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A thiol-inducible and quick-response DNA cross-linking agent

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ABSTRACT

Three new 2,4-dinitrobenzenesulfonyl derivatives **1**–**3** were successfully prepared for the first time using a simple process. They were efficiently triggered by thiols (glutathione and L-cysteine) to release the corresponding phenol derivatives (**4**–**6**) within 5 minutes. The quick response of **1**–**3** toward thiols was determined by ^{1}H NMR and HPLC. Moreover, our results indicated that **1** could induce DNA cross-linking in the presence of glutathione, probably due to the quinone methide formation of phenol intermediate **4** followed by departure of 2,4-dinitrobenzenesulfonyl group.

Antitumor agents that target DNA, such as nitrogen mustard, mitomycin C, and psoralen, prevent the melting of DNA double strands, disrupt cell maintenance and transcription,[1] and finally lead to cell death. However, the side effects due to nonspecific targeting limit the clinical application of these agents. Therefore, induced DNA cross-linking agents are playing increasingly important roles in cancer therapy.[2] Among these agents, quinone methides (QMs) are important intermediates for the ultimate cytotoxins responsible for the antitumor activity.[3] Moreover, they can be generated by many methods, including photochemical, fluoride-induced activation, thermal digestion, oxidation, and H_2O_2-induced reactions.[4] Unfortunately, most of these methods increase the occurrence of side effects due to undesirably high reaction temperatures, the requirement for additional reagents and acidic or basic conditions, long reaction times, and the inaccessibility of precursors.[5] One efficient strategy to reduce the toxicity of inducible quinone methide precursors would be the production of quick-response reactions toward the bioavailable molecule under tumor-specific conditions. Glutathione (GSH) is the most abundant free thiol in the cell[6] and has been shown to be more abundant in tumors than in the corresponding normal tissue.[7] Moreover, GSH has been reported to efficiently activate 2,4-dinitrobenzenesulfonyl derivatives.[8] Our group recently demonstrated that an anticancer prodrug coupled with a 2,4-dinitrobenzenesulfonyl group could be triggered by GSH and release the chemotherapeutic agent mechlorethamine.[9] Considering that QM intermediate could be induced by a phenol quaternary ammonium structure[4f], we design and investigate new inducible reactivities of the 2,4-dinitrobenzenesulfonyl compounds **1**–**3** (Fig. 1).

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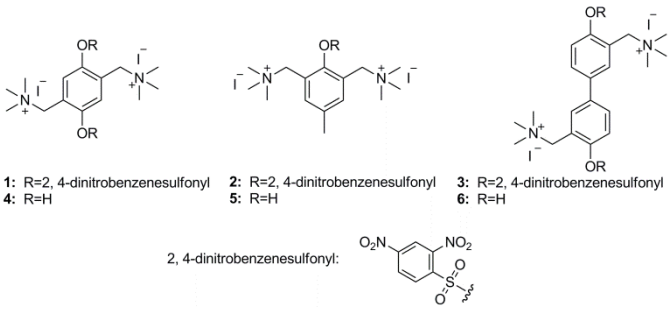
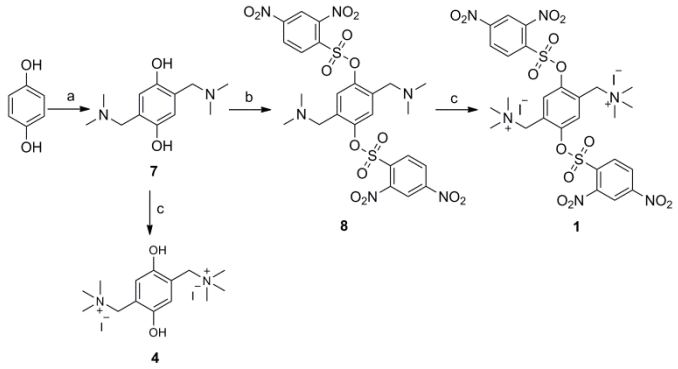


Figure 1. The structure of compounds **1**–**6**.

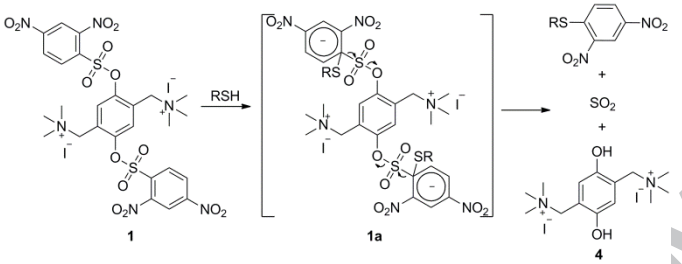
New compounds **1**–**3** and three known compounds **4-6** were synthesized starting from the corresponding commercially available phenol derivatives according to the procedure reported previous.[10] For example, compound **7** was obtained by Mannich reaction with hydroquinone as the starting material (Scheme 1). Treatment of **7** with 2,4-dinitrobenzene sulfonyl chloride yielded **8**, quaternarization of which was carried out by reaction with methyl iodide to prepare compound **1**. Quaternarization of **7** yielded compound **4**.

