On the Synthesis of Quinone-based BODIPY Hybrids: New Insights on Antitumor Activity and Mechanism of Action in Cancer Cells

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Abstract: Fluorescent quinone-based BODIPY hybrids were synthesised and characterised by NMR analysis and mass spectrometry. We measured their cytotoxic activity against cancer and normal cell lines, performed mechanistic studies by lipid peroxidation and determination of reduced (GSH) and oxidized (GSSG) glutathione, and imaged their subcellular localisation by confocal microscopy. Cell imaging experiments indicated that nor-β-lapachone-based BODIPY derivatives might preferentially localise in the lysosomes of cancer cells. These results assert the potential of hybrid quinone-BODIPY derivatives as promising prototypes in the search of new potent lapachone antitumor drugs.

Keywords: Quinone; BODIPY; Lapachone; Cancer; Subcellular localization.
1. Introduction

Over the years, medicinal chemists have been improving several chemical strategies for the discovery of new drugs.\(^1\) For example, enzymatic targets have been used in molecular docking studies for computational drug design to predict the chemical structure of potential inhibitors.\(^2\) Looking for new directions, late stage functionalization of drug-like molecules arises as a modern and elegant strategy in drug discovery programs, as recently reviewed by Cernak and coworkers.\(^3\) Another strategy stems from the inspiration of natural products. Compounds isolated from diverse natural sources can be used as starting points to generate novel structures with varied biological activities.\(^4\) As discussed by Newman and Cragg,\(^5\) natural products still have a major role in the design of new antitumor drugs.

The search for antitumor drugs is a big challenge with multiple questions.\(^6\) Some of the key questions are associated to the mechanism of action of cytotoxic drugs and the subcellular organelles where drugs preferentially accumulate. These questions can be generally answered by bioimaging experiments\(^7\) as well as \textit{in vitro} biological assays.\(^8\)

Lapachones are naturally occurring naphthoquinones and among the most studied quinones due their potent antitumor activity.\(^9\) Lately, diverse lapachone derivatives have been reported as potent cytotoxic drugs against different cancer cell lines.\(^10\) In this regard, advances in the synthesis of lapachones with potent antitumor activity have been accomplished via modification of the A- and C-rings,\(^11\) with recent progress being achieved by da Silva Júnior,\(^12\) Pinto,\(^13\) Hong,\(^14\) Ferreira,\(^15\) Bonifazi,\(^16\) among others\(^17\) (Scheme 1A).
As studied by the Boothman group, the mechanism of action of most β-lapachones is related to the destruction of cancer cells with elevated levels of NAD(P)H:quinone oxidoreductase 1 (NQO1).\textsuperscript{18,19,20} Recently, Ohayon and coworkers\textsuperscript{21} have shed some light on the possibility that β-lapachones might act non-reversibly as inhibitors of deubiquitinases. The therapeutic effect of β-lapachones could be also related to the oxidation of ubiquitin specific peptidase 2 (USP2), as a likely downstream effect of reactive oxygen species (ROS) generation.

In this context, unraveling the mechanism of action of antitumor lapachone derivatives is a recurring challenge for the scientific community. In the last few years, our group has dedicated great efforts to prepare fluorescent lapachones by hybridization of the quinoidal moiety with fluorescent compounds, such as the benzothiadiazole structure (Scheme 1B).\textsuperscript{22} Fluorescent lapachones allow us to answer critical questions related to their mechanism of pharmacological action, including subcellular localization studies in live cells.\textsuperscript{23} Recently, our group has described the first fluorescent lapachone-BODIPY hybrid as well as biological studies, including cytotoxic activity in different cancer cell lines and cell imaging experiments (Scheme 1B).\textsuperscript{24}

Herein, we have developed a new chemical platform to prepare new lapachone derivatives coupled to the fluorescent BODIPY core, which has excellent photophysical properties.\textsuperscript{24b} Lapachones exhibit potent antitumor activity due to their ability to act on multiple targets, as we have recently demonstrated.\textsuperscript{25} This family of quinoidal derivatives can be divided into α-lapachones (para-quinones) and β-lapachones (ortho-quinones). We have demonstrated that both α- and β-lapachones display cytotoxic activity in cancer cells.\textsuperscript{26} In the present work, we have synthesized a small collection of α- and β-lapachone derivatives (Scheme 1C) and evaluated their cytotoxicity in human
cancer and non-cancerous cell lines. Furthermore, we have also prepared fluorescent BODIPY-lapachones to examine the subcellular localization of lapachone-based compounds in cancer cells and any derived effects in their cytotoxic activity.

Scheme 1. Overview of lapachone derivatives and the design of quinone-based BODIPY hybrids.
2. Results and discussion

The synthesis of the quinone-based BODIPY hybrids 8-11 was accomplished by a convergent synthetic route,\textsuperscript{24} using a classical copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reaction.\textsuperscript{27} We used an alkyne-containing BODIPY and azide-containing quinones to assemble fluorescent, hybrid quinoidal-BODIPY molecules. We started the preparation of quinone-containing BODIPYs by the synthesis of boron-dipyrromethene with a terminal alkyne for subsequent CuAAC reaction. There are numerous reports on synthetic approaches to generate BODIPY scaffold with bespoke spectral properties.\textsuperscript{28} Among them, we used the methodology from the Dehaen group with minor modifications\textsuperscript{30} for preparing compound 3. With regards to the azides 4-7, we initially prepared 3-azido-nor-α-lapachone (4) and 4-azido-α-lapachone (5) from lapachol. The methodology for the synthesis of 4 and 5 is well described in the literature.\textsuperscript{31,32} Cyclic lapachones were obtained by insertion of bromine atoms and nucleophilic substitution with sodium azide to afford the respective clickable analogues 4 and 5.

