In Situ Generated Novel $^1$H MRI Reporter for β-Galactosidase Activity Detection and Visualization in Living Tumor Cells

Shuo Gao, Lei Zhao, Zhiqiang Fan, Vikram D. Kodibagkar, Li Liu, Hanqin Wang, Hong Xu, Mingli Tu, Bifu Hu, Chuanbin Cao, Zhenjian Zhang and Jian-Xin Yu

1Center of Translational Medicine, Fifth School of Medicine/Suizhou Central Hospital, Hubei University of Medicine, Suizhou, China, 2School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ, United States, 3Department of Radiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, United States, 4Biomedical Research Institute, Hubei University of Medicine, Shiyan, China

For wide applications of the lacZ gene in cellular/molecular biology, small animal investigations, and clinical assessments, the improvement of noninvasive imaging approaches to precisely assay gene expression has garnered much attention. In this study, we investigate a novel molecular platform in which alizarin 2-O-β-D-galactopyranoside AZ-1 acts as a lacZ gene/β-gal responsive $^1$H-MRI probe to induce significant $^1$H-MRI contrast changes in relaxation times $T_1$ and $T_2$ in situ as a concerted effect for the discovery of β-gal activity with the exposure of Fe$^{3+}$. We also demonstrate the capability of this strategy for detecting β-gal activity with lacZ-transfected human MCF7 breast and PC3 prostate cancer cells by reaction-enhanced $^1$H-MRI $T_1$ and $T_2$ relaxation mapping.

Keywords: β-galactosidase detection, responsive Fe-based $^1$H-MRI agent, $T_1$ and $T_2$ relaxation mapping, in vitro $^1$H-MRI imaging, lacZ gene reporter, synthesis

INTRODUCTION

Due to various advantages such as stability, high turnover rate, and ease of conjugation, the lacZ gene-encoding β-galactosidase (β-gal) has been broadly used in cellular/molecular biology, small animal studies, clinical trials with assays of clonal insertion, transcriptional activation, and protein expression and interaction (Kruger et al., 1999; Haberkorn et al., 2005; Razgulin et al., 2011; Yang et al., 2019). Moreover, overexpressed β-gal has been identified as a vital enzyme biomarker related to cell senescence and cancer progression (Chatterjee et al., 1979; Alam et al., 1990; Dimri et al., 1995; Paradis et al., 2001; Pacheco-Rivera et al., 2016; Lozano-Torres et al., 2017; Sharma and Leblanc, 2017; Kim et al., 2018; Wang et al., 2019; Li et al., 2020b; Gao et al., 2020; Qiu et al., 2020). Thus, β-gal activity detection has been exploited with diverse techniques including colorimetric assays (James et al., 2000; Browne et al., 2010; Zeng et al., 2012; Yeung et al., 2013; Chen et al., 2016; Hu Q. et al., 2021).
β-Galactosidase prompts the hydrolysis of β-D-galactopyranoside by cleavage of its β-anomeric C-O linkage between β-D-galactopyranose and aglycone; the hydrolysis reactivity of β-D-galactopyranosides to β-gal is completely dependent upon the aglycone structure. However, the structure activity relationship of the aglycones in β-D-galactopyranosides vs. β-gal is not yet clear (Juers et al., 2012; Duo et al., 2017). Therefore, further exploration is still highly needed to discover effective β-gal substrates for functional molecular imaging probes. We believe that the traditional histopathological methods of assaying β-gal activity might be the fruitful resources for developing novel imaging agents for the assessment of lacZ gene expression. In reviewing the histopathological literature, we noticed that the well-established β-gal substrate alizarin 2-O-β-D-galactopyranoside AZ-1 (Figure 1) is readily hydrolyzed by β-gal to release aglycone alizarin, which chelates with ferric iron Fe3+ to form an intense dark violet Fe complex (James et al., 2000). By comparison of the structural characteristics of the Fe3+-alizarin complex with Fe3+-based 1H-MRI contrast agents (Davies et al., 1996; Richardson et al., 1999; Schwert et al., 2002; Schwert et al., 2005; Haas and Franz, 2009; Yu et al., 2012a; Yu et al., 2012b; Gulaka et al., 2013; Li et al., 2013; Yu et al., 2013; Kuznik and Wyskocka, 2016), we speculated that the Fe3+-alizarin complex could function as an Fe3+-based 1H-MRI contrast agent. If so, the well-established β-gal substrate AZ-1 could work as a lacZ gene or β-gal 1H-MRI reporter. Upon delivery and cleavage at lacZ-transfected or β-gal-overexpressed tumor cells with the presence of Fe3+, the paramagnetic Fe complex could be spontaneously formed in situ and specifically produced the 1H MRI contrast effect while localizing and accumulating 1H-MRI signals at the β-gal activity site. Figure 1 depicts the Fe3+-alizarin complex generated in situ for the 1H-MRI detection of β-gal activity. We now demonstrate the use of exploiting AZ-1 to assess β-gal activity in vitro with lacZ-transfected human MCF7 breast and PC3 prostate cancer cells by 1H MRI $T_1$ and $T_2$ relaxation mapping.

**FIGURE 1** | The proposed mechanism of an in situ generated stable Fe3+-alizarin complex for 1H MRI detection of β-gal activity.
RESULTS AND DISCUSSION

Verification of the Fe$^{3+}$–Alizarin Complex as an $^1$H-MRI Contrast Agent

Alizarin is 1,2-dihydroxy-9,10-anthraquinone with a tricyclic aromatic planar structure and chelates with Fe$^{3+}$ to form a thermodynamically stable octahedral Fe$^{3+}$–alizarin (1:3) complex at physiological pH conditions with the stability constant $\log \beta = 32.21$ (Das et al., 1995; Das et al., 2002). To explore the MRI signal–enhancing capability of the Fe$^{3+}$–alizarin complex, the spin–lattice relaxation time $T_1$ and spin–spin relaxation time $T_2$ of the Fe$^{3+}$–alizarin complex were measured with a 4.7 T MR scanner using a saturation recovery spin echo sequence and multi-spin echo sequence with varying repetition times (TRs) and echo times (TEs), respectively. The images were acquired using a 3-cm diameter solenoid coil (home-built) with $4 \times 4$ cut section of a 96-well plate containing the different concentrations of alizarin and ferric ammonium citrate (FAC) mixed solutions in 1:1 (V/V′) DMSO/PBS (0.1 M, pH = 7.4) at 37°C in 4 h.

### FIGURE 2

The $^1$H-MRI of an Fe$^{3+}$–alizarin complex. MRI acquisition parameters: $^1$H-MRI, 200 MHz, matrix size: 128 × 128, FOV: 40 mm × 40 mm, slice thickness: 2 mm; receiver bandwidth: 20 kHz, $T_1$-map: saturation recovery spin-echo sequence, TR = 200, 400, 800, 1,000, 2,000, 3,000, and 6,000 ms, respectively, TE = 15 ms; $T_2$-map: multi-echo SE sequence, TE = 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, and 160 ms, respectively, TR = 2000 ms. (A) Control, FAC (15.0 mM); (B) alizarin (2.5 mM), FAC (15.0 mM); (C) alizarin (6.0 mM) and FAC (15.0 mM); (D) alizarin (9.0 mM), FAC (15.0 mM) in 1:1 (V/V′), and DMSO/PBS (0.1 M, pH = 7.4) at 37°C in 4 h.

