Supporting Information for

Bis-Michael Acceptors as Novel Probes to Study the Keap1/Nrf2/ARE Pathway

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Table S1. Fold-induction of NQO1 by qPCR

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fold-induction at: $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 µM</td>
</tr>
<tr>
<td></td>
<td>$R^1 = H$</td>
</tr>
<tr>
<td>Sulfaphane</td>
<td>0.81 ± 0.08</td>
</tr>
<tr>
<td>7</td>
<td>2.02 ± 0.15</td>
</tr>
<tr>
<td>8</td>
<td>1.62 ± 0.45</td>
</tr>
<tr>
<td>11</td>
<td>1.96 ± 0.72</td>
</tr>
<tr>
<td>15</td>
<td>0.63 ± 0.19</td>
</tr>
<tr>
<td>17</td>
<td>0.57 ± 0.18</td>
</tr>
<tr>
<td>18</td>
<td>3.59 ± 0.11</td>
</tr>
<tr>
<td>19</td>
<td>5.56 ± 1.64</td>
</tr>
<tr>
<td>23</td>
<td>2.78 ± 0.77</td>
</tr>
<tr>
<td>27</td>
<td>2.48 ± 0.09</td>
</tr>
<tr>
<td>30</td>
<td>5.97 ± 0.73</td>
</tr>
<tr>
<td>31</td>
<td>3.18 ± 0.17</td>
</tr>
<tr>
<td>32</td>
<td>8.38 ± 0.62</td>
</tr>
<tr>
<td>33</td>
<td>14.97 ± 4.77</td>
</tr>
<tr>
<td>34</td>
<td>14.38 ± 2.12</td>
</tr>
</tbody>
</table>

