui, L.; Busch, T. M.; Glickson, J.; Luo, Q.; Chance, B.; Zheng, G. Redox Ratio of Mitochondria as an Indicator for the Response of Photodynamic Therapy. *J. Biomed. Opt*. **2004**, *9* (4), 772−778.

(10) Chen, Y.; Graham, A.; Potter, W.; Morgan, J.; Vaughan, L.; Bellnier, D. A.; Henderson, B. W.; Oseroff, A.; Dougherty, T. J.; Pandey, R. K. Bacteriopurpurinimides: Highly Stable and Potent Photosensitizers for Photodynamic Therapy. *J. Med. Chem*. **2002**, *45* (2), 255−258.

(11) Mosinger, J.; Micka, Z. Quantum Yields of Singlet Oxygen of Metal Complexes of *meso*-Tetrakis(sulphonatophenyl)porphine. *J. Photochem. Photobiol.* **1997**, *107*, 77−82.

(12) Chen, Y.; Sumlin, A.; Morgan, J.; Dougherty, T. J.; Gryshuk, A.; Oseroff, A.; Henderson, B. W.; Dougherty, T. J.; Pandey, R. K. Synthesis and Photosensitizing Efficacy of Isomerically Pure Bacteriopurpurinimide. *J. Med. Chem.* **2004**, *47* (20), 4814−4817.

(13) Dolmans, D. E. J. G. J.; Kadambi, A.; Hill, J. S.; Waters, C. A.; Robinson, B. C.; Walker, J. P.; Fukumura, D.; Jain, R. K. Vascular Accumulation of a Novel Photosensitizer, MV6401, Causes Selective Thrombosis in Tumor Vessels after Photodynamic Therapy. *Cancer Res*. **2002**, *62*, 2151−2156.

(14) Ciulla, T. A.; Criswell, M. H.; Snyder, W. J. Small, W., IV. Photodynamic Therapy with PhotoPoint Photosensitizer MV6401, Indium Chloride Methyl Pyropheophorbide, Achieves Selective Closure of Rat Corneal Neovacularisation and Rabbit Choriocapillaris. *Br. J. Ophthalmol.* **2005**, *89*, 113−119.

JM050039K