Alizarin β-D-Galactopyranoside Synthesis

After the Fe$^{3+}$–alizarin complex was shown to be an $^1$H-MRI contrast agent, we therefore began the β-D-galactopyranosylation with alizarin at the phenolic hydroxyl groups. Previously, James et al. (2000) reported a modified method for the synthesis of AZ-1, involving the reaction of alizarin potassium salt with acetobromo-α-D-galactose via the nucleophilic substitution procedure followed by deacetylation mediated by the aqueous NaOH solution, but the yield was low (14%). We observed that
the phenolic hydroxyl groups at 1,2-positions of alizarin have excellent site-reaction selectivity due to the various electronic deficiency/stERICally hindered effects (Mahal et al., 2011) and apparently different $pK_a$ values: $pK_{a(2-OH)} = 5.98 \pm 0.05$, whereas $pK_{a(1-OH)} = 9.88 \pm 0.05$ (Das et al., 1995; Das et al., 2002), which suggested that the phase-transfer catalysis method at pH 8–9 could provide regio- and stereoselective synthesis of AZ-1, as exploited previously for $\beta$-gal $^{19}$F-MRS/MRI reporters (Yu et al., 2005; Kodibagkar et al., 2006; Yu et al., 2006; Yu and Mason, 2006; Liu et al., 2007; Yu et al., 2008a; Yu et al., 2008b; Yu et al., 2012b; Yu et al., 2013; Yu et al., 2017). To the well-stirred solution of alizarin in $\text{CH}_2\text{Cl}_2$-$\text{H}_2\text{O}$ (pH 8.0–9.0) using tetrabutylammonium bromide (TBAB) as a catalyst at 50°C, an equimolar amount of 2, 3, 4, and 6-tetra-O-acetyl-$\alpha$-$D$-galactopyranosyl bromide was dropped under N$_2$ atmosphere afforded alizarin 1,2-di-O-2', 3', 4', 6'-tetra-O-acetyl-$\beta$-$D$-galactopyranoside AZ-M2 at 62% yield. Deacetylation accomplished the target molecule alizarin 1,2-di-O-$\beta$-$D$-galactopyranoside AZ-2 with 94% yield. Figure 3 illustrates the structures of AZ-1/AZ-M1 and AZ-2/AZ-M2. As expected, the free di-$\beta$-$D$-galactopyranoside AZ-2 is soluble in PBS (0.1 M, pH = 7.4) at the concentration of 15 mM; meanwhile, the free mono-$\beta$-$D$-galactopyranoside AZ-1 unlikely requires the addition of DMSO for the same concentration. The structures of AZ-M1/AZ-1 and AZ-M2/AZ-2 were confirmed by NMR and HRMS data. The molecular/quasimolecular ions of AZ-M1 and AZ-1, as well as AZ-M2 and AZ-2, showed the introduction of one and two galactopyranosyl units to AZ-M1/AZ-1 and AZ-M2/AZ-2, respectively, in which the $\beta$-$D$-galactopyranoside configuration was determined by $1H/13C$ NMR data of the anomeric protons at $\delta_{H-1'} = \delta_{H-1''} = 4.90$–5.30 ppm and their coupling constants $J_{1',2'} = J_{1'',2''} \approx 8.0$ Hz while maintaining the corresponding anomeric C-1'/C-1'' at $\delta_{C-1'} = \delta_{C-1''} = 99.5$–104.0 ppm in accordance (Supporting Information, Supplementary Figures S4–S13), which are in agreement with the typical characteristics for the identification of the anomeric $\beta$-$D$-configuration (Yu et al., 2005; Yu et al., 2006; Yu and Mason, 2006; Kodibagkar et al., 2006; Liu et al., 2007; Yu et al., 2008a; Yu et al., 2008b; Yu et al., 2012b; Yu et al., 2013; Yu et al., 2017).

**$\beta$-Gal Reactivity**

AZ-1 has been identified as a highly sensitive substrate for the demonstration of $\beta$-gal in a range of Gram-negative bacteria under incubation at 37°C in air for 18 h (James et al., 2000). However, none of the existing data have shown the kinetics of AZ-1 vs. $\beta$-gal, which is crucial for further in vivo imaging applications. The absorption spectra of AZ-1 and AZ-2 solutions in 1:1 (V/V) DMSO/PBS (0.1 M, pH = 7.4) with and without $\beta$-gal (E801A) at 20–22°C indicated that upon reactions of AZ-1 and AZ-2 with $\beta$-gal, a new absorption around 520 nm, corresponding to the in situ released alizarin mono-/dianions, appeared and increased gradually. Hence, the absorbance measurements at 520 nm following the enzymatic
FIGURE 4 | The kinetic hydrolysis time courses of alizarin β-D-galactopyranosides AZ-1 (▲) and AZ-2 (●) with β-gal. Absorbance measurements at λ = 520 nm following the addition of β-gal (E801A, 3 units) to solutions of AZ-1, AZ-2 each (5.0 mM) in 1:1 (V/V′) DMSO/PBS (0.1 M, pH = 7.4) at 20–22°C in different time points; The time courses of alizarin β-D-galactopyranosides AZ-1 (○) and AZ-2 (●) each (5.0 mM) in 1:1 (V/V′) DMSO/PBS (0.1 M, pH = 7.4) at 20–22°C in different time points without β-gal (E801A).

FIGURE 5 | 1H-MRI detection of β-gal activity. 1H-MRI acquisition: using the same parameters as in Figure 2. (A) alizarin 2-O-β-D-galactopyranoside AZ-1 (9.0 mM) and FAC (15.0 mM); (B) alizarin 2-O-β-D-galactopyranoside AZ-1 (4.0 mM), FAC (15.0 mM), and β-gal (E801A, 5 units); (C) alizarin 2-O-β-D-galactopyranoside AZ-1 (6.0 mM), FAC (15.0 mM), and β-gal (E801A, 5 units); (D) alizarin 2-O-β-D-galactopyranoside AZ-1 (9.0 mM), FAC (15.0 mM), and β-gal (E801A, 5 units) in 1:1 (V/V′) DMSO/PBS (0.1 M, pH = 7.4) at 37°C in 4 h.
reaction of AZ-1 and AZ-2 with β-gal (E801A) at 20–22°C in different time points showed that both AZ-1 and AZ-2 are very reactive to β-gal (E801A) with varying hydrolytic rates at $V_{(AZ-1)} = 93.3$ and $V_{(AZ-2)} = 133.3 \mu M/min/unit$, respectively (Figure 4).

Also, the absorption spectra of AZ-1 and AZ-2 by reaction with other enzymes α-galactosidase (Sigma G7163) and β-glucuronidase (Sigma G8295) at 20–22°C showed that both AZ-1 and AZ-2 remained essentially stable over the period of 60 min, verifying their specificity for reaction to β-gal.