$^a$ no toxicity was observed at these concentrations
Table S2. % Mortality of all compounds evaluated in the LDH assay \(^a\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Mortality at:</th>
<th>1 (\mu)M</th>
<th>20 (\mu)M</th>
<th>100 (\mu)M</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>(R_1 = \text{Hex})</td>
<td>Not tested</td>
<td>2.39 ± 0.06</td>
<td>4.23 ± 0.11</td>
</tr>
<tr>
<td>4</td>
<td>(R_1 = \text{Me})</td>
<td>Not tested</td>
<td>4.04 ± 0.29</td>
<td>Not tested</td>
</tr>
<tr>
<td>7</td>
<td>(R_1 = \text{H})</td>
<td>Not tested</td>
<td>0.85 ± 0.26</td>
<td>1.91 ± 0.43</td>
</tr>
<tr>
<td>8</td>
<td>(R_1 = \text{Hex})</td>
<td>Not tested</td>
<td>0.49 ± 0.85</td>
<td>1.93 ± 0.47</td>
</tr>
<tr>
<td>9</td>
<td>(R_1 = \text{Me})</td>
<td>Not tested</td>
<td>1.33 ± 0.35</td>
<td>3.23 ± 1.49</td>
</tr>
<tr>
<td>10</td>
<td>(R_1 = \text{allyl})</td>
<td>Not tested</td>
<td>2.00 ± 0.25</td>
<td>3.54 ± 0.70</td>
</tr>
<tr>
<td>11</td>
<td>(R_1 = \text{Bn})</td>
<td>Not tested</td>
<td>1.25 ± 0.23</td>
<td>1.04 ± 0.80</td>
</tr>
<tr>
<td>12</td>
<td>(R_1 = \text{propargyl})</td>
<td>Not tested</td>
<td>1.33 ± 0.35</td>
<td>7.26 ± 3.12</td>
</tr>
<tr>
<td>13</td>
<td>(R_1 = \text{CH}_2\text{CF}_3)</td>
<td>Not tested</td>
<td>3.25 ± 0.93</td>
<td>11.43 ± 0.03</td>
</tr>
<tr>
<td>14</td>
<td>(R_1 = \text{CH}_2\text{CO}_2\text{t-Bu})</td>
<td>Not tested</td>
<td>1.68 ± 0.32</td>
<td>0.86 ± 0.74</td>
</tr>
<tr>
<td>15</td>
<td>(R_1 = \text{CH}_3\text{CN})</td>
<td>Not tested</td>
<td>-1.09 ± 0.20</td>
<td>-0.94 ± 0.02</td>
</tr>
<tr>
<td>16</td>
<td>(R_1 = \text{CH}_2\text{C(O)Me})</td>
<td>Not tested</td>
<td>-0.68 ± 0.04</td>
<td>-0.26 ± 0.27</td>
</tr>
<tr>
<td>17</td>
<td>(R_1 = (\text{CH}_2)_2\text{CO}_2\text{Me})</td>
<td>Not tested</td>
<td>0.44 ± 0.06</td>
<td>-0.31 ± 0.01</td>
</tr>
<tr>
<td>18</td>
<td>(R_2 = \text{CO}_2\text{All})</td>
<td>Not tested</td>
<td>0.49 ± 0.85</td>
<td>16.80 ± 0.09</td>
</tr>
<tr>
<td>19</td>
<td>(R_2 = \text{CO}_2\text{t-Bu})</td>
<td>Not tested</td>
<td>-1.25 ± 0.23</td>
<td>15.63 ± 0.43</td>
</tr>
<tr>
<td>20</td>
<td>(R_2 = \text{CO}_3\text{H})</td>
<td>Not tested</td>
<td>1.19 ± 0.29</td>
<td>1.12 ± 0.21</td>
</tr>
<tr>
<td>23</td>
<td>(R_2 = \text{Cl})</td>
<td>Not tested</td>
<td>1.19 ± 0.29</td>
<td>1.12 ± 0.21</td>
</tr>
<tr>
<td>24</td>
<td>(R_2 = \text{I})</td>
<td>Not tested</td>
<td>1.19 ± 0.29</td>
<td>1.12 ± 0.21</td>
</tr>
<tr>
<td>25</td>
<td>(R_2 = \text{CH}_2\text{OH})</td>
<td>Not tested</td>
<td>0.85 ± 0.26</td>
<td>1.91 ± 0.43</td>
</tr>
<tr>
<td>26</td>
<td>(R_2 = \text{CH}_2\text{O})</td>
<td>Not tested</td>
<td>2.28 ± 0.29</td>
<td>4.37 ± 1.07</td>
</tr>
<tr>
<td>21</td>
<td>(R_3 = \text{Me})</td>
<td>Not tested</td>
<td>1.07 ± 0.35</td>
<td>2.63 ± 0.60</td>
</tr>
<tr>
<td>27</td>
<td>(R_3 = \text{H})</td>
<td>Not tested</td>
<td>1.07 ± 0.35</td>
<td>4.31 ± 2.28</td>
</tr>
<tr>
<td>28</td>
<td>(R_3 = \text{Me})</td>
<td>Not tested</td>
<td>1.19 ± 0.29</td>
<td>1.12 ± 0.21</td>
</tr>
<tr>
<td>29</td>
<td>(R_4 = \text{CH}_3)</td>
<td>Not tested</td>
<td>2.28 ± 0.29</td>
<td>24.39 ± 1.16</td>
</tr>
<tr>
<td>30</td>
<td>(R_4 = (\text{CH}_2)_3)</td>
<td>Not tested</td>
<td>2.62 ± 0.01</td>
<td>35.61 ± 12.02</td>
</tr>
<tr>
<td>31</td>
<td>(R_4 = (\text{CH}_2)_4)</td>
<td>Not tested</td>
<td>2.68 ± 0.05</td>
<td>Not tested</td>
</tr>
<tr>
<td>32</td>
<td>(R_4 = (\text{CH}_2)_5)</td>
<td>Not tested</td>
<td>1.36 ± 0.27</td>
<td>Not tested</td>
</tr>
<tr>
<td>33</td>
<td>(R_4 = \text{CH}_2\text{OH})</td>
<td>Not tested</td>
<td>0.80 ± 0.04</td>
<td>Not tested</td>
</tr>
<tr>
<td>34</td>
<td>(R_4 = \text{CH}_2\text{OH})</td>
<td>Not tested</td>
<td>3.48 ± 0.27</td>
<td>11.78 ± 1.65</td>
</tr>
<tr>
<td>35</td>
<td>(R_4 = \text{CH}_2\text{O})</td>
<td>Not tested</td>
<td>3.91 ± 0.06</td>
<td>9.43 ± 0.61</td>
</tr>
<tr>
<td>36</td>
<td>(R_4 = \text{CH}_2\text{O})</td>
<td>Not tested</td>
<td>3.38 ± 2.01</td>
<td>15.24 ± 2.21</td>
</tr>
</tbody>
</table>