The synthesis of azides 6 and 7 was conducted following methodologies described by our group.\textsuperscript{25,26,33} Compound 6 was prepared from C-allyl lawsone upon reaction with iodine to obtain the cyclized product, which underwent nucleophilic substitution with sodium azide. Finally, the last clickable derivative 7 was obtained from nor-lapachol, previously synthesized from lapachol, by the well-established Hooker oxidation method.\textsuperscript{34} Nor-lapachol was transformed into the key intermediate 3-bromo-nor-β-lapachone, which was reacted with sodium azide to render 3-azide-nor-β-lapachone (7). Once the azido-quinones 4-7 and the alkyne BODIPY 3 were obtained,
we readily prepared a small collection of **quinone-based BODIPY hybrids** (8-11) in good yields (Scheme 2).

![Scheme 2. Synthesis of quinone-based BODIPY hybrids 8-11.](image)

Lapachone-based BODIPYs 8-11 were evaluated for in vitro cytotoxicity using an MTT assay in five human cancer cell lines (i.e. PC3, SF295, MDA-MB435, SW620 and HCT-116) and two murine non-cancer (L929 and V79) cells. As previously reported, the compounds 8-11 were classified according to their cytotoxicity against cancer cells as highly active (IC\textsubscript{50} < 2 µM), moderately active (2 µM < IC\textsubscript{50} < 10 µM), or inactive (IC\textsubscript{50} > 10 µM). As shown in Table 1, no active para-quinones (8-10) were
identified. We have reported α-lapachone derivatives with potent cytotoxicity against cancer cells prepared by C-ring modification and insertion of arylamino groups at positions C-3 (nor-α-lap) and C-4 (α-lap).\textsuperscript{36} In fact, molecular hybridization\textsuperscript{37} between α-lapachones and 1,2,3-triazole groups has been reported as an efficient strategy for antitumour compounds.\textsuperscript{22} Herein, the same approach rendered hybrids compounds (8-10) that were not active against any the tested cancer cell lines, with IC\textsubscript{50} values round 8 μM for all compounds.

On the other hand, compound 11 exhibited significant cytotoxicity against all human cancer cell lines with IC\textsubscript{50} values in the low micromolar range (i.e. 0.3 to 0.9 μM, Table 1). This observation is in line with previous reports of ortho-quinones (i.e. β-lapachone) being more active than the corresponding para-quinones, such as α-lapachone.\textsuperscript{38} Moreover, Abreu and collaborators\textsuperscript{39} have shown that the radicals generated during the reduction of β-lapachones reduction confer more stability to reduced ortho-quinone derivatives when compared to their reduced para-isomers. As a result, ortho-quinones, such as the hybrid derivative 11, might present better redox cycling ability, which is the ability of a molecule to undergo repeated reduction and oxidation, producing more free radicals than compounds 8-10 (para-quinones), which is in agreement with the absence of cytotoxicity determined for 8-10.

\textbf{Table 1.} Cytotoxic activity expressed by IC\textsubscript{50} (μM, 95% CI) of compounds 8-11 in cancer\textsuperscript{a} and normal cells\textsuperscript{b} after 72 h. Values obtained by nonlinear regression from three independent experiments and compared to the antitumor drug doxorubicin (DOXO).

<table>
<thead>
<tr>
<th>Compd</th>
<th>PC3</th>
<th>SF295</th>
<th>MDA-MB435</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>&gt; 8.10</td>
<td>&gt; 8.10</td>
<td>&gt; 8.10</td>
</tr>
</tbody>
</table>

\textsuperscript{a}cancer cell lines

\textsuperscript{b}normal cells
The lapachone derivative 11 presented high activity in all the cancer cell lines evaluated, showing the strongest effect in human glioblastoma cells (SF295) with an $IC_{50}$ value around 300 nM. This result represents an improvement in the potency of previously described lapachone-based BODIPY derivatives. The results against non-tumor cells showed that 11 presented $IC_{50}$ values in V79 and L929 cells in the range 0.92-0.65 µM. These results indicate that 11 was slightly less cytotoxic against non-tumor cells, with selectivity indexes (i.e. ratio of cytotoxicities between cancer and normal cells) around 3. Whereas further optimization of the lapachone structure would be needed to enhance these selectivity indexes, these values are in line with those of doxorubicin, a clinically used antitumor drug and one of the most potent and important antitumor quinones.

<table>
<thead>
<tr>
<th>Compd</th>
<th>SW620</th>
<th>HCT-116</th>
<th>L929</th>
<th>V79</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>&gt; 7.83</td>
<td>&gt; 7.83</td>
<td>&gt; 7.83</td>
<td></td>
</tr>
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<td></td>
</tr>
<tr>
<td>11</td>
<td>0.38</td>
<td>0.28</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.35-0.41)</td>
<td>(0.17-0.40)</td>
<td>(0.67-0.91)</td>
<td></td>
</tr>
<tr>
<td>DOXO</td>
<td>0.05</td>
<td>0.45</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.02-0.07)</td>
<td>(0.41-0.49)</td>
<td>(0.89-0.98)</td>
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</tr>
<tr>
<td>8</td>
<td>&gt; 8.10</td>
<td>&gt; 8.10</td>
<td>&gt; 8.10</td>
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</tr>
<tr>
<td>9</td>
<td>&gt; 7.83</td>
<td>&gt; 7.83</td>
<td>&gt; 7.83</td>
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<tr>
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<td>&gt; 8.19</td>
<td>&gt; 8.19</td>
</tr>
<tr>
<td>11</td>
<td>0.68</td>
<td>0.32</td>
<td>0.92</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>(0.60-0.75)</td>
<td>(0.25-0.38)</td>
<td>(0.75-1.00)</td>
<td>(0.51-0.72)</td>
</tr>
<tr>
<td>DOXO</td>
<td>0.29</td>
<td>0.21</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>(0.26-0.37)</td>
<td>(0.18-0.23)</td>
<td>(0.19-0.24)</td>
<td>(0.21-0.28)</td>
</tr>
</tbody>
</table>

