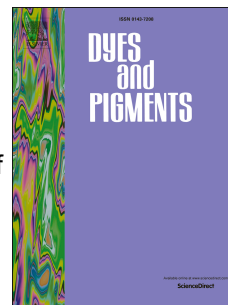


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# Hydrophobic resorufamine derivatives: potent and selective red fluorescent probes of the endoplasmic reticulum of mammalian cells

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## ABSTRACT

The endoplasmic reticulum (ER) of eukaryotic cells plays critical roles in the processing of secreted and transmembrane proteins. Defects in these functions are associated with a wide range of pathologies. To image this organelle, cells are often treated with fluorescent ER-Tracker dyes. Although these compounds are selective, existing red fluorescent probes of the ER are costly glibenclamide derivatives that inhibit ER-associated sulphonylurea receptors. To provide simpler and more cost-effective red fluorescent probes of the ER, we synthesized amino analogues of the fluorophore resorufin. By varying the polarity of linked substituents, we identified hexyl resorufamine (HRA) as a novel hydrophobic (cLogD (pH 7.4) = 3.8) red fluorescent (Ex. 565 nm; Em. 614 nm in ethanol) molecular probe. HRA is exceptionally bright in organic solvents (quantum yield = 0.70), it exclusively localizes to the ER of living HeLa cells as imaged by confocal microscopy, it is effective at concentrations as low as 100 nM, and it is non-toxic under these conditions. To examine its utility, we used HRA to facilitate visualization of small molecule-mediated release of a GFP-GPI fusion protein from the ER into the secretory pathway. HRA represents a potent, selective, and cost-effective probe for imaging and labeling the ER.

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