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# Pinkment: a synthetic platform for the development of fluorescent probes for diagnostic and theranostic applications†

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Reaction-based fluorescent-probes have proven successful for the visualisation of biological species in various cellular processes. Unfortunately, in order to tailor the design of a fluorescent probe to a specific application (*i.e.* organelle targeting, material and theranostic applications) often requires extensive synthetic efforts and the synthetic screening of a range of fluorophores to match the required synthetic needs. In this work, we have identified Pinkment-OH as a unique “plug-and-play” synthetic platform that can be used to develop a range of ONOO<sup>−</sup> responsive fluorescent probes for a variety of applications. These include theranostic-based applications and potential material-based/bioconjugation applications. The as prepared probes displayed an excellent sensitivity and selectivity for ONOO<sup>−</sup> over other ROS. *In vitro* studies using HeLa cells and RAW 264.7 macrophages demonstrated their ability to detect exogenously and endogenously produced ONOO<sup>−</sup>. Evaluation in an LPS-induced inflammation mouse model illustrated the ability to monitor ONOO<sup>−</sup> production in acute inflammation. Lastly, theranostic-based probes enabled the simultaneous evaluation of indomethacin-based therapeutic effects combined with the visualisation of an inflammation biomarker in RAW 264.7 cells.

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## Introduction

There is a growing need for new and effective diagnostic tools that can evaluate biomarkers involved in inflammatory based diseases.<sup>1–6</sup> Inflammation is the innate defence mechanism of the body that recognises damaged cells, pathogens and

infections. The inflammatory response often results in the generation of reactive oxygen species/reactive nitrogen species (ROS/RNS), which are involved in the functional regulation of M1 and M2 macrophages.<sup>7,8</sup> The M1 pro-inflammatory phenotype is induced by lipopolysaccharide (LPS), which triggers the generation of ROS from NADPH using NADPH oxidase (NOX).<sup>9</sup> This production of ROS regulates an array of cellular events including the activation of the nuclear factor kappa-B (NF-κB), the production of cytokines and cell survival whereas, high levels of ROS are associated with programmed cell death, *i.e.* apoptosis.<sup>7,10–14</sup> The high sensitivity and high spatial and temporal resolution of fluorescent probes allow us to visualise these key cellular events. Our group and others have focused on the fluorescence-based detection of ROS/RNS such as ONOO<sup>−</sup>, H<sub>2</sub>O<sub>2</sub> and HOCl.<sup>1,15–21</sup> To achieve the selective detection of a particular ROS requires the careful consideration of both fluorophore and reactive motif. In this regard, resorufin is a particularly attractive fluorophore due to its red shifted fluorescence and easy to functionalise scaffold. Pioneering work led by Chang *et al.* developed peroxyresorufin-1 (PR1) for H<sub>2</sub>O<sub>2</sub> detection whereby resorufin is masked with boronic esters.<sup>22,23</sup> Boronic esters have been identified as a relevant sensing group for both H<sub>2</sub>O<sub>2</sub> and ONOO<sup>−</sup> detection. However, in an environment with both species present, boronic esters preferentially react with ONOO<sup>−</sup> due to the inherent faster reactivity of ONOO<sup>−</sup> in comparison to H<sub>2</sub>O<sub>2</sub>.<sup>24</sup> Previously, we have

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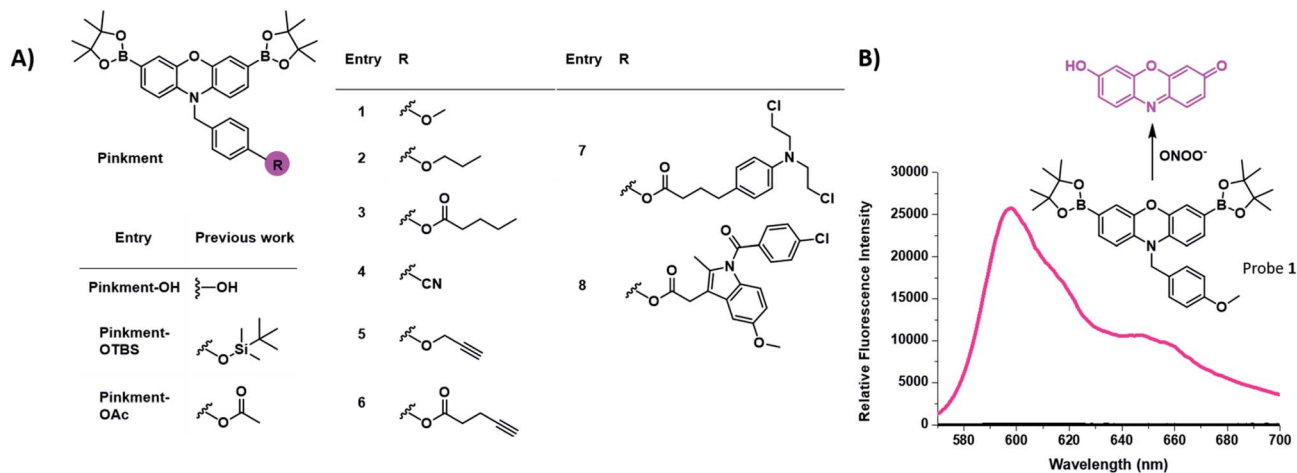


