Water soluble distyryl-boradiazaindacenes as efficient photosensitizers for photodynamic therapy†

Serdar Atilgan,[a] Zeynep Ekmekci,[a] A. Lale Dogan,[b] Dicle Guc[b] and Engin U. Akkaya*[a]

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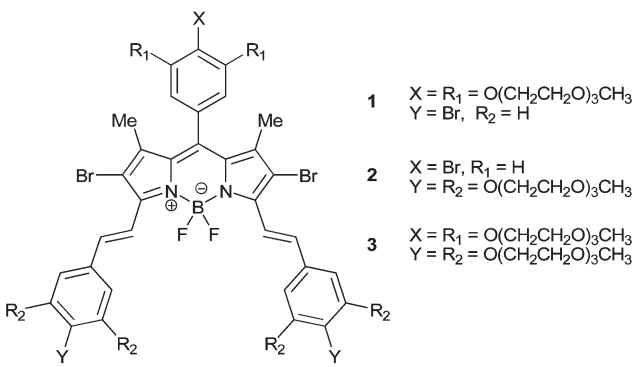
We introduce a novel class of water soluble, extended conjugation boradiazaindacene dyes which are efficient singlet oxygen generators and have spectacular photoinduced cytotoxicity when excited in the "therapeutic window" of the electromagnetic spectrum.

Photodynamic therapy (PDT) is a noninvasive method of treating malignant tumors[1] and age-related macular degeneration,[2] and is particularly promising in the treatment of multidrug-resistant (MDR) tumors.[3] The PDT strategy is based on the preferential localization of certain photosensitizers in tumor tissues upon systemic administration. The sensitizer is then excited with red or near infrared (NIR) light, generating reactive oxygen species (ROS) including singlet oxygen ($^1O_2$) and thus irreversibly damaging tumor cells. Current practice of PDT is limited to a few functionalized porphyrins, however these compounds are not considered to be ideal drugs for use in PDT. Among the limitations, the most prominent is the low extinction coefficient of porphyrins in the body's therapeutic window (650–800 nm, low absorptivity region in typical mammalian tissues). Therefore, there is a significant impetus to develop novel and more efficient sensitizers for use in PDT. Partially reduced porphyrins are an alternative.[4] As non-porphyrin photosensitizers, texaphyrins,[5] phthalocyanines,[6] squaraines,[7] chalcogenopyrylium dyes,[8] azaboradiazaindacenes[9] and perylenediimides[10] have been suggested. There is also a recent report[11] of a diiodo-substituted boradiazaindacene (BODIPY) as a sensitizer, but it requires excitation outside of the therapeutic window.

Among the requirements for a successful photosensitizer, in addition to long wavelength excitability, singlet oxygen generation capacity and good solubility characteristics are of paramount importance. In many sensitizer systems, to overcome the hydrophobicity of the dye, micellar drug formulations are used, but unfortunately such emulsifying agents have been reported to elicit anaphylactic reactions *in vivo*.[12] Therefore, water solubility remains an important issue.

Boradiazaindacenes (BODIPY dyes or difluoroboradipyrrines) are well known fluorescent dyes[13] with emerging applications in light harvesting systems[14] and chemosensors.[15] In recent years, there have been exciting reports[16] demonstrating their versatile chemistry. Boradiazaindacenes with methyl substituents on 3 or 5 positions were previously shown to undergo condensation reactions with aldehydes to yield longer wavelength absorbing dyes (100 nm red shifted) with intramolecular charge transfer (ICT) characteristics.[16e,17] The extended conjugation in these dyes moves the absorption peak to 590–600 nm. Incorporation of a second styryl group would result in further red shifts in the absorption spectrum. There has been a single example of such a boradiazaindacene,[18] but it was not obtained by the modification of a boradiazaindacene core, instead styryl-pyrroles had to be synthesized and then converted into boradiazaindacene dyes. Recently, we discovered[16c] a direct method of conversion of the boradiazaindacene dyes into distyryl-substituted boradiazaindacenes. These dyes have strong absorptions in the 650–680 nm region. In order to transform these novel dyes into potential PDT reagents, we wanted to incorporate two structural modifications. First, to facilitate the intersystem crossing *via* the heavy atom effect, bromine substituents were placed. Secondly, to improve water solubility, without compromising organic solubility which is essential for chromatographic manipulations, we introduced a number of amphiphilic triethyleneglycol moieties into the parent structure. It is also known that oligoethyleneglycol moieties confer cell permeability and tumor targeting characteristics on photosensitizers.[19] Thus, three dyes shown in Scheme 1 were targeted. The syntheses are straightforward, starting with the known 3,4,5-trihydroxybenzaldehyde derivative[20] or 4-bromobenzaldehyde in the case of compound **2**. Boradiazaindacene dyes, obtained using standard procedures, were brominated at the 2 and 6 positions using NBS and the free radical initiator AIBN. Electrophilic aromatic substitution using Br_2 did not yield the desired compounds in acceptable yields, although there is literature precedence for similar compounds.[21] The key step is the final double condensation leading to the target molecules. The absorption