*PC3 prostate carcinoma, SF295 glioblastoma, MDA-MB435 melanoma, and SW620 and HCT-116 colon carcinomas cell lines. b*Chinese hamster V79 and mouse L929 cells.
Quinones are highly redox active molecules leading to formation of reactive oxygen species (ROS), which cause oxidative stress by oxidising lipids, proteins and DNA. Lipid peroxidation is a well-established mechanism of cellular damage, and it can be used as an intracellular indicator of oxidative stress. The measurement of malondialdehyde (MDA), which is one of the most abundant products from lipid peroxidation, is a highly sensitive assay to quantitatively evaluate the formation of lipid peroxides in cells. We determined the levels of MDA in three cancer cell lines (prostate, glioma and colon) and one non-tumor cell line (murine fibroblast) 24 h after exposure of compound 11, and observed that the levels of MDA in all cancer cells as well as normal fibroblasts evaluated were significantly higher than in the controls (Figure 1). In addition, when comparing the equal concentrations of compound 11 between cell lines used in this current study, there is no more sensitive strain than another in relation to the extent of byproduct of lipid peroxidation, thiobarbituric acid reactive substances (TBARS) formation. Moreover, the pretreatment of the cells with N-acetylcysteine (NAC) showed a strong protective effect by preventing lipid peroxidation mainly by promoting GSH synthesis by NAC, and by its ROS scavenger ability highlighting the prooxidant mechanism linked to cancer and non-cancer cells death. The role of ROS as an important colaborative factor in cell death was well correlated by variation of thiols content after tested compound exposure. Oxidative stress burden usually correlates with cellular thiol levels or vice versa cellular thiol/disulfide ratio is a well-accepted indicator of the redox state of a cell. Naphthoquinones with higher redox potential showed stronger cytotoxicity, presumably because of stronger electrophilicity against thiols and because their GSH conjugates are more readily reduced to semiquinones which activate oxygen.

To determine whether oxidative stress induced by compound 11 is accompanied by changes in total glutathione, the reduced (GSH), oxidized glutathione
(GSSG) and GSH/GSSG ratio were measured. After 24 h exposure, compound 11 significantly reduced (p<0.05) the total content of glutathione of all cell cultures (Table 2) The decrease in total amount of glutathione after compound 11 exposure was accompanied by an increase in GSSG levels (p<0.05) in relation to non-treated cultures. Due to observed compound 11 exposure effects on both reduced and oxidized glutathione the ratio of GSH/GSSG was decreased. On the other hand, in all cultures pre-exposed to NAC before quinone treatment the level of total glutathione was kept nearly or slightly above at the control value.

Figure 1. Determination of MDA levels (TBARS assay) in human cancer cells (PC3, SF295 and HCT-116) and in non-cancer mouse fibroblasts (L929) after 24 h-incubation with compound 11 or 10 µM H$_2$O$_2$ (positive control) in the presence or absence of NAC
(5 mM). *p < 0.05 as compared to control by ANOVA followed by Tukey's test. Values as means ± s.e.m. for three independent experiments in triplicate.

However, the neutralization of ROS by pretreatment with NAC suggests that an alternative mechanism different from ROS generation might be involved in the cytotoxic effect of compound 11, as stated in Table 3, in co-treated (GSH-OEt + naphthoquinones) cancer and non-cancer cells, GSH-OEt only reduced the sensitivity of compound 11 exposure but not abrogated it, which pointed that another mechanism differently of ROS generation capacity may be involved in its cytotoxic effects. Quinonoid compounds can potentially damage multiple macromolecules in cells, such as DNA, resulting in single or double strand breaks or the formation of oxidative nucleobases such as 8-oxo-7,8-dihydroguanine, thymine and uracil glycol.\textsuperscript{44} In order to assess this possibility, we performed comet assays, which are consistent with the reported DNA damage potential attributed to quinonoid molecules.\textsuperscript{45} Compound 11 caused DNA strand breaks after 24 h exposure in all three cancer cell lines as well as in murine fibroblasts. In relation to the antioxidant potential of NAC, it is known that NAC can regulate of oxidative stress related gene expression, having an antagonistic effect on oxidative injuries.\textsuperscript{47} However, as shown in Figure 2, NAC pretreatment did not prevent DNA damage but only resulted in slightly lower levels of fragmented DNA. Altogether, these results suggest that the high cytotoxicity of compound 11 is not exclusively limited to the generation of ROS generation. The results of ongoing studies in our laboratories will be reported in due course.
Table 2. Effects of compound 11 on intracellular thiols after 24 h with or without NAC pretreatment.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Concentration</th>
<th>Total (µg/mg protein)</th>
<th>GSH (µg/mg protein)</th>
<th>GSSG (µg/mg protein)</th>
<th>GSH/GSSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC3</td>
<td>Controla</td>
<td>0.1% DMSO</td>
<td>4.23 ± 0.75</td>
<td>3.04 ± 0.51</td>
<td>1.11 ± 0.12</td>
<td>2.69 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>NAC</td>
<td>5 mM</td>
<td>4.74 ± 0.50</td>
<td>3.53 ± 0.33</td>
<td>1.18 ± 0.21</td>
<td>2.87 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>5 µM</td>
<td>5.53 ± 0.81</td>
<td>1.35 ± 0.10</td>
<td>2.16 ± 0.15</td>
<td>0.37 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>11 plus NACb</td>
<td>5 µM</td>
<td>4.26 ± 0.55</td>
<td>3.13 ± 0.22</td>
<td>1.07 ± 0.15</td>
<td>2.88 ± 0.33</td>
</tr>
<tr>
<td>SF295</td>
<td>Control</td>
<td>0.1% DMSO</td>
<td>4.51 ± 0.83</td>
<td>3.16 ± 0.21</td>
<td>1.31 ± 0.55</td>
<td>2.34 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>NAC</td>
<td>5 mM</td>
<td>4.86 ± 0.71</td>
<td>3.81 ± 0.55</td>
<td>1.02 ± 0.10</td>
<td>3.61 ± 0.81</td>
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<tr>
<td></td>
<td>11</td>
<td>5 µM</td>
<td>3.44 ± 0.10</td>
<td>1.31 ± 0.16</td>
<td>2.09 ± 0.21</td>
<td>0.39 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>11 plus NACb</td>
<td>5 µM</td>
<td>4.72 ± 0.22</td>
<td>3.47 ± 0.55</td>
<td>1.21 ± 0.10</td>
<td>2.82 ± 0.10</td>
</tr>
<tr>
<td>HCT-116</td>
<td>Control</td>
<td>0.1% DMSO</td>
<td>5.02 ± 0.91</td>
<td>3.74 ± 0.55</td>
<td>1.19 ± 0.37</td>
<td>3.09 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>NAC</td>
<td>5 mM</td>
<td>5.38 ± 0.45</td>
<td>4.09 ± 0.61</td>
<td>1.23 ± 0.33</td>
<td>3.26 ± 0.21</td>
</tr>
<tr>
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<td>3.81 ± 0.56</td>
<td>1.78 ± 0.25</td>
<td>2.01 ± 0.22</td>
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<td>11 plus NACb</td>
<td>5 µM</td>
<td>5.12 ± 0.11</td>
<td>3.71 ± 0.25</td>
<td>1.38 ± 0.33</td>
<td>2.63 ± 0.22</td>
</tr>
<tr>
<td>L929</td>
<td>Control</td>
<td>0.1% DMSO</td>
<td>4.87 ± 0.25</td>
<td>3.76 ± 0.51</td>
<td>1.05 ± 0.33</td>
<td>3.55 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>NAC</td>
<td>5 mM</td>
<td>5.43 ± 0.81</td>
<td>3.89 ± 0.75</td>
<td>1.52 ± 0.10</td>
<td>2.52 ± 0.33</td>
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<tr>
<td></td>
<td>11</td>
<td>5 µM</td>
<td>3.50 ± 0.15</td>
<td>1.15 ± 0.21</td>
<td>2.27 ± 0.33</td>
<td>0.33 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>11 plus NACb</td>
<td>5 µM</td>
<td>5.21 ± 0.33</td>
<td>3.89 ± 0.21</td>
<td>1.24 ± 0.25</td>
<td>3.08 ± 0.50</td>
</tr>
</tbody>
</table>