Fig. 1 (A) Chemical structure of the resorufin-based probes for the sensing of  $\text{ONOO}^-$ , including the previously reported **Pinkment-OH** “plug and play” scaffold and dual analyte probes **Pinkment-OTBS** and **Pinkment-OAc**.<sup>10</sup> (B) Unexpected fluorescence turn on response of **1** in the presence of  $\text{ONOO}^-$ .

demonstrated PR1's ability to preferentially detect  $\text{ONOO}^-$  over  $\text{H}_2\text{O}_2$  *in vitro*.<sup>25</sup> Consequently, we decided to investigate functionalized synthetic derivatives of PR1.<sup>26</sup> This led to the development of **Pinkment-OH** for the design of dual analyte AND-logic probes, **Pinkment-OTBS** ( $\text{ONOO}^-$  “AND” fluoride) and **Pinkment-OAc** ( $\text{H}_2\text{O}_2$  “AND” esterase) using **Pinkment-OH** (Fig. 1) as a synthetic starting point. These results revealed the potential of **Pinkment-OH** to be used as a synthetic platform for the development of  $\text{ONOO}^-$  selective fluorescence probes with additional sensing, targeting or drug units. Here, we have serendipitously discovered that the benzyl unit of our ROS **Pinkment** fluorescent probe can be functionalized with a functional unit of choice without compromising ROS selectivity. As a result, **Pinkment-OH** was successfully shown as a synthetic platform to develop  $\text{ONOO}^-$  selective fluorescent probes with additional functional units (Fig. 1).

## Results and discussion

Initially, our focus was on continuing the development of “AND”-based logic-gates for biological application.<sup>1,17,26</sup> This led to the elaboration of probe **1**, which was accessed in a simple three step synthesis (Scheme S1†). Unexpectedly, we discovered that **1** “turned on” in the sole presence of  $\text{ONOO}^-$  (Fig. 1B and S1†). This led to the development of **Pinkment** probes **2** and **3** to further confirm this observation. These probes were accessible from the synthetic platform **Pinkment-OH**, whose 6-step synthesis has been previously reported by our group.<sup>26</sup> Nucleophilic substitution by **Pinkment-OH** using 1-bromopropane and pentanoyl chloride respectively gave **2** and **3** in moderate yields: 50% and 51% respectively (Scheme S2 and S3†). Probes **2** and **3** showed good selectivity towards  $\text{ONOO}^-$  over other ROS species (Fig. S2–S8†). Surprisingly, the probes demonstrated a high sensitivity towards  $\text{ONOO}^-$  requiring concentrations in the low micromolar range. Both **2** and **3** displayed increased solubility in comparison to **1**. We decided to further explore this

unexpected result by introducing a terminal nitrile group. Probe **4** was accessible in a facile three-step synthesis (Scheme S4†) in the same manner as **1**. Again, good selectivity and sensitivity for  $\text{ONOO}^-$  was observed (Fig. S2–S8†). From these results, we realized that the **Pinkment** benzyl unit can be functionalized with any unit of choice without compromising the ROS selectivity. Thus, we rationalized that **Pinkment-OH** offers a unique platform for the design of  $\text{ONOO}^-$  selective fluorescence based probes that can be tailored towards a range of applications.<sup>27–29</sup> This led to the development of alkyne-based **Pinkment** probes **5** and **6** that have potential to be used in “click” chemistry.<sup>29</sup> These probes were accessed from **Pinkment-OH** and prepared in moderate yields: 48% and 47% for **5**, and **6** respectively (Scheme S5 and S6†). Fluorescence studies of **5** and **6** established good sensitivity and selectivity towards  $\text{ONOO}^-$  over other ROS (Fig. S2–S8†).