Scheme 1

[a]*Department of Chemistry, Middle East Technical University, Ankara, TR-06531, Turkey. E-mail: akkayaeu@metu.edu.tr; Fax: 90 312 210-3200; Tel: 90 312 210-5126*
[b]*Department of Basic Oncology, Oncology Institute, Hacettepe University, Sihhiye, Ankara, TR-06100, Turkey*

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and emission characteristics of the dyes were studied. Alkoxystyryl substituents in **2** and **3** introduced larger red shifts in both absorption and emission spectra compared to the 4-bromostyryl substituent in **1** (Fig. 1). The effect of concentration on the absorbance spectra was studied. The dye **3**, with an absorption peak at 660 nm (ε = 102,000 M^{-1} cm^{-1}) and nine triethyleneglycol arms, showed no signs of aggregation in buffered aqueous solutions even at mM concentrations. The singlet oxygen generation efficiency was studied using the singlet oxygen trap 1,3-diphenylisobenzofuran (DPBF). In order to facilitate comparison to previously reported sensitizers, the activity was studied in 2-propanol. Even at very low concentration levels of 9 nM dyes (**1–3**) and under relatively weak red LED irradiation at 625 nm, remarkable efficiency was observed (ESI). No degradation of the trap was observed either in the dark or with rigorously deaerated solutions. Encouraged by these observations, we tested the most promising sensitizer **3** (considering solubility characteristics) on K562 human erythroleukemia cells. A standard MTT assay was used to quantify cytotoxicity. Cells which were kept in the dark either with or without the sensitizer at 37 °C in a humidified incubator (5% CO_2) showed no decrease in viability. However, in the presence of the sensitizer **3** and under red LED irradiation at 625 nm at 2.5 mW cm^{-2} fluence rate for 4 hours, followed by an incubation period of an additional 20 h, very large decreases in cell viability were observed (Fig. 2). The EC_{50} value (median effective concentration; concentration required for 50% of the maximum possible effect) under these conditions was less than 200 nM. A longer incubation period following irradiation is known to further decrease cell viability. A two color staining experiment with acridine orange and propidium iodide under a fluorescence microscope reveals that under LED irradiation and in the presence of the sensitizer, the membrane integrity is compromised and preferential staining with propidium iodide (PI) takes place, resulting in red fluorescence emission (Fig. 3).

In conclusion, we have demonstrated that novel distyryl-boradiazaindacene dyes with bromo substituents on the fluorochrome π-system are very efficient singlet oxygen generators. In addition, these water soluble photosensitizers were shown to have spectacular photoinduced cytotoxicity at very low concentrations and even under low fluence rate LED irradiation. Dark toxicity was nil at the concentration range studied. Structure–activity fine tuning of the sensitizer with further *in vitro* and *in vivo* studies is likely to result in highly promising reagents for use in PDT. Our work to these ends is in progress.

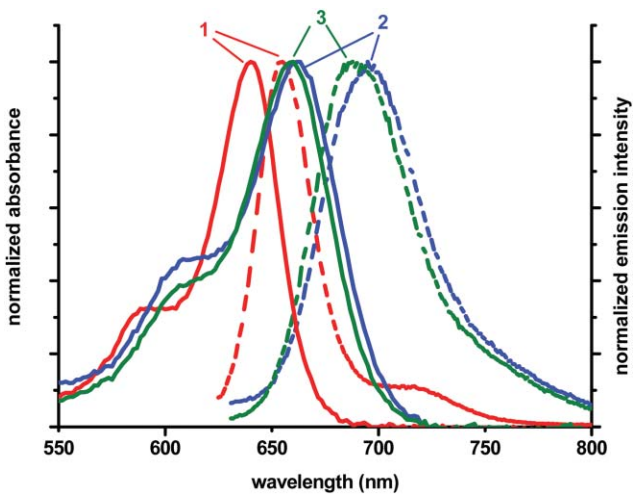

Fig. 1 Normalized absorption (solid lines) and emission spectra (dashed lines) of sensitizers (**1–3**) in ethanol.