*a Negative controls treated with vehicle (0.1% DMSO); b cells were pretreated for 24 h with NAC (5 mM); *p < 0.05 as compared to control by ANOVA followed by Tukey's test. Values as means ± s.e.m. for three independent experiments in triplicate.
**Figure 2.** Effects of compound 11 on DNA strand breaks index after 24 h of exposure using alkaline comet assay exposure in the presence or absence of NAC (5 mM). *p < 0.05 as compared to control by ANOVA followed by Tukey's test. Data are presented as mean values ± SEMs for three independent experiments in triplicate.

**Table 3.** IC\textsubscript{50} values (μM) for compounds 8-11 after 24 h of exposure in different cells in the presence or absence of reduced GSH.

<table>
<thead>
<tr>
<th>Compound</th>
<th>GSH-OEt</th>
<th>PC3</th>
<th>SF295</th>
<th>HCT-116</th>
<th>L929</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>+</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
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<tr>
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<td>-</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
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<td>9</td>
<td>+</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
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<td></td>
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<tr>
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<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
</tr>
</tbody>
</table>
Finally, in order to analyse the subcellular localization studies of the lapachone-
BODIPY hybrid 11, we measured its photophysical properties. However, compound 11
presents a very low fluorescence quantum yield, which limits its capabilities for
fluorescence cell imaging (Table 4 and Figures S16 and S17 in ESI). As reported for
other BODIPY dyes, the amino group in the position 5 of the BODIPY core can
cause significant quenching of the fluorophore via photoinduced electron transfer (PeT),
making it unsuitable for imaging studies. **Whereas the quenching effect is highly
pronounced in compound 11, the extent of PeT quenching is dependent on the structure
of the quinone-based BODIPY core and the amino substituents.** In view of these results,
we synthesized the BODIPY-alkyne 14 as described in the literature and conjugated it
to the quinone 7 by CuAAC reaction to render the hybrid BODIPY-quinone 15. We
measured the photophysical properties of compound 15, and as expected, determined its
excitation and emission maxima in the green visible range, with high extinction
coefficients and fluorescence quantum yields (Table 4 and Figure S1 in ESI), being a
suitable fluorescent probe for live-cell imaging.

<table>
<thead>
<tr>
<th></th>
<th>11 -</th>
<th>11 +</th>
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</thead>
<tbody>
<tr>
<td>4.47 ± 0.73</td>
<td>15.83 ± 1.15</td>
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</tr>
<tr>
<td>1.96 ± 0.21</td>
<td>9.81 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>5.28 ± 0.55</td>
<td>14.25 ± 2.10</td>
<td></td>
</tr>
<tr>
<td>5.91 ± 0.10</td>
<td>12.41 ± 1.75</td>
<td></td>
</tr>
</tbody>
</table>

*a Cell-permeable ethyl ester analogue of reduced GSH (15 mM).
With compound 15 in hands, we tested its cytotoxicity in the same five cancer cell and non-cancer cell lines. Compound 15 displayed antiproliferative effects only in PC3 prostate and HCT-116 colon cell lines with IC\textsubscript{50} values equal to 2.39 (2.14-2.65) and 2.85 (2.65-3.07) µM, respectively. For cancer cells (SF295, MDA-MB435 and SW620) and normal cells (L929 and V79) IC\textsubscript{50} values were determined to be > 7.72 µM. Given the differences in cytotoxicity, we assessed the subcellular localization of compound 15 in PC3 cancer cells. Fluorescence confocal microscopy experiments revealed the preferential accumulation of 15 in the subcellular lysosomal compartments, showing strong co-localization with commercially available LysoTracker Red but not with MitoTracker Red dyes (Figure 3). Under these conditions, compound 11 was not
detectable under the fluorescence microscope due to its low quantum yield (Figure S18 in ESI).

Table 4. Photophysical data for compounds 11 and 15.

