

Synthesis, Photophysical, Electrochemical, Tumor-Imaging, and Phototherapeutic Properties of Purpurinimide-N-substituted Cyanine Dyes Joined with Variable Lengths of Linkers

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Abstract

ABSTRACT: Purpurinimide methyl esters, bearing variable lengths of N-substitutions, were conjugated individually to a cyanine dye with a carboxylic acid functionality. The results obtained from *in vitro* and *in vivo* studies showed a significant impact of the linkers joining the phototherapeutic and fluorescence imaging moieties. The photosensitizer–fluorophore conjugate with a PEG linker showed the highest uptake in the liver, whereas the conjugate linked with two carbon units showed excellent tumor-imaging and PDT efficacy at 24 h postinjection. Whole body imaging and biodistribution studies at variable time points portrayed enhanced fluorescent uptake of the conjugates in the tumor compared to that in the skin. Interestingly, the conjugate with the shortest linker and the one joining with two

carbon units showed faster clearance from normal organs, e.g., the liver, kidney, spleen, and lung, compared to that in tumors. Both imaging and PDT efficacy of the conjugates were performed in BALB/c mice bearing Colon26 tumors. Compared to the others, the short linker conjugate showed poor tumor fluorescent properties and as a corollary does not exhibit the dual functionality of the photosensitizer–fluorophore conjugate. For this reason, it was not evaluated for *in vivo* PDT efficacy. However, in Colon26 tumor cells (*in vitro*), the short linker was highly effective. Among the conjugates with variable linkers, the rate of energy transfer from the purpurinimide moiety to the cyanine moiety increased with decreasing linker length, as examined by femtosecond laser flash photolysis measurements. No electron transfer from the purpurinimide moiety to the singlet excited state of the cyanine moiety or from the singlet excited state of the cyanine moiety to the purpurinimide moiety occurred as indicated by a comparison of transient absorption spectra with spectra of the one-electron oxidized and one-electron reduced species of the conjugate obtained by spectroelectrochemical measurements.