**1H-MRI Detection of β-Gal Activity**

The $T_1$ and $T_2$ maps and relaxation time values were measured with a $4 \times 4$ cut section of 96-well plate containing various concentrations of AZ-1 and AZ-2 (4.0–9.0 mM) together with a fixed concentration of FAC (15.0 mM), respectively, in 1:1 (V/V) DMSO/PBS (0.1 M, pH = 7.4) with or without β-gal (E801A). In the AZ-1/FAC solution at 37°C in 4 h in the absence of β-gal, relaxation times were determined to be $T_1 = 368 \pm 6$ and $T_2 = 134 \pm 1$ ms. In comparison, in the presence of β-gal (E801A, 5 units) in the mixture solution of AZ-1 and FAC at 37°C in 4 h, pronounced shortening in relaxation times $T_1$ and $T_2$ caused by the Fe$^{3+}$–alizarin complex generated in situ was observed at $T_1 = 138 \pm 3, 115 \pm 4$, and $84 \pm 5$ ms, whereas $T_2 = 74 \pm 1, 54 \pm 1$, and $44 \pm 5$ ms, correlating with the increasing concentrations of AZ-1 from 4.0, 6.0 and 9.0 mM, respectively (Figure 5). However, the more soluble and reactive AZ-2, exhibiting significant advantages for in vivo 1H-MRI applications, produced very unexpected results under similar procedures at the same conditions. In the absence of β-gal at 37°C in 4 h, the AZ-2/FAC solution, as the control, yielded surprisingly reduced $T_1 = 230 \pm 11$ and $T_2 = 98 \pm 1$ ms (Figure 6). However, in the presence of β-gal (E801A, 5 units), the mixture solutions of AZ-2/FAC gave rise to an insignificant decrease in $T_1 = 220 \pm 7, 198 \pm 11$, and $177 \pm 5$ ms and $T_2 = 95 \pm 1, 78 \pm 2$, and $72 \pm 1$ ms (AZ-2 concentrations at 4.0, 6.0, and 9.0 mM, respectively, Figure 6), indicating the much less Fe$^{3+}$–alizarin complex formed in situ during the β-gal hydrolysis. Comparing the interactions between AZ-1/AZ-2 and Fe$^{3+}$ with their relaxivities to FAC, we attributed that the larger differences of AZ-2/FAC to FAC solution ($T_1$: 230 ± 11 vs. 389 ± 6 ms and $T_2$: 98 ± 1 vs. 143 ± 1 ms; alternatively, $\Delta R_1 = 1.78 \text{s}^{-1}$ and $\Delta R_2 = 3.21 \text{s}^{-1}$) than AZ-1/FAC solution ($T_1$: 368 ± 6 vs. 389 ± 6 ms, $T_2$: 134 ± 1 vs. 143 ± 1 ms; alternatively, $\Delta R_1 = 0.15 \text{s}^{-1}$, $\Delta R_2 = 0.47 \text{s}^{-1}$) were risen from the formation of the much stronger and more stable molecular tweezer complex AZ-2/Fe$^{3+}$ due to the adjacent $3',4',6'-\text{OH}$ and $3''',4''',6'''-\text{OH}$ located at the same side of 1,2-
di-O-β-D-galactopyranosyl rings in the favored configuration for chelation of Fe^{3+} (Supplementary Figure S3 in the Supporting Information) (Davies et al., 1996; Richardson et al., 1999; Schwert et al., 2002; Schwert et al., 2005; Haas and Franz, 2009; Coskuner et al., 2011; Kuznik and Wyskocka, 2016) which thus simultaneously hindered the reaction with β-gal (E801A) and slowed down the release of alizarin as well as the in situ generation of the Fe^{3+}-alizarin complex. These were confirmed by mixing solutions of AZ-2 and β-gal (E801A) first for hydrolysis, and then followed by adding FAC for

FIGURE 7 | MCF7-lacZ transfection. (A) Western blot: protein extracts from two transfected MCF7-lacZ cell lines (lanes 2,4), together with MCF7-WT cells (lanes 1,3,5) showing intense bands for β-gal activity in MCF7-lacZ cells and none in MCF7-WT cells; (B) MCF7-WT/MCF7-lacZ cells stained by X-gal and S-gal: deep blue (X-gal) and black (S-gal) staining confirming the intense lacZ expression in MCF7-lacZ cells with essentially no β-gal activity in MCF7-WT cells. Regional magnification x100; (C) β-Gal assay for activity quantification in MCF7-lacZ cells: 1.0 unit corresponding to the hydrolysis of 1.0 μmol/min O-nitrophenyl β-D-galactopyranoside, β-gal activity was increasingly associated with the number of MCF7-lacZ cells.

FIGURE 8 | In vitro 1H-MRI detection of lacZ gene expression in PC3-lacZ cells. 1H-MRI acquisition: 1H-MRI, 400 MHz, matrix size: 256 x 128, FOV: 48 mm x 24 mm, gradient echo imaging: TE = multiple values 3-30 ms, TR = 100 ms, flip angle = 10° (A) T_{2^*} maps: A mixture of alizarin 2-O-β-D-galactopyranosides AZ-1 (10.0 mM) and FAC (10.0 mM) with 5 x 10^{5} PC3-WT or PC3-lacZ cells was placed in the interlayer between 1% low-gelling temperature agarose in a 10-mm NMR tube, and then incubated for 4 h at 37°C under 5% CO_{2}/air with 95% humidity, T_{2^*}(AZ-1/PC3-WT/FAC) = 96 ± 23 ms (top row) and T_{2^*}(AZ-1/PC3-lacZ/FAC) = 26 ± 14 ms (bottom row), respectively; (B) relative signal intensity changes at different echo times (TEs) from (A), essentially no signal loss with PC3-WT cells (●) but a significant signal loss with PC3-lacZ cells (●); (C) PC3-WT/PC3-lacZ cells staining by X-gal, S-gal, and AZ-1: deep blue (X-gal), black (S-gal), and dark violet (AZ-1) staining confirming an intense lacZ expression in PC3-lacZ cells with essentially no β-gal activity in PC3-WT cells. Regional magnification x100; (D) cytotoxicity: PC3-WT/PC3-lacZ cells viability vs. AZ-1 in various concentrations in 1:1 (V/V') DMSO/PBS (0.1 M, pH = 7.4) at 37°C under 5% CO_{2}/air with 95% humidity for 72 h, PC3-WT cells (in blue), and PC3-lacZ cells (in red).
complexation at 37°C in 2 h for each step with the same concentrations as in Figure 6. A significant decrease in relaxation times $T_1$ and $T_2$ was seen at $T_1 = 133 \pm 1$, $110 \pm 2$, and $78 \pm 2$ ms and $T_2 = 73 \pm 2, 51 \pm 3$, and $41 \pm 1$ ms, which were very close to expectations based on AZ-1/FAC $T_1$ and $T_2$ data as shown in Figure 5.

**In Vitro $^1$H-MRI Detection of lacZ Transfection in Human Tumor Cells**

The recombinant vector pCMVlacZ has been successfully created and used to stably transfect human prostate cancer PC3-lacZ cells from PC3-wild-type (WT) cells (Liu et al., 2007). Accordingly, human breast cancer MCF7-lacZ cells were stably transfected from MCF7 wild-type (WT) cells: the β-gal activity and quantification in MCF7-lacZ cells were verified on the basis of Western blot, X-gal and S-gal staining, and the β-gal assay (Figure 7).