First, the inducible reactivity was investigated. To examine all intermediates formed, treatment of **1**–**3** with L-cysteine (L-Cys), which is the active group of GSH, was carried out in a mixture of D_2O and DMSO-d_6 by ^{1}H NMR analysis. However, the reaction was so fast that compounds **1**–**3** were completely consumed within 5 minutes (Fig. S1 of the Supplementary file) and converted to the final product, corresponding to the phenol derivatives **4**–**6** (Fig. 1). Moreover, MS analysis of the reaction mixture of compounds **1**–**3** with L-Cys also showed the release of the corresponding phenol derivatives (Fig. S2-S4 of the Supplementary file). According to the previously reported mechanism of 2, 4-dinitrobenzenesulfonyl group as leaving

group upon reaction with glutathione (GSH)[8d], the reaction was triggered by L-Cys and produced the intermediates **1a**–**3a**, which were immediately converted to the phenol derivatives **4**–**6** through elimination of SO_2 (Scheme 2, Scheme S1 of the Supplementary file).



Scheme 1. Synthesis of compounds **1** and **4**: a) 33% aqueous $(CH_3)_2NH$, 37% formaldehyde aqueous solution, 30°C for 30 min, then 90-95°C 2 h; b) 2,4-dinitrobenzene sulfonyl chloride, K_2CO_3, THF, RT; c) CH_3I, CH_3CN, RT.



Scheme 2. The mechanism of the reaction of compound **1** and L-Cys.

Although sulfonyl esters were hydrolyzed to some extent under physiological conditions (Fig. S5-S7 of the Supplementary file), ^{1}H NMR analysis suggested that compounds **1**–**3** could also release the corresponding phenol derivatives **4**–**6** within 5 minutes in the presence of L-Cys or GSH (Fig. S8 of the Supplementary file). HPLC and MS analysis were performed to further evaluate the quick response of **1**–**3** toward thiols. Of several substances tested, coumarin was chosen as the most appropriate internal standard (IS) in this assay because it was stable and did not interfere with the product of the inducible reactions. The results indicated that the thioether (2-amino-3-[*S*-(2,4-dinitrobenzene)]propionic acid, $[M+H^+]$=288, t≈17 min) was generated within 2 minutes after addition of compounds **1**–**3** (Fig. S9 of the Supplementary file). Moreover, the percentage of thioether compared with that of coumarin did not change markedly over a period of 4.5 hours (Table S1 of the Supplementary file). The reaction mixture without L-Cys or compounds **1**–**3** had a light yellow or achromatic color. Interestingly, the phosphate buffer of L-Cys immediately showed a brilliant red color after the addition of compounds **1**–**3,** and it showed an orange color 1 minute later (Fig. 2, Fig. S10 of the Supplementary file), indicating the reactions of compounds **1**–**3** with L-Cys. Taken together, these results indicated that the inducible reaction of compounds **1**–**3** by L-Cys was instantaneous, and these three compounds could be used as quick-response agents toward thiols.

To obtain further insight into the selectivity of the reaction, we studied the inducible activity of **1** toward other amino acids, such as L-arginine, L-proline, *β*-alanine, glycine, and L-valine (Fig. S11 of the Supplementary file). The results indicated that the production of **4** was not triggered by other amino acids, suggesting that the activation of **1** to release **4** was highly selective for cellular thiols (GSH and L-Cys) over other amino acids.