<table>
<thead>
<tr>
<th>Compound</th>
<th>λ_{abs.}(nm)</th>
<th>λ_{abs.}(nm)</th>
<th>ε (M^{-1}cm^{-1})</th>
<th>QY^{a}</th>
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<tbody>
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<td>495</td>
<td>530</td>
<td>30,300</td>
<td>0.03</td>
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<tr>
<td>15</td>
<td>498</td>
<td>511</td>
<td>68,800</td>
<td>0.82</td>
</tr>
</tbody>
</table>

^{a} Determined as relative to fluorescein in basic EtOH (QY: 0.97).

Mefloquine, a known antimalarial and antitumoral molecule, has been reported to accumulate in the subcellular lysosomes of acute myeloid leukemia cells, where it exhibits high cytotoxicity due to lysosomal disruption.\textsuperscript{50} This observation led us to evaluate the compound 15 in 2 leukemia cancer cell lines (HL-60 and Jurkat cells). Notably, 15 presented high activity in these cell lines with IC\textsubscript{50} values in the low micromolar range [for HL-60 cells, IC\textsubscript{50} = 0.92 (0.90-1.05) and for Jurkat cells, IC\textsubscript{50} = 0.94 (0.86-0.98) µM]. Furthermore, because compound 15 showed very low cytotoxicity in non-cancer cells (i.e. L929 and V79), its selectivity index was over 8.5 (i.e. ~7-fold higher than compound 11). Finally, we also assessed the effect of compound 15 on intracellular thiols in HL-60 and Jurkat cancer cell lines (Table 5). Remarkably, a weaker protective effect was observed for cells pretreated with NAC, suggesting a different mechanism of action between both lapachone-BODIPY hybrids. The ROS-independent cytotoxic effect of compound 15 is in line with the subcellular localization studies and its potential activity in lysosomal disruption of leukemia cancer cells.
Figure 3. Fluorescence confocal microscopy of PC3 cells upon incubation with compound 15 as well as Mitotracker Red (top panel) and LysoTracker Red (bottom panel). Images arranged from left to right as: green fluorescence (15), red fluorescence (MitoTracker/LysoTracker), merged green/red channels and brightfield images. Scale bar: 10 μm.

Table 5. Effects of compound 15 on intracellular thiols after 24 h of cell exposure with or without NAC pretreatment.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Concentration</th>
<th>Intracellular thiols (µg/mg protein)</th>
<th>( \text{GSH} )</th>
<th>( \text{GSSG} )</th>
<th>( \text{GSH/GSSG} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-60</td>
<td>Control( ^a )</td>
<td>0.1% DMSO</td>
<td>3.61 ± 0.22</td>
<td>3.01 ± 0.11</td>
<td>0.66 ± 0.17</td>
<td>4.53 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>NAC( ^b )</td>
<td>5 mM</td>
<td>3.85 ± 0.81</td>
<td>3.03 ± 0.25</td>
<td>0.75 ± 0.90</td>
<td>4.02 ± 0.61</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5 µM</td>
<td>3.01 ± 0.44</td>
<td>0.72 ± 0.20( ^* )</td>
<td>2.18 ± 0.10( ^* )</td>
<td>0.31 ± 0.05( ^* )</td>
</tr>
<tr>
<td></td>
<td>15 plus NAC( ^c )</td>
<td>5 µM</td>
<td>2.83 ± 0.71( ^* )</td>
<td>1.19 ± 0.10( ^* )</td>
<td>1.47 ± 0.33( ^* )</td>
<td>0.76 ± 0.10( ^* )</td>
</tr>
<tr>
<td>Jurkat</td>
<td>Control( ^a )</td>
<td>0.1% DMSO</td>
<td>3.87 ± 0.55</td>
<td>3.02 ± 0.21</td>
<td>0.78 ± 0.33</td>
<td>3.81 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>NAC( ^b )</td>
<td>5 mM</td>
<td>4.13 ± 0.25</td>
<td>3.25 ± 0.17</td>
<td>0.81 ± 0.55</td>
<td>3.97 ± 1.17</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5 µM</td>
<td>3.28 ± 0.60( ^* )</td>
<td>0.60 ± 0.33( ^* )</td>
<td>2.57 ± 0.10( ^* )</td>
<td>0.21 ± 0.11( ^* )</td>
</tr>
<tr>
<td></td>
<td>15 plus NAC( ^c )</td>
<td>5 µM</td>
<td>3.11 ± 0.05( ^* )</td>
<td>1.58 ± 0.55( ^* )</td>
<td>1.44 ± 0.51( ^* )</td>
<td>1.08 ± 0.33( ^* )</td>
</tr>
</tbody>
</table>
3. Conclusions

We have synthesized and characterized a small collection of novel quinone-based BODIPY hybrids of the natural products lapachol and lawsone. All compounds were evaluated in cancerous and non-cancerous cell lines, and we identified two nor-β-lapachone hybrids (11 and 15) with potent cytotoxic activity. Mechanistic studies for both compounds suggest that the action of compound 11 may be related to the generation of reactive oxygen species whereas the fluorescent lapachone 15 may exert its cytotoxic action in subcellular lysosomal organelles. This study provides new structure-activity relationships in the preparation of biologically active lapachone derivatives as well as new insights in the potential mechanism of action for their cytotoxic activity.

4. Experimental Section

4.1. Chemistry

Melting points were obtained on Thomas Hoover and are uncorrected. Analytical grade solvents were used. Column chromatography was performed on silica gel (SilicaFlash G60 UltraPure 60-200 µm, 60 Å). $^1$H and $^{13}$C NMR were recorded at room temperature using a Bruker AVANCE DRX400, in the solvents indicated, with TMS as
internal reference. Chemical shifts (δ) are given in ppm. Electron-impact mass spectra (70 eV) were obtained using a VG Autospec apparatus (Micromass, Manchester, UK). Absorption spectra were obtained on a Varian Cary 100 spectrophotometer at room temperature in the solvents described above. Fluorescence spectra were obtained on a Varian Cary Eclipse spectrofluorimeter with a xenon arc lamp as the light source while using an excitation wavelength (λ_{exc}) corresponding to a higher absorption band. In all experiments, a quartz cuvette was employed with a 1 cm optical path length. Azide derivatives 4-7 and BODIPY 14 were synthesized as previously reported in the literature.25,26,30-33 Structures of the novel compounds 3, 8-11 and 15 were determined by 1H and 13C NMR. Electrospray ionization mass spectra were also obtained to confirm compound identities.