Given AZ-2 showed much better aqueous solubility and reactivity to β-gal, the stabilized molecular tweezer complexation AZ-2/Fe$^{3+}$ obstructed its implementation spreading to effective $^1$H-MRI assessment of β-gal. So, AZ-1 with a significant signal loss either on $T_1$ or $T_2$ upon β-gal hydrolysis was prompted for the further in vitro $^1$H-MRI evaluation. As an initial demonstration for in vitro $^1$H-MRI detection of β-gal with lacZ-transfected human cancer cells, we first acquired $T_2^*$ maps on pair mixtures of AZ-1 (10.0 mM) with PC3-WT cells ($5 \times 10^5$) and PC3-lacZ cells ($5 \times 10^5$), respectively, in the presence of FAC (10.0 mM) layered between agarose after incubation 4 h at 37°C under 5% CO$_2$/air with 95% humidity. Significant differences confined within the layers were observed between PC3-WT and PC3-lacZ cells at different echo times (Figure 8A), in which there was essentially no signal loss with PC3-WT cells but a remarkable signal decrease with PC3-lacZ cells upon increasing echo times (TEs) (Figure 8B). The relaxation time $T_2^*$ was determined to be $T_2^*_{(AZ-1/PC3/FAC)} = 96 \pm 23$ ms in PC3-WT cells, while $T_2^*_{(AZ-1/PC3-lacZ/FAC)} = 26 \pm 14$ ms in PC3-lacZ cells. Again, the β-gal activity was verified based on X-gal, S-gal, and AZ-1 staining (dark violet) (Figure 8C), with each staining method consistently showing intense lacZ expression in PC3-lacZ cells with essentially no β-gal activity in PC3-WT cells.

The cytotoxicity of AZ-1 was studied with PC3-WT and PC3-lacZ cells in PBS (0.1 M, pH = 7.4) incubated 72 h at 37°C under...
5% CO₂/air with 95% humidity. Cell viability assays showed that neither AZ-1 nor alizarin showed toxicity to PC3 cells, for AZ-1 viability exceeded 96% for both PC3-WT and PC3-lacZ cells at all concentrations tested (Figure 8D).

Furthermore, in vitro ¹H-MRI acquisition of AZ-1 (10.0 mM) with PC3-WT cells (5 × 10⁵) and PC3-lacZ cells (5 × 10⁵) in the presence of FAC (10.0 mM) was performed in a 1:1 (V/V) DMSO/PBS (0.1 M, pH = 7.4) solution. A pronounced signal decrease in the relaxation time T₁ was observed between PC3-WT (T₁(PC3-WT/FAC) = 245 ± 9 ms) and PC3-lacZ cells (T₁(PC3-lacZ/FAC) = 82 ± 7 ms) after incubation 4 h at 37°C under 5% CO₂/air with 95% humidity.

Similarly, after incubation of AZ-1 (10.0 mM) with MC7-WT cells (5 × 10⁵) and MC7-lacZ cells (5 × 10⁵), in the same conditions as the previous study, the relaxation times were observed to be T₁(AZ-1/MC7-WT/FAC) = 223 ± 11 ms and T₁(AZ-1/MC7-lacZ/FAC) = 97 ± 12 ms in MC7-WT cells, whereas T₁(AZ-1/MC7-lacZ/FAC) = 75 ± 7 ms and T₂(AZ-1/MC7-lacZ/FAC) = 45 ± 9 ms made for MC7-lacZ cells (Figures 9A,B). The T₁ and T₂ values are shown as bars adjacent to T₁ and T₂ maps; both illustrated significant differences after the reaction with β-gal at ΔR₁ = 8.85 s⁻¹ and ΔR₂ = 11.91 s⁻¹. Staining by X-gal, S-gal, and AZ-1 (dark violet) (Figure 9C) all displayed an intense lacZ expression in MC7-lacZ cells but with essentially no β-gal activity in MC7-WT cells. Cell viability assays indicated that both AZ-1 and the released aglycone alizarin have no toxicity to MC7 cells upon the viability above 95% to MC7-WT and MC7-lacZ cells at all concentrations tested for 72 h (Figure 9D).

Currently, a Gd-based contrast agent–enhanced ¹H-MRI has been widely applied for medical diagnosis, offering a noninvasive way to generate anatomical and physiological information while maintaining high spatial and temporal resolution (Terreno et al., 2010; Haris et al., 2015; Wahsner et al., 2019). An Fe-based ¹H MRI contrast agent, different from the Gd³⁺-based 1H MRI contrast agent with very strong relaxivity, exhibited much shorter relaxation times because of the formation of Fe complexes with the complete coordination of Fe³⁺, eliminating the possibility of inner-sphere to directly coordinate water, leaving outer-sphere and second-sphere coordination water molecules as the only pathways for relaxation (Davies et al., 1996; Richardson et al., 1999; Schwert et al., 2002; Schwert et al., 2005; Haas and Franz, 2009; Kuznik and Wysocka, 2016). However, an Fe-based contrast agent enhanced ¹H-MRI has now become a viable alternative because Fe³⁺ is extensively present in the tissues of the human body and is involved in transport, storage, compartmentalization, and excretion mechanisms, while Gd³⁺ is not naturally present in human biochemistry (Beutler, 2004; Weber et al., 2006; Kaplan and Kaplan, 2009; Theil and Goss, 2009). Particularly, cancer cells need a significant amount of Fe³⁺ for rapid replication, so endogenously abundant Fe³⁺ in tumors has been recognized as a molecular target for chemotherapeutic treatments through depleting cancer cellular Fe³⁺ to disrupt cancer cell proliferation and inhibit tumor growth (Fe³⁺-chelation therapy) (Buss et al., 2003; Richardson, 2005). In this study, we introduced exogenous Fe³⁺ with the ultimate goal of developing this approach to hunt the elevated Fe³⁺ level in tumors for the ¹H-MRI signal generation. Indeed, alizarin has a very high thermodynamic stability constant logβ = 32.21 (Das et al., 1995; Das et al., 2002), indicating its capability of capturing Fe³⁺ from tumor to produce the Fe³⁺–alizarin complex in situ while simultaneously generating the ¹H-MRI signal enhancement (Richardson et al., 1999; Davies et al., 1996; Schwert et al., 2002; Schwert et al., 2005; Haas and Franz, 2009; Kuznik and Wysocka, 2016). Moreover, alizarin has been known to inhibit human cytomegalovirus replication, HIV-1 RT-associated RDDP, and integrase activities (Esposito et al., 2011). Furthermore, alizarin is the core part of anthraquinones, which constitute numerous antitumor drugs widely applied in the treatment of various neoplasms such as Adriamycin and daunorubicin, and their coordination with Fe³⁺ was shown to diminish cardiotoxicity while improving the antitumor activity in chemotherapy and maintain sound radiosensitizing properties in radiotherapy (Lown, 1993; Nowak and Tarasiuk, 2012; Malik and Müller, 2016). Therefore, this novel molecular platform also indicates the potential for cancer therapy and imaging by utilizing the β-gal responsive turn on pathway to selectively deplete tumor Fe³⁺, resulting in cancer cell cycle arrest and apoptosis while generating ¹H-MRI contrast enhancement, thereby providing insight into the lacZ gene expression, development, location, and magnitude.

