proper wavelength of light will produce a measurable fluorescent signal through a FRET interaction. Quantification of the signal intensity provides a means for quantifying agonist activity of test compounds. In a similar manner, the presence of an antagonist will block activation of the GPCR signaling pathway, decreasing phosphorylation of the GFP-tagged substrate. This will decrease the intensity of the FRET signal produced upon irradiation with light, thus providing a method for measuring antagonist activity of test compounds.[52]

Amplified Luminescent Proximity Homogeneous Assay (AlphaScreen™)

The need to develop assay capable of avoiding background fluorescence from assay components, reagents, plates, and other materials also lead to the exploration of methods that could produce fluorescent signals in the absence of a FRET interaction. The prospect of inducing fluorescent signals for the purposes of monitoring biological processes through the generation of singlet oxygen was first introduced in by Ullman in 1994.[54] Originally referred to as luminescent oxygen channeling assay (LOCI), reagents and assay kits that employ this technology are now readily available for drug discovery purposes under the name AlphaScreen™ and are marketed by Perkin Elmer. In some ways, the AlphaScreen™ is similar to FRET and TRFRET assay systems, as all three systems depend on the interaction of a donor and acceptor for the production of a measurable signal. Unlike FRET and TRFET, however, irradiation of the donor does not lead to an energy transfer to the acceptor followed by light emission. AlphaScreen™ technology employs the coupling of the photosensitizer phthalocyanine, which produces singlet oxygen (an excited state oxygen) upon irradiation at 680 nm, and thioxene derivatives, which emit light (520–620 nm) in the presence of singlet oxygen (Figure 4.23).[55]

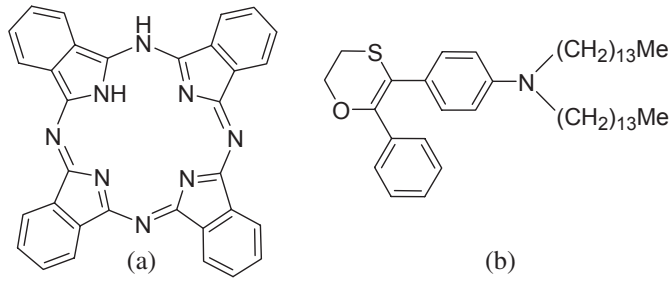

FIGURE 4.23 AlphaScreen™ assays depend on phthalocyanine derivatives (a) ability to generate singlet oxygen upon absorption of light and the reaction of thioxene derivatives (b) with singlet oxygen coupled with the emission of light.