We then turned our attention to assessing the imaging capacity of probes **2** and **3** and the potential “click” based probes **5** and **6** in cells and live animals. To demonstrate their suitability as imaging tools, all four probes were evaluated for cellular toxicity in murine RAW 264.7 macrophages using a MTS cell proliferation assay. Probes **2**, **3**, **5** and **6** were incubated at different concentrations ranging from 5 to 40  $\mu\text{M}$  for 24 h (Fig. S9†). Probes **2**, **5** and **6** were found to be non-toxic. In contrast, probe **3** decreased the cell viability of RAW 264.7 macrophages by 40% at a concentration of 40  $\mu\text{M}$  compared to control conditions. As a result, **3** was not taken forward for further cell studies since high concentrations of the probe are required for *in vivo* studies.

Probes **2**, **5** and **6** were shown to be non-toxic, and were evaluated with exogenous  $\text{ONOO}^-$ , using SIN-1 (500  $\mu\text{M}$ ) in RAW 264.7 macrophages (Fig. 2A and S10†). Each probe alone demonstrated minimal fluorescence in cells, the addition of SIN-1 led to a significant enhancement in intracellular fluorescence at a wavelength corresponding to the dye, resorufin, therefore, suggesting the intracellular reaction of the probe with



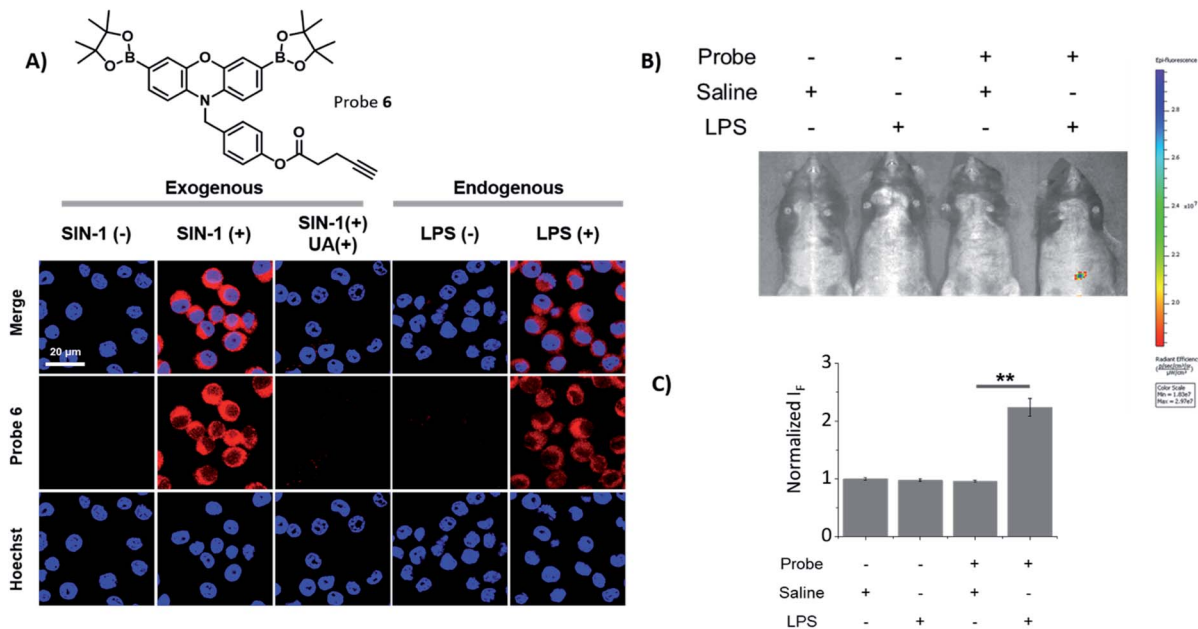


Fig. 2 (A) Confocal imaging of RAW 264.7 macrophages treated with probe 6 (20  $\mu$ M, 30 min) in the presence and absence of SIN-1 (500  $\mu$ M, 30 min) and uric acid (100  $\mu$ M, 2 h) or LPS (1  $\mu$ g mL<sup>-1</sup>, 24 h) as indicated. Fluorescence data was collected using  $\lambda_{ex}$  = 559 nm and  $\lambda_{em}$  = 580–650 nm, respectively. The cell nuclei was stained using Hoechst 33342 and fluorescence collected at  $\lambda_{ex}$  = 405 nm and  $\lambda_{em}$  = 450–480 nm. Scale bar = 20  $\mu$ m.  $N$  = 3. (B) Intraperitoneal injection of male C57BL/6J mice with probe 6 (200  $\mu$ M) or saline in the absence and presence of LPS (2 mg mL<sup>-1</sup> in saline) with  $\lambda_{ex}$  = 535 nm and  $\lambda_{em}$  = 600 nm.  $N$  = 3. (C) Quantification of (B) C57BL/6J male mice treated with probe 6 (200  $\mu$ M) or saline in the absence and presence of LPS (2 mg mL<sup>-1</sup> in saline) with  $\lambda_{ex}$  = 550 nm and  $\lambda_{em}$  = 580–620 nm. Error bars represent s. d. with \*\* $p$   $\leq$  0.01.  $N$  = 3. Normalised fluorescence intensities were calculated using the saline solution fluorescence intensities.