CONCLUSION

In this study, we present a novel responsive molecular platform for β-gal activity detection using ¹H-MRI, in which the ¹H-MRI signal enhancement is specifically generated, localized, and accumulated in situ at the β-gal activity site. In conjunction with this design, we have successfully produced and characterized alizarin 2-O-β-D-galactopyranoside AZ-1 and alizarin 1,2-di-O-β-D-galactopyranoside AZ-2. We have also demonstrated the feasibility of using AZ-1 by spontaneous in situ formation of paramagnetic Fe³⁺–alizarin complex to assess the β-gal activity in solution with Fe³⁺ ions existence by ¹H-MRI T1 and T2/T2* relaxation mapping. ¹H-MRI clearly showed the significant differences in both T₁ and T₂ data together to gain the additional certainty in imaging evaluation and detection reliability of β-gal activity.

EXPERIMENTAL

General Methods

NMR spectra were recorded on a Varian Unity INOVA 400 spectrometer (400 MHz for ¹H, 100 MHz for ¹³C). ¹H and ¹³C chemical shifts are referenced to TMS as an internal standard with CDCl₃ or DMSO-d₆ as solvents, and chemical shifts are given in ppm. All compounds were characterized by NMR at 25°C. Mass spectra were obtained by positive and negative ESI-MS using a Micromass Q-TOF hybrid quadrupole/time-of-flight instrument (Micromass UK Ltd.). Absorption spectra were taken on a UV-2700 UV-Vis Shimadzu spectrophotometer.
Solutions in organic solvents were dried with anhydrous sodium sulfate and concentrated in vacuo below 45°C. 2, 3, 4, 6-Tetra-O-acetyl-α-D-galactopyranosyl bromide was purchased from the Sigma Chemical Company. β-Gal (E801A) was purchased from the Promega (Madison, WI, United States), and enzymatic reactions were performed at 37°C in the PBS solution (0.1 M, pH = 7.4). Column chromatography was performed on silica gel (200–300 mesh), and silica gel GF254 used for analytical TLC was purchased from the Aldrich Chemical Company. The detection was affected by spraying the plates with 5% ethanolic H2SO4 (followed by heating at 110°C for 10 min) or by direct UV illumination of the plate. The purity of the final products was determined by HPLC with ≥95%.

Alizarin 2-O-2′, 3′, 4′, 6′-Tetra-O-Acetyl-β-D-Galactopyranoside AZ-M1

A solution of 2, 3, 4, 6-tetra-O-acetyl-α-D-galactopyranosyl bromide (1.23 g, 3.0 mmol) in CH2Cl2 (15 ml) was added dropwise to a vigorously stirred CH2Cl2-H2O biphasic mixture (pH 8–9) of alizarin (0.72 g, 3.0 mmol) and tetrabutylammonium bromide (TBAB) (322 mg, 1.0 mmol) in CH2Cl2-H2O (30 ml, 1:1 V/V) around 1 h at 50°C under N2 atmosphere, with stirring continued for an additional 4–5 h until TLC showed that the reaction was complete. The product was extracted with CH2Cl2 (4 × 30 ml) and subsequently washed (H2O), dried (Na2SO4), and evaporated under reduced pressure to give a syrup, which was purified by column chromatography on silica gel to give alizarin 2-O-2′, 3′, 4′, 6′-tetra-O-acetyl-β-D-galactopyranoside AZ-M1.

Alizarin 1,2-Di-O-2′, 3′, 4′, 6′-Tetra-O-Acetyl-β-D-Galactopyranoside AZ-M2

A solution of 2, 3, 4, 6-tetra-O-acetyl-α-D-galactopyranosyl bromide (2.71 g, 6.6 mmol) in CH2Cl2 (30 ml) was added dropwise to a vigorously stirred CH2Cl2-H2O biphasic mixture (pH 10–11) of alizarin (0.72 g, 3.0 mmol) and TBAB (322 mg, 1.0 mmol) in CH2Cl2-H2O (30 ml, 1:1 V/V) around 1 h at 55°C under N2 atmosphere, with stirring continued for an additional 4–5 h until TLC showed that the reaction was complete. The product was extracted with CH2Cl2 (4 × 40 ml) and subsequently washed (H2O), dried (Na2SO4), and evaporated under reduced pressure to give a syrup, which was purified by column chromatography on silica gel to give alizarin 1,2-di-O-2′, 3′, 4′, 6′-tetra-O-acetyl-β-D-galactopyranoside AZ-M2.

Alizarin 2-O-β-D-Galactopyranoside AZ-1 and Alizarin 1,2-Di-O-β-D-Galactopyranoside AZ-2

General procedure: A solution of alizarin 2-O-2′, 3′, 4′, 6′-teta-O-acetyl-β-D-galactopyranoside AZ-M1 or alizarin 1,2-di-O-2′, 3′, 4′, 6′-tetra-O-acetyl-β-D-galactopyranoside AZ-M2 (1.30 g) in anhydrous MeOH (120 ml) containing 0.5 M NH3 was vigorously stirred from 0°C to room temperature overnight until TLC showed that the reaction was complete and then evaporated to dryness in vacuo. Chromatography of the crude syrup on silica gel with ethyl acetate-methanol afforded the corresponding alizarin 2-O-β-D-galactopyranoside AZ-1 and alizarin 1,2-di-O-β-D-galactopyranoside AZ-2 in high yields.

MRI

MRI studies were performed using a 4.7T horizontal bore magnet or a 9.4T vertical bore magnet equipped with a Varian INOVA Unity system (Palo Alto, CA, United States). T1 and T2 (or T2*) maps were acquired using a spin echo (or gradient echo) sequence with varying repetition times (TRs) or echo times (TEs), respectively. The raw data were acquired using a centric k-space reordering scheme, followed by the phase encoding steps with higher phase encoding gradient amplitudes. Data acquisition parameters of the FLASH readout were TR/TE/Flip angle = 10 ms/5 ms/10°. The standard multi-echo Carr–Purcell–Meiboom–Gill pulse sequence was used for measuring T2 from a single echo train. The T1 and T2* maps were obtained on a voxel-by-voxel basis using a nonlinear least-squares fit equation M = M0e−TR/TE from the images taken at each echo time. Images were reconstructed and analyzed by using MatLab (MathWorks, Natick, MA).

lacZ Transfection in Human Tumor Cells

The E. coli lacZ gene (from pSV-β-gal vector, Promega, Madison, WI, United States) was inserted into a high expression human cytomegalovirus (CMV) immediate early enhancer/promoter vector phCMV (Gene Therapy Systems, San Diego, CA, United States), producing a recombinant vector phCMV/lacZ. This was used to transfect wild-type MCF7 (human breast cancer) and PC3 (human prostate cancer) cells (ATCC, Manassas, VA, United States) using GenePORTER2 (Gene Therapy Systems, Gentian, Inc., San Diego, CA, United States). The highest β-gal expressing colony was selected using the antibiotic G418 disulfate (800 μg/ml, Research Products International Corp, Mt Prospect, IL, United States), and G418 (200 μg/ml) was also included for routine culture. The cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM, Mediatech Inc., Herndon, VA, United States) containing 10% fetal bovine serum (FBS, 0.1 M, pH = 7.4, Atlanta Biologicals, Inc., Lawrenceville, GA, United States) with 100 units/ml of penicillin and 100 units/ml streptomycin, and cultured in a humidified 5% CO2 incubator at 37°C. The β-gal activity of lacZ-transfected tumor cells was measured using a β-Gal Assay Kit with o-nitrophenyl-β-D-galactopyranoside (Promega, Madison, WI, United States) and confirmed by X-gal or S-gal staining. Cells were fixed in PBS plus 0.5% glutaraldehyde (5 min) and rinsed in PBS prior to staining. Staining was performed using standard procedures for 2 h at 37°C in PBS plus 1 mg/ml X-gal (Sigma, St. Louis, MO, United States), 1 mM MgCl2, 5 mM K3Fe(CN)6, and 5 mM K4Fe(CN)6 or with 1.5 mg/ml 5-gal (Sigma, St. Louis, MO, United States) and 2.5 mg/ml FAC.
Western Blot
The protein extracted from the wild-type and lacZ-expressing MCF7 and PC3 cancer cells was quantified using the Bradford method by a protein assay (Bio-Rad, Hercules, CA, United States). Protein (30 μg) was added to each well, separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to a polyvinylidene fluoride membrane. A primary monoclonal anti-β-gal antibody (Promega, Madison, WI, United States) and anti-actin antibody (Sigma, St. Louis, MO, United States) were used as probes at a dilution of 1:5,000, with the reacting protein detected using a horseradish peroxidase-conjugated secondary antibody and ECL detection (Amersham, Piscataway, NJ, United States).