**Procedure for the synthesis of BODIPY 3.** To a solution of dichlorinated BODIPY (215 mg, 0.639 mmol) in acetonitrile (15 mL) under stirring at room temperature, propargylamine (102 µL, 1.6 mmol, 2.5 eq) was added. After 1 hour under stirring at room temperature TLC control showed full conversion of the starting material. Solvent was evaporated under reduced pressure and, after purification via silica column chromatography (C6H14/DCM, 3:2-1:3), the desired product 3 (201 mg, 0.56 mmol, 89% yield) was obtained. 1H NMR (400 MHz, CDCl3) δ: 7.49-7.43 (m, 5H), 6.93 (d, 1H, J = 4 Hz), 6.49 (br, 1H), 6.39 (d, 1H, J = 4 Hz), 6.28 (d, 1H, J = 4 Hz), 6.21 (d, 1H, J = 4 Hz), 4.17 (dd, 2H, J = 2.4 and 3.6 Hz), 2.38 (t, 1H, J = 2.4 Hz). 13C NMR (100 MHz, CDCl3) δ: 169.3, 135.9, 133.7, 133.2, 132.0, 131.2, 130.3, 129.5, 128.3, 121.6, 113.2, 110.9, 78.8, 34.1. EI/MS (m/z) [M+H]+: 356.0. Calcd for [C_{18}H_{14}BClF_2N_3]^+: 356.0.
General procedure to prepare quinone-based BODIPY hybrids 8-11 and 15. In a round bottom flask, the respective azide derivatives (0.5 mmol), alkyne BODIPYs (0.6 mmol), and 10 mL of CH$_3$CN were added. The reaction mixture was stirred until complete solubilization of the reagents after which, CuI (10% per mole) was added. The system was kept under inert atmosphere (Ar) until complete consumption of BODIPY and monitored by TLC until its completion. The solvent from the crude was evaporated under reduced pressure and it was purified by column chromatography on silica-gel, using eluents with an increasing polarity gradient mixture of hexane and ethyl acetate.

Nor-α-lapachone-based BODIPY (8). Compound 8 was obtained as a brown solid after 18 h of reaction (258 mg, 83% yield); mp 173-175 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ: 8.16-8.14 (m, 1H), 8.05-8.03 (m, 1H), 7.75-7.73 (m, 2H), 7.59 (s, 1H), 7.48-7.44 (m, 5H), 6.86 (d, 1H, $J$ = 4.8 Hz), 6.82 (s, 1H), 6.35 (d, 1H, $J$ = 3.8 Hz), 6.29 (d, 1H, $J$ = 4.8 Hz), 6.18 (d, 1H, $J$ = 3.8 Hz), 6.00 (s, 1H), 4.73 (d, 2H, $J$ = 6.1 Hz), 1.74 (s, 3H), 1.16 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ: 180.9, 179.8, 177.8, 144.6, 135.9, 134.8, 135.9, 134.8, 133.6, 133.5, 131.9, 131.7, 130.5, 130.3, 129.3, 128.2, 127.2, 126.8, 126.5, 121.9, 121.1, 118.1, 94.3, 67.8, 40.4, 27.5, 21.0. EI/HRMS (m/z) [M+Na]$^+$: 647.1551. Calcd for [C$_{32}$H$_{24}$BClF$_2$N$_6$O$_3$Na]$^+$: 647.1557.

α-Lapachone-based BODIPY (9). Compound 9 was obtained as a brown solid after 18 h of reaction (229 mg, 72% yield); mp 171-173 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ: 8.12-8.10 (m, 1H), 7.95-7.93 (m, 1H), 7.69-7.67 (m, 3H), 7.47-7.42 (m, 5H), 6.87 (d,
$^1$H, $J = 4.4$ Hz), 6.81 (sl, 1H), 6.35-6.32 (m, 2H), 6.17 (d, 1H, $J = 3.6$ Hz), 5.78 (t, 1H, $J = 6.6$ Hz), 4.74 (d, 2H, $J = 6$ Hz), 2.72 (dd, 1H, $J = 14.6$ and 6.8 Hz), 2.40 (dd, 1H, $J = 14.6$ and 6.8 Hz), 1.50 (s, 3H), 1.35 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 182.8, 179.3, 161.9, 156.2, 143.8, 135.9, 134.5, 133.7, 133.5, 132.7, 131.9, 131.8, 131.1, 131.3, 129.4, 128.3, 126.7, 126.4, 122.3, 120.9, 115.1, 112.9, 111.2, 79.3, 50.2, 40.5, 39.3, 26.9, 26.2. EI/HRMS ($m/z$) [M+Na]$^+$: 661.1704. Calcd for [C$_{33}$H$_{26}$BClF$_2$N$_6$O$_3$Na]$^+$: 661.1714.

Nor-$\alpha$-lapachone-based BODIPY derivative (10). Compound 10 was obtained as a brown solid after 18 h of reaction (268 mg, 88% yield); mp 165-168 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 7.94 (d, 1H, $J = 7.6$ Hz), 7.91 (d, 1H, $J = 7.6$ Hz), 7.84 (s, 1H), 7.61 (t, 1H, $J = 7.6$ Hz), 7.55 (t, 1H, $J = 7.6$ Hz), 7.48-7.45 (m, 5H), 7.09 (sl, 1H), 6.85 (d, 1H, $J = 4.8$ Hz), 6.41 (d, 1H, $J = 4.8$ Hz), 6.33 (d, 1H, $J = 4$ Hz), 6.16 (d, 1H, $J = 4$ Hz), 5.40 (m, 1H), 4.82 (d, 2H, $J = 5.2$ Hz), 4.78 (d, 2H, $J = 6.4$ Hz), 3.41-3.34 (m, 1H), 3.04-2.97 (m, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 181.7, 177.3, 161.9, 159.1, 144.7, 135.9, 134.2, 133.7, 133.4, 133.1, 132.6, 132.2, 131.9, 131.3, 130.3, 129.8, 129.3, 128.3, 126.2, 125.2, 124.2, 123.7, 120.4, 112.8, 111.4, 83.2, 53.0, 40.0, 30.2. EI/HRMS ($m/z$) [M+Na]$^+$: 633.1386. Calcd for [C$_{31}$H$_{22}$BClF$_2$N$_6$O$_3$Na]$^+$: 633.1401.