ONOO<sup>-</sup> and their suitability for use as fluorescence-based probes. The SIN-1 generated fluorescence signal was then evaluated with the ONOO<sup>-</sup> scavenger, uric acid.<sup>30</sup> As expected, uric acid attenuated the fluorescent increase that was induced by SIN-1 for all probes, thus confirming the ONOO<sup>-</sup> mediated increase in fluorescence intensity. Next, we evaluated the capability of 2, 5 and 6 to detect endogenous ONOO<sup>-</sup> in LPS primed RAW 264.7 macrophages. All three probes were shown to detect endogenous ONOO<sup>-</sup> in LPS primed RAW 264.7 macrophages (Fig. 2A and S11<sup>†</sup>), confirming their promise for the imaging of LPS-induced inflammatory responses. In addition, HeLa and A549 cell lines treated with or without SIN-1 were used to illustrate the versatility of the **Pinkment** probes (Fig. S12 and S13<sup>†</sup>).

Encouraged by these cell imaging results, we used a known LPS-induced inflammation mouse model<sup>31</sup> for the *in vivo* detection of ONOO<sup>-</sup> (Fig. 2B). The injection of LPS (2 mg mL<sup>-1</sup> in saline) to the abdominal region of mice followed by the injection of 6 (200  $\mu$ M) led to its fluorescence activation. The quantified fluorescence intensity in the probe(+)/LPS(+) group was significantly larger than that in the probe(+)/LPS(-) group (Fig. 1C), demonstrating the potential of using 6 for the monitoring of ONOO<sup>-</sup> *in situ* during acute inflammation.

In order to follow our current interest in theranostics,<sup>32</sup> we then turned our attention towards the potential of **Pinkment-OH** for the design of fluorescence-based drug releasing probes. Therefore, we used the drugs chlorambucil and indomethacin to afford two distinct theranostic probes 7 and 8, respectively

(Fig. 1A). Chlorambucil is used to treat chronic lymphatic leukemia<sup>33</sup> and indomethacin is used as a non-steroidal anti-inflammatory drug (NSAID).<sup>34,35</sup> Both 7 and 8 were easily accessible from **Pinkment-OH** (Scheme S7 and S8<sup>†</sup>).

Mass spectrometry confirmed and validated the simultaneous release of each drug and fluorescent resorufin dye (Fig. S14 and S15<sup>†</sup>). Therefore, the enhancement in the fluorescence intensity over time indicates the release of each drug. As such, time-dependent fluorescence experiments with 7 and 8 in the presence of ONOO<sup>-</sup> were performed to illustrate the time dependence of the drug release. These experiments revealed a maximum fluorescence response after  $\sim$ 10 min (Fig. S16<sup>†</sup>).