Cytotoxicity
The cytotoxicity for the free β-D-galactopyranoside AZ-1 and the released aglycone alizarine was assessed in both wild-type and lacZ-expressing MCF7 and PC3 cells using a colorimetric CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (MTS) (Promega, Madison, WI, United States). Assays were performed in triplicate using 24-well plates seeded with 10³ cells per well in 500 μL of RPMI-1640 without phenol red and supplemented with 10% FCS and 2 mM glutamine (Urano et al., 2005; Kamiya et al., 2007).

DATA AVAILABILITY STATEMENT
The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS
J-XY: conceived and designed the study, analyzed the data, and wrote the manuscript. SG, LZ, and ZF: synthesized, purified the compounds, and performed most part of NMR experiments. VK: conducted ¹H-MRI experiments with PC3-lacZ cells and demonstrated the feasibility for detection of the lacZ gene expression. LL: conducted lacZ transfection in tumor cells and validated the β-gal activity. HW: assisted in toxicity evaluation. HX: helped with structural characterization. MT, BH, CC, and ZZ: assisted in processing data.

FUNDING
This research was supported in part by the Research/Development Grants (2016QD)ZR01 and HBMUPI201808) from the Biomedical Research Institute, fifth School of Medicine/Suizhou Central Hospital, Hubei University of Medicine, and the Natural Science Foundation of China (21877028).

SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2021.709581/full#supplementary-material

REFERENCES
Alam, J., and Cook, J. L. (1990). Reporter Genes: Application to the Study of Mammalian Gene Transcription. Anal. Biochem. 188, 245–254. doi:10.1016/0003-2697(90)90601-5
Arenda, F., Singh, J. B., Gianolio, E., Stefania, R., and Aime, S. (2011). β-Gal Gene Expression MRI Reporter in Melanoma Tumor Cells. Design, Synthesis, Andin Vitroand VivoTesting of a Gd(III) Containing Probe Forming a High Relaxivity, Melanin-like Structure upon β-Gal Enzymatic Activation. Bioconjug. Chem. 22, 2625–2635. doi:10.1021/bc200486j
Asanuma, D., Sakabe, M., Kamiya, M., Yamamoto, K., Hiratake, J., Ogawa, M., et al. (2015). Sensitive β-galactosidase-targeting Fluorescence Probe for Visualizing Small Peritoneal Metastatic Tumours In Vivo. Nat. Commun. 6, 6463–6470. doi:10.1038/ncomms7463
Aresta, B., N. E., Brown, G., Scott, E. W., and Walter, G. A. (2010). LacZ as a Genetic Reporter for Real-Time MRI. Magn. Reson. Med. 63, 745–753. doi:10.1002/mrm.22235
Bertozi, C. R., and Kiesling, L. L. (2001). Chemical Glycobiology. Science 291, 2337–2346. doi:10.1126/science.1059820
Beutler, E. (2004). CELL BIOLOGY: 'Pumping' Iron: The Proteins. Science 306, 2051–2053. doi:10.1126/science.1107224
Broome, A.-M., Ramamurthy, G., Lavik, L., Kigett, A., Kinstlinger, I., and Basilion, J. (2015). Optical Imaging of Targeted β-Galactosidase in Brain Tumors to Detect EGFR Levels. Bioconjug. Chem. 26, 660–668. doi:10.1021/bc500897y
Brown, N. K., Huang, Z., Dockrell, M., Hashmi, P., and Price, R. G. (2010). Evaluation of New Chromogenic Substrates for the Detection of Coliforms. J. Appl. Microbiol. 108, 1828–1838. doi:10.1111/j.1365-2672.2009.04588.x
Burke, H. M., Gunnalaugsson, T., and Scanlan, E. M. (2015). Recent Advances in the Development of Synthetic Chemical Probes for Glycosidase Enzymes. Chem. Commun. 51, 10576–10588. doi:10.1039/c5cc02793d
Buss, J., Torti, F., and Torti, S. (2003). The Role of Iron Chelation in Cancer Therapy. Cmc 10, 1021–1034. doi:10.2174/0929967033457638
Celen, S., Deroose, C., Groot, T. d., Chitneni, S. K., Gijsbers, R., Debyser, Z., et al. (2008). Synthesis and Evaluation of 18F- and ¹¹C-Labeled Phenyl-Galactopyranosides as Potential Probes for In Vivo Visualization of LacZ Gene Expression Using Positron Emission Tomography. Bioconjug. Chem. 19, 441–449. doi:10.1021/bc700216d
Chang, Y.-T., Cheng, C.-M., Su, Y.-Z., Lee, W.-T., Hsu, J.-S., Liu, G.-C., et al. (2007). Synthesis and Characterization of a New Bioactivated Paramagnetic Gadolinium(III) Complex [Gd(DOTA-FPG)(H2O)] for Tracing Gene Expression. Bioconjug. Chem. 18, 1716–1727. doi:10.1021/bc700191s
Chatterjee, S. K., Bhattacharya, M., and Barlow, J. J. (1979). Glycosyltransferase and Glycosidase Activities in Ovarian Cancer Patients. Cancer Res. 39, 1943–1951.
Chen, J., Jackson, A. A., Rotello, V. M., and Nugen, S. R. (2016). Colorimetric Detection of Escherichia coli Based on the Enzyme-Induced Metallization of Gold Nanorods. Bioconjug. Chem. (2015). Synthesis and Characterization of a New Bioactivated Paramagnetic Gadolinium(III) Complex [Gd(DOTA-FPG)(H2O)] for Tracing Gene Expression. Bioconjug. Chem. 18, 1716–1727. doi:10.1021/bc700191s
Chatterjee, S. K., Bhattacharya, M., and Barlow, J. J. (1979). Glycosyltransferase and Glycosidase Activities in Ovarian Cancer Patients. Cancer Res. 39, 1943–1951.
Chen, J., Jackson, A. A., Rotello, V. M., and Nugen, S. R. (2016). Colorimetric Detection of Escherichia coli Based on the Enzyme-Induced Metallization of Gold Nanorods. Bioconjug. Chem. 18, 1716–1727. doi:10.1021/bc700191s
Chen, X., Zhang, X., Ma, X., Zhang, Y., Gao, G., Liu, J., Zhang, X., et al. (2018). Ratiometric Fluorescent Probes with a Self-Immolative Spacer for Real-Time Detection of β-galactosidase and Imaging in Living Cells. Analytica Chim. Acta 1033, 193–198. doi:10.1016/j.aca.2018.05.071
Chen, X., Zhang, X., Ma, X., Zhang, Y., Gao, G., Liu, J., Zhang, X., et al. (2019). Novel Fluorescent Probe for Rapid and Ratiometric Detection of β-galactosidase and Live Cell Imaging. Talanta 192, 308–313. doi:10.1016/j.talanta.2018.09.061
Coskuner, O., and Gonzalez, C. A. (2011). in Metallic Systems: A Quantum Chemists Perspective, Chapter 3, Carbohydrate and Trivalent Iron Ion