\(^a\) The data are reported as Mean ± SEM based on two separated experiments.
Methyl 8-oxo-1,4-dioxaspiro[4.5]decane-7-carboxylate (2)

$^1\text{H}$ NMR spectrum

$^{13}\text{C}$ NMR spectrum
Methyl 9-methyl-8-oxo-1,4-dioxaspiro[4.5]decane-7-carboxylate

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 9-hexyl-8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate (3)

$^1$H NMR spectrum

13C NMR spectrum
Methyl 9-methyl-8-oxo-1,4-dioxaspiro[4.5]decane-7-carboxylate

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 9-methyl-8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate (4)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
1-(2-methylprop-1-en-1-yl)pyrrolidine
$^1$H NMR spectrum

4,4-dimethylcyclohex-2-enone (22)
$^1$H NMR spectrum
4,4-dimethylcyclohexanone (5)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 5,5-dimethyl-2-oxocyclohexanecarboxylate (6)
\(^1\)H NMR spectrum
Methyl 3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (7)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 5-hexyl-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (8)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 3,3,5-trimethyl-6-oxocyclohex-1-enecarboxylate (9)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 5-allyl-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (10)
$^1$H NMR spectrum
Methyl 5-benzyl-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (11)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 3,3-dimethyl-6-oxo-5-(prop-2-yn-1-yl)cyclohex-1-enecarboxylate (12)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 3,3-dimethyl-6-oxo-5-(2,2,2-trifluoroethyl)cyclohex-1-enecarboxylate (13)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 5-(2-(tert-butoxy)-2-oxoethyl)-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (14)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 5-(cyanomethyl)-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (15)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 3,3-dimethyl-6-oxo-5-(2-oxopropyl)cyclohex-1-enecarboxylate (16)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 5-(3-methoxy-3-oxopropyl)-3,3-dimethyl-6-oxocyclohex-1-ene carboxylate (17)

\[^{1}H\text{ NMR spectrum}\]

\[^{13}C\text{ NMR spectrum}\]
Allyl 3,3-dimethyl-6-oxocyclohex-1-ene-carboxylate (18)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
 tert-Butyl 3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (19)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
3,3-dimethyl-6-oxocyclohex-1-enecarboxylic acid (20)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 6-hydroxy-3,3-dimethylcyclohex-1-enecarboxylate (21)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
2-chloro-4,4-dimethylcyclohex-2-enone (23)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
2-iodo-4,4-dimethylcyclohex-2-enone (24)
$^1$H NMR spectrum

2-(hydroxymethyl)-4,4-dimethylcyclohex-2-enone (25)
$^1$H NMR spectrum
(3,3-dimethyl-6-oxocyclohex-1-en-1-yl)methyl 5,5-dimethyl-2-oxocyclohexanecarboxylate (26)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 5,5-dimethyl-2-oxocyclohex-3-enecarboxylate (27)
$^1$H NMR spectrum
Methyl 1,5,5-trimethyl-2-oxocyclohex-3-enecarboxylate (28)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Methyl 3,3-dimethyl-6-oxocyclohexa-1,4-dienecarboxylate (29)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Dimethyl 5,5'-methylenebis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (30)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Dimethyl 5,5'-{(propane-1,3-diy)bis(3,3-dimethyl-6-oxocyclohex-1-ene)carboxylate} (31)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Dimethyl 5,5'-((butane-1,4-diyl)bis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (32)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
(E)-dimethyl 5,5’-(but-2-ene-1,4-diyl)bis(3,3-dimethyl-6-oxocyclohex-1-ene-carboxylate) (33)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Dimethyl 5,5'-(but-2-yne-1,4-diyl)bis(3,3-dimethyl-6-oxocyclohex-1-ene-carboxylate) (34)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
Bis(methyl 8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate) methylene (35)

$^1$H NMR spectrum

![H NMR spectrum](image)

$^{13}$C NMR spectrum

![C NMR spectrum](image)
Bis(methyl 8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate) propylene (36)

$^1$H NMR spectrum

$^{13}$C NMR spectrum
(3,3-dimethyl-6-oxocyclohex-1-en-1-yl)methyl 3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (37)

$^1$H NMR spectrum

$^{13}$C NMR spectrum