Fluorescence studies were carried out including ROS selectivity, H<sub>2</sub>O<sub>2</sub> titration and ONOO<sup>-</sup> titration studies (Fig. S17–S21<sup>†</sup>) and demonstrated high sensitivity towards these inflammation-based biomarkers. Following these initial studies, we evaluated both 7 and 8 in RAW 264.7 macrophages towards exogenous ONOO<sup>-</sup> detection (Fig. S22<sup>†</sup>). The presence of SIN-1 significantly enhanced the intracellular fluorescence of 7 and 8, confirming the applicability of the probes *in vitro*. Despite 7 displaying significant promise, the creation of an appropriate model system to differentiate between cancerous and healthy cells would require a significant amount of development and as such was beyond the scope of this current research. Therefore, only 8 was further evaluated, since its cellular behaviour was easier to monitor. Endogenous ONOO<sup>-</sup> was also detected by 8 in RAW 264.7 macrophages (Fig. 3A). Indomethacin, a NSAID, is an effective and non-selective



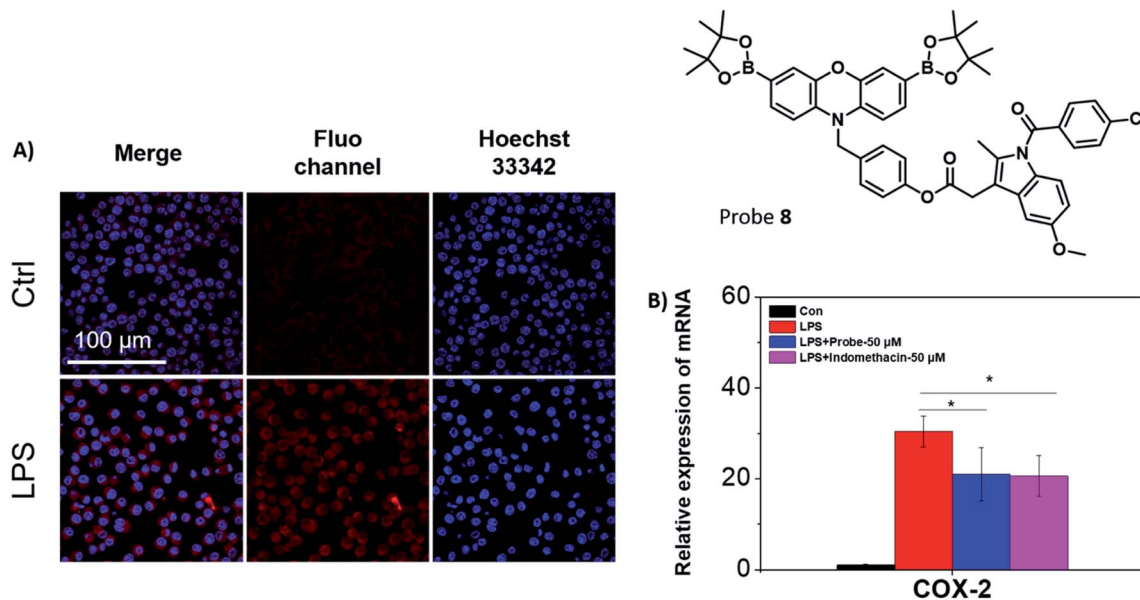


Fig. 3 (A) Confocal imaging of RAW 264.7 macrophages treated with LPS ( $1 \mu\text{g mL}^{-1}$ , 24 h) and then loaded with **8** ( $20 \mu\text{M}$ , 30 min) as indicated. Fluorescence data was collected using  $\lambda_{\text{ex}} = 559 \text{ nm}$  and  $\lambda_{\text{em}} = 580\text{--}650 \text{ nm}$ , respectively. The cell nuclei were stained using Hoechst 33342 and fluorescence collected at  $\lambda_{\text{ex}} = 405 \text{ nm}$  and  $\lambda_{\text{em}} = 450\text{--}480 \text{ nm}$ . Scale bar =  $100 \mu\text{m}$ .  $N = 3$ . (B) Effect of **8** on LPS-induced COX-2 gene expression in RAW 264.7 macrophages. Cells were treated with LPS alone ( $1 \mu\text{g mL}^{-1}$ ) or together with **8** for 24 h. Indomethacin was set as a positive control, and the relative mRNA level of COX-2 gene was normalized by GAPDH ( $*p < 0.05$ ).  $N = 4$ .

inhibitor of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), of which COX-2 is mainly responsible for the inflammatory response.<sup>36</sup> The therapeutic effects on the LPS-induced inflammatory responses in RAW 264.7 macrophages were further investigated using **8**. RAW 264.7 macrophages were treated with LPS and the expression of the pro-inflammatory gene (COX-2) was investigated using qRT-PCR in the presence or absence of **8** (Fig. 3B).<sup>37</sup> The mRNA level of COX-2 decreased in the presence of **8** ( $50 \mu\text{M}$ ) in comparison to the LPS-induced group. A similar effect to the LPS-induced group was observed with indomethacin alone. This suggests that **8** can monitor ONOO<sup>-</sup> production in acute inflammation, and in addition, reduce the inflammatory response by releasing indomethacin.