β-Galactosidase Reporters
β-Galactosidase Reporters

Dewk, R. A. (1996). Glycobiology: Toward Understanding the Function of Sugars. *Chem. Rev.* 96, 683–720. doi:10.1021/cr900428b

Esposito, F., Kharlamova, T., Distinto, S., Zinzula, L., Cheng, Y. C., Dutschman, G., et al. (2011). Alizarin Derivatives as New Dual Inhibitors of the HIV-1 Reverse Transcriptase-Associated DNA Polymerase and RNase H Activities Effective Also on the RNase H Activity of Non-nucleoside Resistant Reverse Transcriptases. *FEBS J.* 278, 1441–1457. doi:10.1111/j.1742-4658.2011.08057.x

Feng, F., Liu, L., and Wang, S. (2009). Water-Soluble Conjugated Polyelectrolyte-Based Fluorescence Enzyme Coupling Protocol for Continuous and Sensitive β-Galactosidase Detection. *Macromol. Chem. Phys.* 210, 1188–1193. doi:10.1002/macp.200900264

Fernández-Cuervo, G., Tucker, K. A., Malm, S. W., Jones, K. M., and Pagel, M. D. (2016). Diamagnetic Imaging Agents with a Modular Chemical Design for Quantitative Detection of β-Galactosidase and β-Glucuronidase Activities with CatalyCEST MRI. *Bioconjug. Chem.* 27, 2549–2557. doi:10.1021/acs.bcc.6b00100

Fernández-Tejada, A., Cañada, F. J., and Jiménez-Barbero, J. (2015). Recent Developments in Synthetic Carbohydrate-Based Diagnostics, Vaccines, and Therapeutics. *Chem. Eur. J.* 21, 10616–10628. doi:10.1002/chem.201500831

Fu, W., Yan, C., Zhang, Y., Ma, Y., Guo, Z., and Zhu, W.-H. (2019). Near-Infrared Aggregation-Induced Emission-Active Probe Enables In Situ and Long-Term Tracking of Endogenous β-Galactosidase Activity. *Front. Chem.* 7, 291. doi:10.3389/fchem.2019.00291

Gao, G. (2008). Eu/Tb Luminescence for Alkaline Phosphatase and β-galactosidase Assay in Hydrogels and on Paper Devices. *J. Mater. Chem.* B 6, 2143–2150. doi:10.1039/b702657a

Green, O., Gnaim, S., Blau, R., Eldar-Boock, A., Satchi-Fainaro, R., and Shabat, D. (2017). Near-Infrared Dioxetan Luminesophores with Direct Chemiluminescence Emission Mode. *J. Am. Chem. Soc.* 139, 13243–13248. doi:10.1021/jacs.7b04846

Gu, K., Qu, W., Guo, Z., Yan, C., Zhu, S., Yao, D., et al. (2019). An Enzyme-Activatable Probe Liberating αLGens: On-Site Sensing and Long-Term Tracking of β-galactosidase in Ovarian Cancer Cells. *Chem. Sci.* 10, 398–405. doi:10.1039/c9sc02466g

Gu, K., Xu, Y., Li, H., Guo, Z., Zhu, S., Zhu, S., et al. (2016). Real-Time Tracking and In Vivo Visualization of β-Galactosidase Activity in Colorectal Tumor with a Ratiometric Near-Infrared Fluorescent Probe. *J. Am. Chem. Soc.* 138, 5334–5340. doi:10.1021/jacs.6b01705
Kamiya, M., Asanuma, D., Kuranaga, E., Takeishi, A., Sakabe, M., Miura, M., et al. (2011). β-Galactosidase Fluorescence Probe with Improved Cellular Accumulation Based on a Spirocyclic Rhodol Scaffold. J. Am. Chem. Soc. 133, 12960–12963. doi:10.1021/ja204781n

Kamiya, M., Kobayashi, H., Hama, Y., Koyama, Y., Bernardo, M., Nagano, T., et al. (2009). An Enzymatically Activated Fluorescence Probe for Targeted Tumor Imaging. J. Am. Chem. Soc. 129, 3918–3929. doi:10.1021/ja087710a

Kaplan, C. D., and Kaplan, J. (2009). Iron Acquisition and Transcriptional Regulation. Chem. Rev. 109, 4536–4552. doi:10.1021/cr900167b

Kim, E.-J., Podder, A., Maiti, M., Lee, J. M., Chung, B. G., and Bhuniya, S. (2018). A Novel Molecular Magnetic Resonance Imaging. J. Am. Chem. Soc. 140, 15591–15598. doi:10.1021/jacs.8b05560

Kong, X., Li, M., Dong, B., Yin, Y., Song, W., and Lin, W. (2019). An Important Tool for Metastasis Research and Evaluation of New Cancer Therapies. Cancer Metastasis Rev. 37, 285–294.

Kruger, A., Schirrmacher, V., and Khokha, R. (1999). The Bacterial lacZ Gene: An Important Tool for Metastasis Research and Evaluation of New Cancer Therapies. Cancer Metastasis Rev. 17, 1475–1471. doi:10.1021/acs.analchem.9b03951

Li, X., Qiu, W., Li, J., Chen, X., Hu, Y., Gao, Y., et al. (2020b). First-generation Species-Selective Chemical Probes for Fluorescence Imaging of Human Senescence-Associated β-galactosidase Activity. J. Am. Chem. Soc. 142, 7129–7130. doi:10.1021/jacs.9b10319

Li, X., Zhang, Z., Yu, Z., Magnussen, J., and Yu, J.-X. (2013). Novel Molecular Platform Integrated Iron Chelation Therapy for 1H-MRI Detection of β-Galactosidase Activity. Mol. Pharmaceutics 10, 1360–1367. doi:10.1021/mp300627t

Li, Y., Ning, L., Yuan, F., Zhang, T., Zhang, J., Xu, Z., et al. (2020). Activatable Formation of Emissive Excimers for Highly Selective Detection of β-Galactosidase Activity. J. Am. Chem. Soc. 142, 5733–5740. doi:10.1021/jacs.9b11196

Lilley, L. M., Kamper, S., Caldwell, M., Chia, Z. K., Ballweg, D., Vistain, L., et al. (2020). Self-Immolative Activation of β-Galactosidase-Responsive Probes for In Vivo MR Imaging in Mouse Models. Angew. Chem. Int. Ed. 59, 388–394. doi:10.1002/anie.201909933