Nor-$\beta$-lapachone-based BODIPY (11). Compound 11 was obtained as a brown solid after 48 h of reaction (227 mg, 73% yield); mp 190-193 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 7.96 (d, 1H, $J = 7.2$ Hz), 7.83-7.79 (m, 2H), 7.73 (t, 1H, $J = 8.9$ Hz), 7.65 (t, 1H, $J = 7.8$ Hz), 7.46-7.42 (m, 5H), 6.89 (sl, 1H), 6.84 (d, 1H, $J = 4$ Hz), 6.34 (sl, 2H),
6.14 (sl, 1H), 6.00 (sl, 1H), 4.74 (d, 2H, \( J = 4.4 \) Hz), 1.77 (s, 3H), 1.17 (s, 3H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \): 180.2, 174.9, 171.4, 161.8, 144.3, 136.0, 134.6, 133.7, 133.2, 132.4, 131.9, 131.6, 130.3, 120.8, 129.3, 128.3, 126.8, 125.6, 122.3, 120.5, 112.8, 111.4, 111.4, 95.9, 67.1, 40.4, 27.7, 21.2. EI/HRMS (\( m/z \)) [M+Na]\(^+\): 647.1521. Calcd for \([\text{C}_{32}\text{H}_{24}\text{BClF}_2\text{N}_6\text{O}_3\text{Na}]^+\): 647.1557.

**Fluorescent nor-\(\beta\)-lapachone-based BODIPY (15).** Compound 15 was obtained as a red solid after 48 h of reaction (226 mg, 70% yield); mp 176-179 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \): 8.21 (d, 1H, \( J = 7.2 \) Hz), 7.83 (t, 1H, \( J = 7.2 \) Hz), 7.79-7.73 (m, 2H), 7.64 (s, 1H), 7.18 (d, 2H, \( J = 8.0 \) Hz), 7.09 (d, 2H, \( J = 8.0 \) Hz), 6.03 (s, 1H), 5.98 (s, 2H), 5.24 (d, 2H, \( J = 4.0 \) Hz), 2.56 (s, 6H), 1.80 (s, 3H), 1.41 (s, 6H), 1.22 (s, 3H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \): 180.1, 174.6, 171.4, 158.9, 155.5, 143.9, 143.2, 141.7, 135.0, 133.6, 131.9, 131.7, 130.2, 129.4, 127.9, 126.7, 125.7, 122.5, 121.3, 115.6, 111.2, 96.0, 67.2, 62.3, 27.8, 21.3, 14.6. EI/HRMS (\( m/z \)) [M+Na]\(^+\): 670.2401. Calcd for \([\text{C}_{36}\text{H}_{32}\text{BF}_2\text{N}_5\text{O}_4\text{Na}]^+\): 670.2413.

**4.2 Biological Data**

**Inhibition of cell proliferation – MTT tests.** Cell growth was quantified by the ability of living cells to reduce the yellow dye MTT to a purple formazan product. Cytotoxicity was checked on different human cancer cell lines (HL-60 and Jurkat leukemias, PC3 prostate carcinoma, SF295 glioblastoma, MDA-MB435 melanoma, SW620 and HCT-116 colon carcinomas cell lines), and two non-cancer murine fibroblasts (Chinese hamster V79 and mouse L929 cells). All cancer cells were obtained from the National
Cancer Institute in Bethesda, MD, USA. The V79 cells were kindly provided by Dr. Henriques JAP (Federal University of Rio Grande do Sul, Porto Alegre, Brazil), and L929 cells were purchased from Rio de Janeiro Cell Bank (BCRJ, Rio de Janeiro, Brazil). For the experiments, cells were plated in 96-well plates (0.7 \times 10^5 to 0.3 \times 10^6 cells/well for cancer cells and 0.5 \times 10^5 cells/well for murine fibroblasts), and tested compounds, dissolved in DMSO (0.1%), was then added to each well, followed by incubation for 24 h at concentrations ranging from 0.04 to 40.92 \mu M or 72 h at concentrations ranging from 0.04 to 8.19 \mu M. In some experiments, the contribution of ROS to the cytotoxicity of tested quinones was assessed by cells co-treated with GSH-OEt (15 mM). Our preliminary experiments showed that the ethyl ester of GSH was not cytotoxic and provided a more efficient protection than GSH at the same concentration (15 mM). In fact, GSH is not readily transported into most cells. Thus, in the MTT experiments, we used GSH ethyl ester, which is more lipophilic, readily taken up by cells and hydrolyzed to GSH by cellular nonspecific esterases.\textsuperscript{51} Afterward, the plates were centrifuged and the medium replaced by fresh medium (150 \mu L) containing 0.5 mg/mL MTT. Three hours later, the MTT formazan product was dissolved in 150 \mu L DMSO, and absorbance was measured using a multiplate reader (Spectra Count, Packard, Ontario, Canada). Drug effect was quantified as the percentage of control absorbance of the reduced dye at 595 nm. Doxorubicin (0.001-1.06 \mu M) was used as positive control. Experiments were carried out in triplicate and repeated at least three times.