## Conclusions

The ability of the **Pinkment** scaffold to be functionalised with any unit of choice without compromising the overall ROS selectivity, opens up new possibilities for the design of highly specific ONOO<sup>-</sup> probes that can be used in a variety of applications. In this work, we have successfully illustrated the applicability of **Pinkment**-based probes for diagnostic and theranostics applications. Our probes displayed good selectivity and sensitivity towards ONOO<sup>-</sup> over a range of other ROS. Cellular studies with the **Pinkment** probes led to the identification of alkyne-functionalised **Pinkment** probe **6** as a suitable candidate for *in vivo* studies using an inflammatory mouse model. These promising results led us to design potential theranostic probes **7** and **8** with candidate **8** displaying promising properties *in vitro*. We believe this work demonstrates **Pinkment-OH** as a useful synthetic platform to enable the rapid

development of a ONOO<sup>-</sup> fluorescent probe that can be tailored to the needs of the chemical biologist. In particular, the alkyne **Pinkment** probes offer the possibility of attaching any desired unit *via* click chemistry. Therefore, we anticipate that the **Pinkment** scaffold can be further elaborated for the development of dual analyte, organelle targeting and theranostic probes for a range of diagnostic and theranostic applications.

## Conflicts of interest

There are no conflicts to declare.

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## Notes and references

- X. L. Hu, N. Kwon, K. C. Yan, A. C. Sedgwick, G. R. Chen, X. P. He, T. D. James and J. Yoon, *Adv. Funct. Mater.*, 2020, **30**, 1907906.
- D. Wu, A. C. Sedgwick, T. Gunnlaugsson, E. U. Akkaya, J. Yoon and T. D. James, *Chem. Soc. Rev.*, 2017, **46**, 7105–7123.
- S. Erbas-Cakmak, S. Kolemen, A. C. Sedgwick, T. Gunnlaugsson, T. D. James, J. Yoon and E. U. Akkaya, *Chem. Soc. Rev.*, 2018, **47**, 2228–2248.
- A. C. Sedgwick, L. L. Wu, H. H. Han, S. D. Bull, X. P. He, T. D. James, J. L. Sessler, B. Z. Tang, H. Tian and J. Yoon, *Chem. Soc. Rev.*, 2018, **47**, 8842–8880.
- J. Chan, S. C. Dodani and C. J. Chang, *Nat. Chem.*, 2012, **4**, 973–984.
- K. C. Yon, A. C. Sedgwick, Y. Zang, G. R. Chen, X. P. He, J. Li, J. Yoon and T. D. James, *Small Methods*, 2019, **3**, 1900013.
- A. Weidinger and A. V. Kozlov, *Biomolecules*, 2015, **5**, 472–484.
- B. Ghesquiere, B. W. Wong, A. Kuchnio and P. Carmeliet, *Nature*, 2014, **511**, 167–176.
- A. Kumar, S. H. Chen, M. B. Kadiiska, J. S. Hong, J. Zielonka, B. Kalyanaraman and R. P. Mason, *Free Radical Biol. Med.*, 2014, **73**, 51–59.
- B. Halliwell, *Annu. Rev. Nutr.*, 1996, **16**, 33–50.
- H. Wiseman and B. Halliwell, *Biochem. J.*, 1996, **313**, 17–29.
- J. S. Beckman and W. H. Koppenol, *Am. J. Physiol.: Cell Physiol.*, 1996, **271**, C1424–C1437.
- M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur and J. Telser, *Int. J. Biochem. Cell Biol.*, 2007, **39**, 44–84.
- H. Y. Tan, N. Wang, S. Li, M. Hong, X. B. Wang and Y. B. Feng, *Oxid. Med. Cell. Longevity*, 2016, **2016**, 2795090.