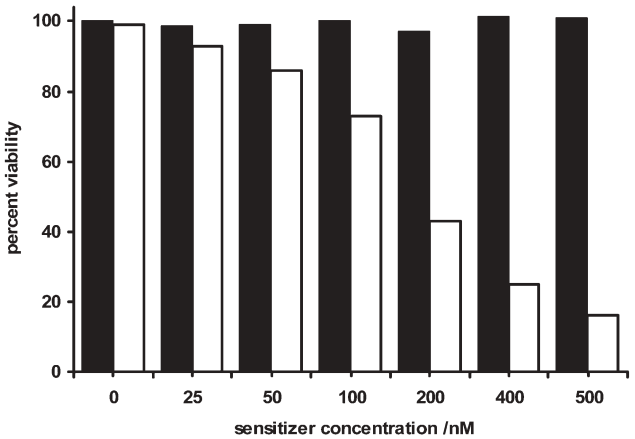

Fig. 2 Percent viability as determined by a standard MTT assay with K562 cells kept in full medium at 37 °C in an incubator, in the presence of varying concentrations of the sensitizer **3**. The black bars show cell viability following 24 h incubation in the dark and the white bars show percent viability at the indicated concentrations under 4 h irradiation with red LED at 2.5 mW cm^{-2} fluence rate, followed by 20 h incubation in the dark at 37 °C. Percent viability values shown here are the averages of 4 runs.

Fig. 3 Fluorescence microscope images of acridine orange (AO) and propidium iodide (PI) stained K562 cells, incubated in full medium with 500 nM sensitizer **3** in the dark (the middle panel); irradiated with red LED at 625 nm for 4 hours, followed by 20 h incubation at 37 °C in the presence of 500 nM sensitizer **3** (right panel); and incubated in the dark for 24 h (left panel). Live cells were preferentially stained with AO (green) and dead cells with PI (red) due to increased cellular permeability.

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Notes and references

1 R. Bonnet, *Chemical Aspects of Photodynamic Therapy*; Gordon and Breach Science: Amsterdam, 2000.
2 R. Bonnet and G. Martinez, *Tetrahedron*, 2001, **57**, 9513–9547.
3 M. A. M. Capella and L. S. J. Capella, *J. Biomed. Sci.*, 2003, **10**, 361–366.
4 (*a*) Y. Chen, A. Graham, W. Potter, J. Morgan, L. Vaughan, D. A. Bellnier, B. W. Henderson, A. Oseroff, T. J. Dougherty and

R. K. Pandey, *J. Med. Chem.*, 2002, **45**, 255–258; (*b*) G. Li, S. K. Pandey, M. P. Dobhal, R. Mehta, Y. Chen, A. Gryshuk, K. Olson, A. R. Oseroff and R. K. Pandey, *J. Org. Chem.*, 2004, **69**, 158–172.

5 (*a*) J. L. Sessler and R. A. Miller, *Biochem. Pharmacol.*, 2000, **59**, 733–739; (*b*) T. D. Mody and J. L. Sessler, *J. Porphyrins Phthalocyanines*, 2001, **5**, 134–142; (*c*) D. J. Magda, Z. Wang, N. Gerasimchuk, W. Wei, P. Anzenbacher and J. L. Sessler, *Pure Appl. Chem.*, 2004, **76**, 365–374.

6 M. E. Rodriguez, F. Moran, A. Bonansea, M. Monetti, D. A. Fernandez, C. A. Strassert, V. Rivarola, J. Awruch and L. E. Dicelio, *Photochem. Photobiol. Sci.*, 2003, **2**, 988–994.

7 (*a*) D. Ramaiah, I. Eckert, K. T. Arun, L. Weidenfeller and B. Epe, *Photochem. Photobiol.*, 2002, **76**, 672–677; (*b*) D. Ramaiah, I. Eckert, K. T. Arun, L. Weidenfeller and B. Epe, *Photochem. Photobiol.*, 2004, **79**, 99–104.

8 M. R. Detty, S. L. Gibson and S. J. Wagner, *J. Med. Chem.*, 2004, **47**, 3897–3915.

9 (*a*) J. Killoran, L. Allen, J. F. Gallagher, W. M. Gallagher and D. F. O'Shea, *Chem. Commun.*, 2002, 1862–1863; (*b*) A. Gorman, J. Killoran, C. O'Shea, T. Kenna, W. M. Gallagher and D. F. O'Shea, *J. Am. Chem. Soc.*, 2004, **126**, 10619–10631; (*c*) S. O. McDonnell, M. J. Hall, L. T. Allen, A. Byrne, W. M. Gallagher and D. F. O'Shea, *J. Am. Chem. Soc.*, 2005, **127**, 16360–16361.