**Lipid peroxidation (TBARS assay).** The extent of tested compound-induced lipid peroxidation was determined by the reaction of thiobarbituric acid (TBA) with
malondialdehyde (MDA), a product formed by lipid peroxidation. The assays were performed according to Salgo and Pryor, with minor modifications. Cells were incubated with compound for 24 h, and after lysis with Tris-HCl (15 mM for 1 h). Two milliliters 0.4 mg/mL trichloroacetic acid, 0.25 M HCl were added to the lysate, which was then incubated with 6.7 mg/mL TBA for 15 min at 100 ºC. The mixture was centrifuged at 750 x g for 10 min. As TBA reacts with other products of lipid peroxidation in addition to MDA, results are expressed in terms of thiobarbituric reactive species (TBARS), which are determined by absorbance at 532 nm. Hydrolyzed 1,1,3,3-tetramethoxypropan was used as the standard. The results were normalized by protein content. In order to evaluate the contribution of ROS on lipid peroxidation extent, cells were pre-treated for 24 h with NAC (5 mM), and after they were exposed to tested compound during 24 h. H$_2$O$_2$ (10 μM) was used as positive control. All experiments were performed in triplicate in three independent experiments.

**Determination of reduced (GSH), oxidized (GSSG) glutathione, and GSG/GSSG ratios.** Total glutathione (GSH + GSSG) was determined by spectrophotometer determination of 5-thio-2-nitrobenzoate (TNB), which was produced from DTNB, according to Akerboom and Sies with minor modification. Briefly, cells were exposed with tested compound (5 μM) during 24 h. Then, cells were washed with ice-cold PBS, and resuspended in 0.1 sodium phosphate (5 mM EDTA, pH 8.0), and sonicated to obtain the cell homogenate. An equal volume of 2 M HClO$_4$-4 mM EDTA was added to the cell extract, and the precipitated proteins were sedimented by centrifugation at 8000 x g for 15 min at 4 ºC. The supernatant was neutralized with 2 M KOH, and the insoluble residue was removed by centrifugation under the same conditions. For the
spectrophotometric determination 910 μL of the cell extract supernatant or of the standard glutathione solution, in the same phosphate-EDTA buffer, were mixed with 50 μL of 4 mg/mL NADPH in 0.5% (w/v) NaHCO₃, 20 μL of 6 U/mL glutathione reductase in phosphate-EDTA buffer, and 20 μL of 1.5 mg/mL DTNB in 0.5% NaHCO₃. The increase in absorbance was measured at 412 nm. The results were normalized by protein content. Total glutathione content was determined as μg/mg protein. For GSSG determination, 4-vinypyridine was added to a final concentration of 0.1% (v/v), and then incubated for 1 h at room temperature. At this concentration, 4-vinypyridine is able to react with all GSH without interfering with GSSG determination. GSH was determined based on the total glutathione and GSSG concentration results. In order to evaluate the contribution of ROS on glutathione modulation content, cells were pre-treated for 24 h with NAC (5 mM), and after they were exposed to tested compound during 24 h. All experiments were performed in triplicate in three independent experiments.

Alkaline comet assay (Single cell gel electrophoresis). The alkaline comet assay was performed as described by Singh et al. with minor modifications, and following the recommendations of the International Workshop on Genotoxicity Test Procedures. At the end of the treatment with 11 (2.5, 5, and 10 μM during 24 h) exposure in the presence or absence of 5 mM NAC (pre-treated for 24 h), cells were washed with ice-cold PBS, detached with 100 μL trypsin (0.15%) and resuspended in complete RPMI medium. Next, 20 μL of cell suspension (~10⁶ cells/mL) were mixed with 0.75% low melting point agarose and immediately spread onto a glass microscope slide precoated with a layer of 1% normal melting point agarose. The agarose was allowed to set at 4°C
for 5 min. The slides were incubated in ice-cold lysis solution (2.5 M NaCl, 10mM Tris, 100mM EDTA, 1% Triton X-100 and 10% DMSO, pH 10.0) at 4°C for a minimum of 1 h to remove cellular proteins, leaving the DNA as “nucleoids.” After the lysis procedure, the slides were placed on a horizontal electrophoresis unit. The unit was filled with fresh buffer (300 mM NaOH and 1mM EDTA, pH>13.0) to cover the slides for 20 min at 4°C to allow DNA unwinding and expression of alkali-labile sites. Electrophoresis was conducted for 20 min at 25 V and 300 mA (0.86 V/cm). After electrophoresis, the slides were neutralized (0.4 M Tris, pH 7.5), stained with ethidium bromide (20 μg/mL) and analyzed using a fluorescence microscope. All the above steps were conducted under yellow light or in the dark to prevent additional DNA damage. Images of 100 randomly selected cells (50 cells from each of two replicate slides) were analyzed for each concentration of test substance. Cells were scored visually and assigned to one of five classes, according to tail size (from undamaged-0, to maximally damaged-4), and a DNA strand breaks index value was calculated for each sample of cells. DNA strand breaks index thus ranged from 0 (completely undamaged: 100 cells x 0) to 400 (with maximum damage: 100 cells x 4).59

**Spectral characterization.** Spectral characterization of BODIPY-prodrug conjugates. Spectroscopic and quantum yield data were recorded on a Synergy HT spectrophotometer (Biotek). Compounds were dissolved at the indicated concentrations and spectra were recorded at r.t. Spectra are represented as means from at least two independent experiments with n = 3. Quantum yields were calculated by measuring the integrated emission area of the fluorescence spectra and comparing it to the area measured for fluorescein in basic EtOH.
Fluorescence confocal microscopy. PC3 cells were grown in DMEM cell culture media supplemented with 10% FBS, antibiotics (100 U mL\(^{-1}\) penicillin and 100 mg mL\(^{-1}\) streptomycin) and 2 mM L-glutamine in a humidified atmosphere at 37 °C with 5% CO\(_2\). Cells were plated on glass chamber slides Lab-Tek™ II (Nunc), incubated with compounds 11 or 15 (5 μM) with or without Tracker dyes at 37 °C for 10 min. Cells were imaged in phenol red-free DMEM under a Zeiss LSM 510 META fluorescence confocal microscope equipped with a live cell imaging stage. Fluorescence and brightfield images were acquired using a 40X oil objective. Fluorophores were excited with 488 nm (compound 15) or 543 nm (Lysotracker Red, Mititacker Red) lasers. All images were analysed and processed with ImageJ.

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Notes and References


Supplementary Material
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