Liu, H.-W., Chen, L., Xu, C., Li, Z., Zhang, H., Zhang, X.-B., et al. (2018). Recent Progresses in Small-Molecule Enzymatic Fluorescent Probes for Cancer Imaging. Chem. Soc. Rev. 47, 71–80. doi:10.1039/c7cs00862g

Liu, L., Kodisbargak, V. D., Yu, J.-X., and Mason, R. P. (2007). 19F-NMR Detection of lacZ Gene Expression via the Enzymic Hydrolysis of 2-fluoro-4-nitrophosphoryl β-D-galactospyranoside In Vivo in PC3 Prostate Tumor Xenografts in the Mouse. J. Am. Chem. Soc. 129, 12960–12963. doi:10.1021/ja073666f

Liu, L., and Mason, R. P. (2010). Imaging β-Galactosidase Activity in Human Tumor Xenografts and Transgenic Mice Using a Chemiluminescent Substrate. PLoS ONE 5, e12024–31. doi:10.1371/journal.pone.0012024

Lowe, A. Y., Huber, M. M., Ahrens, E. T., Rothbacher, U., Moats, R., Jacobs, R. E., et al. (2000). In Vivo visualization of Gene Expression Using Magnetic Resonance Imaging. Nat. Biotechnol. 18, 321–325. doi:10.1038/37378

Lown, J. W. (1993). Anthracrylone and Anthraquinone Anticancer Agents: Current Status and Recent Developments. Pharmacol. Ther. 60, 185–214. doi:10.1016/0163-7258(93)90066-y

Lozano-Torres, B., Estepa-Fernández, A., Rovira, M., Orzáez, M., Serrano, M., Martínez-Mañé, R., et al. (2019). The Chemistry of Senescence. Nat. Rev. Chem. 3, 426–441. doi:10.1038/s41570-019-0108-0

Lozano-Torres, B., Galiana, I., Rovira, M., Garrido, E., Chabí, S., Bernards, A., et al. (2017). An Off-On Two-Photon Fluorescent Probe for Tracking Cell Senescence In Vivo. J. Am. Chem. Soc. 139, 8808–8811. doi:10.1021/jacs.7b08105

Malah, A., Villinger, A., and Langer, P. (2011). Site-selective Arylation of Alizarin and Purpurin Based on Suzuki-Miyaura Cross-Coupling Reactions. Eur. J. Org. Chem. 2011, 2075–2087. doi:10.1002/ejoc.201001497

Malik, E. M., and Müller, C. E. (2016). Anthracrylones as Pharmacological Tools and Drugs. Med. Res. Rev. 36, 705–748. doi:10.1002/med.21391

Mizukami, S., Matsushita, H., Takakawa, R., Sugihara, F., Shirakawa, M., and Kikuchi, K. (2011). 19F MRI Detection of β-galactosidase Activity for Imaging of Gene Expression. Chem. Sci. 2, 1151–1155.

Nakamura, Y., Mochida, A., Nagaya, T., Okumaya, S., Ogata, F., Choyke, P. L., et al. (2017). A Topically-Sprayable, Activatable Fluorescent and Retaining Probe, SPIDER-βGal for Detecting Cancer: Advantages of Anchoring to Cellulor Proteins after Activation. Oncotarget 8, 39512–39521. doi:10.18632/oncotarget.17080

Nowak, R., and Tarasiuk, J. (2012). Anthracrylone Antimour Agents, Doxorubicin, Pirurubacin and Benzoperimidin BPI, Trigger Caspase-3/ caspase-8-dependent Apoptosis of Leukaemia Sensitive HL60 and Resistant HL60/21/VINC and HL60/DOX Cells. Anticancer Drugs 23, 380–392. doi:10.1097/1jod.0b013e328348abab

Oushiki, D., Kojima, H., Takahashi, Y., Komatsu, T., Terai, T., Hanaoka, K., et al. (2009). Appearance of Senescence in Liver Carcinogenesis Induced by Diethylnitrosamine. Toxicol. Lett. 197, 123–131. doi:10.1016/j.toxlet.2015.11.011

Paradis, V., Youssef, N., Dargère, D., Bâ, N., Bonvoust, F., Deschatrette, J., et al. (2001). Replicative Senescence in normal Liver, Chronic Hepatitis C, and Hepatocellular Carcinomas. Hum. Pathol. 32, 327–332. doi:10.1053/hupa.2001.22747

Peng, L., OLeary, C., Li, Y., Zhang, R., Li, K., Feng, G., et al. (2015). A Fluorescent Light-Up Probe Based on AIE and ESPIE Processes for β-galactosidase Activity Detection and Visualization in Living Cells. J. Mater. Chem. B 3, 9168–9172. doi:10.1039/c5tb01938a

Razgulin, A., Ma, N., and Rao, J. (2011). Strategies for β-galactosidase Activity Detection and Imaging of Senescence Cells. J. Biotechnol. 157, 5779–5788.
Zhang, J., Cheng, P., and Pu, K. (2019b). Recent Advances of Molecular Optical Probes in Imaging of β-Galactosidase. Bioconjug. Chem. 30, 2089–2101. doi:10.1021/acs.bioconjchem.9b00391

Zhang, J., Li, C., Dutta, C., Fang, M., Zhang, S., Tiwari, A., et al. (2017). A Novel Near-Infrared Fluorescent Probe for Sensitive Detection of β-galactosidase in Living Cells. Analytica Chim. Acta 968, 97–104. doi:10.1016/j.aca.2017.02.039

Zhang, X.-X., Wu, H., Li, P., Qu, Z.-J., Tan, M.-Q., and Han, K.-L. (2016). A Versatile Two-Photon Fluorescent Probe for Ratiometric Imaging E. coli β-galactosidase in Live Cells and In Vivo. Chem. Commun. 52, 8283–8286. doi:10.1039/c6cc04373a

Zhang, X., Chen, X., Zhang, Y., Liu, K., Shen, H., Zheng, E., et al. (2019). A Near-Infrared Fluorescent Probe for the Ratiometric Detection and Living Cell Imaging of β-galactosidase. Anal. Bioanal. Chem. 411, 7957–7966. doi:10.1007/s00216-019-02181-7

Zhao, X., Yang, W., Fan, S., Zhou, Y., Sheng, H., Cao, Y., et al. (2019). A Hemicyanine-Based Colorimetric Turn-On Fluorescent Probe for β-galactosidase Activity Detection and Application in Living Cells. J. Lumin. 205, 310–317. doi:10.1016/j.jlumin.2018.09.036

Zhou, Z., Bai, R., Munasinghe, J., Shen, Z., Nie, L., and Chen, X. (2017). T1-T2 Dual-Modal Magnetic Resonance Imaging: From Molecular Basis to Contrast Agents. ACS Nano 11, 5227–5232. doi:10.1021/acsnano.7b03075

Zhu, W. H., Chen, J. A., Pan, H., Wang, Z., Gao, J., Tan, J., et al. (2020). Imaging of Ovarian Cancers Using Enzyme Activatable Probes with Second Near-Infrared Window Emission. Chem. Commun. (Camb) 56, 2731–2734. doi:10.1039/c9cd09158k

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Gao, Zhao, Fan, Kodibagkar, Liu, Wang, Xu, Tu, Hu, Cao, Zhang and Yu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.