10 F. Yukruk, A. L. Dogan, H. Canpinar, D. Guc and E. U. Akkaya, *Org. Lett.*, 2005, **7**, 2885–2887.

11 T. Yogo, Y. Urano, Y. Ishitsuka, F. Maniwa and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 12162–12163.

12 (*a*) D. Dye and J. Watkins, *Br. Med. J.*, 1980, **280**, 1353–1353; (*b*) L. B. Michaud, *Ann. Pharmacother.*, 1997, **31**, 1402–1404.

13 (*a*) A. Treibs and F.-H. Kreuzer, *Justus Liebigs Ann. Chem.*, 1968, **718**, 208–223; (*b*) R. P. Haugland, *The Handbook. A Guide to Fluorescent Probes and Labeling Technologies*, 10th edn, Molecular Probes, Inc.: Eugene, 2005.

14 (*a*) A. Burghart, L. H. Thoresen, J. Chen, K. Burgess, F. Bergstrom and L. B. A. Johansson, *Chem. Commun.*, 2000, 2203–2204; (*b*) G. Ulrich, C. Goze, C. M. Guardigli, A. Roda and R. Ziessel, *Angew. Chem., Int. Ed.*, 2005, **44**, 3694–3698.

15 Recent examples: (*a*) B. Turfan and E. U. Akkaya, *Org. Lett.*, 2002, **4**, 2857–2859; (*b*) G. Ulrich and R. Ziessel, *J. Org. Chem.*, 2004, **69**, 2070–2083; (*c*) Y. Gabe, Y. Urano, K. Kikuchi, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2004, **126**, 3357–3367; (*d*) K. Rurack, M. Kollmannsberger, U. Resch-Genger and J. Daub, *J. Am. Chem. Soc.*, 2000, **122**, 968–969; (*e*) A. Coskun and E. U. Akkaya, *J. Am. Chem. Soc.*, 2005, **127**, 10464–10465; (*f*) N. Basaric, M. Baruah, W. Qin, B. Metten, M. Smet, W. Dehaen and N. Boens, *Org. Biomol. Chem.*, 2005, **3**, 2755–2761; (*g*) L. Zeng, E. W. Miller, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2006, **128**, 10–11.

16 (*a*) R. Ziessel, C. Goze, G. Ulrich, M. Cesario, P. Retailleau, A. Harriman and J. P. Rostron, *Chem.–Eur. J.*, 2005, **11**, 7366–7378; (*b*) M. Baruah, W. Qin, R. A. L. Vallee, D. Beljonne, T. Rohand, W. Dehaen and N. Boens, *Org. Lett.*, 2005, **7**, 4377–4380; (*c*) Z. Dost, S. Atilgan and E. U. Akkaya, *Tetrahedron*, 2006, **62**, 8484–8488; (*d*) T. Rohand, M. Baruah, W. Qin, N. Boens and W. Dehaen, *Chem. Commun.*, 2006, 266–268; (*e*) A. Coskun, E. Deniz and E. U. Akkaya, *Org. Lett.*, 2005, **7**, 5187–5189; (*f*) N. Saki, T. Dinc and E. U. Akkaya, *Tetrahedron*, 2006, **62**, 2721–2725.

17 (*a*) R. P. Haugland and H. C. Kang (Molecular Probes Inc.), US Patent 4,774,339, 1988; (*b*) K. Rurack, M. Kollmannsberger and J. Daub, *Angew. Chem., Int. Ed.*, 2001, **40**, 385–387; (*c*) A. Coskun and E. U. Akkaya, *Tetrahedron Lett.*, 2004, **45**, 4947–4949.

18 K. Rurack, M. Kollmannsberger and J. Daub, *New J. Chem.*, 2001, **25**, 289–292.

19 (*a*) M. R. Hamblin, J. L. Miller, I. Rizvi, B. Ortel, E. V. Maytin and T. Hasan, *Cancer Res.*, 2002, **61**, 7155–7162; (*b*) S. K. Sahoo, T. Sawa, J. Fang, S. Tanaka, Y. Miyamoto, T. Akaike and H. Maeda, *Bioconjugate Chem.*, 2002, **13**, 1031–1038.

20 M. A. Oar, J. M. Serin, W. R. Dichtel, J. M. J. Frechet, T. Y. Ohulchanskyy and P. N. Prasad, *Chem. Mater.*, 2005, **17**, 2267–2275.

21 M. Shah, K. Thangaraj, M. L. Soong, L. Wolford, J. H. Boyer, I. R. Politzer and T. G. Pavlopoulos, *Heteroat. Chem.*, 1990, **1**, 389–399.