- A. C. Sedgwick, R. S. L. Chapman, J. E. Gardiner, L. R. Peacock, G. Kim, J. Yoon, S. D. Bull and T. D. James, *Chem. Commun.*, 2017, **53**, 10441–10443.
- A. C. Sedgwick, H. H. Han, J. E. Gardiner, S. D. Bull, X. P. He and T. D. James, *Chem. Commun.*, 2017, **53**, 12822–12825.
- A. C. Sedgwick, H. H. Han, J. E. Gardiner, S. D. Bull, X. P. He and T. D. James, *Chem. Sci.*, 2018, **9**, 3672–3676.
- A. C. Sedgwick, W. T. Dou, J. B. Jiao, L. L. Wu, G. T. Williams, A. T. A. Jenkins, S. D. Bull, J. L. Sessler, X. P. He and T. D. James, *J. Am. Chem. Soc.*, 2018, **140**, 14267–14271.
- L. L. Wu, H. H. Han, L. Y. Liu, J. E. Gardiner, A. C. Sedgwick, C. S. Huang, S. D. Bull, X. P. He and T. D. James, *Chem. Commun.*, 2018, **54**, 11336–11339.
- A. C. Sedgwick, J. E. Gardiner, G. Kim, M. Yevglevskis, M. D. Lloyd, A. T. A. Jenkins, S. D. Bull, J. Yoon and T. D. James, *Chem. Commun.*, 2018, **54**, 4786–4789.
- L. L. Wu, A. C. Sedgwick, X. L. Sun, S. D. Bull, X. P. He and T. D. James, *Acc. Chem. Res.*, 2019, **52**, 2582–2597.
- B. C. Dickinson, C. Huynh and C. J. Chang, *J. Am. Chem. Soc.*, 2010, **132**, 5906–5915.
- E. W. Miller, A. E. Albers, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2005, **127**, 16652–16659.
- A. Sikora, J. Zielonka, M. Lopez, J. Joseph and B. Kalyanaraman, *Free Radical Biol. Med.*, 2009, **47**, 1401–1407.
- M. Weber, A. B. Mackenzie, S. D. Bull and T. D. James, *Anal. Chem.*, 2018, **90**, 10621–10627.
- M. L. Odyniec, A. C. Sedgwick, A. H. Swan, M. Weber, T. M. S. Tang, J. E. Gardiner, M. Zhang, Y. B. Jiang, G. Kociok-Kohn, R. B. P. Elmes, S. D. Bull, X. P. He and T. D. James, *Chem. Commun.*, 2018, **54**, 8466–8469.
- A. Marrocchi, A. Facchetti, D. Lanari, S. Santoro and L. Vaccaro, *Chem. Sci.*, 2016, **7**, 6298–6308.
- Y. P. Chen, Y. L. Xianyu, J. Wu, B. F. Yin and X. Y. Jiang, *Theranostics*, 2016, **6**, 969–985.
- H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
- D. C. Hooper, S. Spitsin, R. B. Kean, J. M. Champion, G. M. Dickson, I. Chaudhry and H. Koprowski, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 675–680.
- D. Lee, S. Khaja, J. C. Velasquez-Castano, M. Dasari, C. Sun, J. Petros, W. R. Taylor and N. Murthy, *Nat. Mater.*, 2007, **6**, 765–769.
- M. L. Odyniec, H. H. Han, J. E. Gardiner, A. C. Sedgwick, X. P. He, S. D. Bull and T. D. James, *Front. Chem.*, 2019, **7**, 775.
- M. J. Reese, D. W. Knapp, K. M. Anderson, J. A. Mund, J. Case, D. R. Jones and R. A. Packer, *PLoS One*, 2018, **13**, e0203517.
- K. Seibert, Y. Zhang, K. Leahy, S. Hauser, J. Masferrer and P. Isakson, in *Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation, and Radiation Injury 2, Pts A and B*, ed. K. V. Honn, S. Nigam and L. J. Marnett, 1997, vol. 400, pp. 167–170.
- A. A. Onischuk, T. G. Tolstikova, I. V. Sorokina, N. A. Zhukova, A. M. Baklanov, V. V. Karasev, G. G. Dultseva, V. V. Boldyrev and V. M. Fomin, *J. Aerosol Med. Pulm. Drug Delivery*, 2008, **21**, 231–243.
- C. Chen, *Nat. Chem. Biol.*, 2010, **6**, 401–402.
- M. Kawashima, N. Ogura, M. Akutsu, K. Ito and T. Kondoh, *J. Oral Pathol. Med.*, 2013, **42**, 499–506.