The DNA cross-linking abilities of compounds **1**–**3** were next investigated in phosphate buffer (pH 7.3) using linearized plasmid DNA (pBR322) by denaturing alkaline agarose gel electrophoresis (Fig. 3).[4a,4b,11] Our previous study indicated that GSH produced no DNA cross-linking.[9] However, treatment of DNA with compound **1** in the presence of GSH induced about 34% DNA cross-linking at 1 mM, similar to that induced by compound **4** (43%) under the same conditions. These results indicated that the designed compound **1** could be considered as a thiol-triggered DNA cross-linking agent through a quinone methide intermediate generated by compound **4**. Rokita and co-workers[12] showed that the adducts of electron-rich *o*-QMs with dA N^6 and dG N1, N^2 remained stable over the course of observation. Moreover, the N7 adduct of dG was easily deglycosylated to form its guanine derivative, which was also stable. Therefore, the DNA cross-linking sites induced by **4** through *o*-QM **4a** and **4b** (Fig. 4) with an electron-donating hydroxyl group most likely occurred at dA and dG. Meanwhile, compounds **2** and **3** showed no DNA cross-linking properties. Peng and colleagues reported that the stable phenol product **6** produced from **3** did not undergo QM formation.[4f] However, the product **5** obtained from **2**, which shared a five-atom bridge between alkylation sites common to N-mustards,[13,14] was shown to be capable of inducing DNA cross-linking.[4b,15] To determine whether QM was generated from compound **2** by triggering with L-Cys, a QM trapping experiment with a large excess of ethyl vinyl ether (EVE) was performed (Scheme 3). Compound **13** (m/z=264.1960) was detected by HPLC and HR-ESIMS when **2** was incubated at 37°C for 24 hours in the presence of L-Cys and EVE, suggesting the formation of QM. The lack of DNA cross-link formation by compound **2** was probably due to two factors. First, due to the unstablity of **2** in phosphate buffer compared to **1** and **3** (Fig. S5-S7 of the Supplementary file), the QM generated from **2** could initially react with a range of cellular components, such as H_2O and L-Cys, to form kinetic products (Scheme S2 of the Supplementary file). Second, electronic perturbation of QM markedly influences the stability and, in turn, alters the kinetics and product profile of the QM reaction with deoxynucleosides. A previous study showed that a related adduct with an electron-donating methyl group is very labile and regenerates its QM.[12]

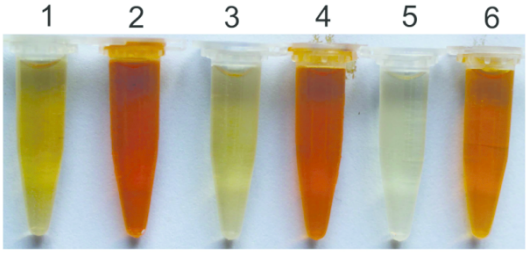


Figure 2. Analysis of color change during the reaction of compound **1** with L-Cys in phosphate buffer (pH 7.3): (1) 5 mM **1**; (2) 5 mM **1** + 10 mM L-Cys; (3) 2.5 mM **1**; (4) 2.5 mM **1** + 5 mM L-Cys; (5) 1 mM **1**; (6) 1 mM **1** + 2 mM L-Cys.

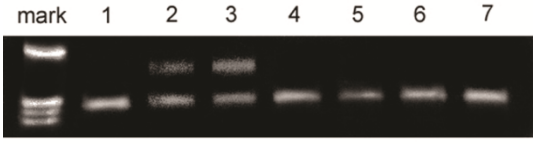
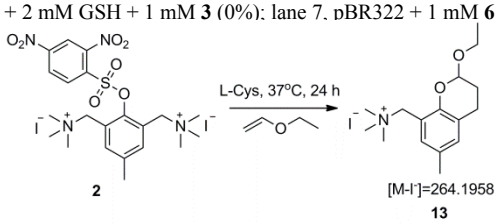


Figure 3. DNA cross-linking ability of compounds **1-6** in phosphate buffer (pH 7.3). Lane 1, pBR322 (control); lane 2, pBR322 + 2 mM GSH + 1 mM **1**

(cross-linking yield 34%); lane 3, pBR322 + 1 mM **4** (43%); lane 4, pBR322 + 1 mM GSH + 1 mM **2** (0%); lane 5, pBR322 + 1 mM **5** (0%); lane 6, pBR322 + 2 mM GSH + 1 mM **3** (0%); lane 7, pBR322 + 1 mM **6** (0%).



Scheme 3. Trapping reaction in the presence of EVE.

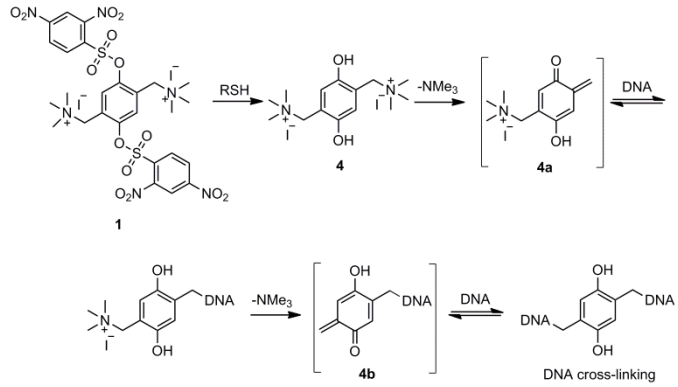


Figure 4. Tandem QM generation and DNA cross-linking formation induced by **1** upon RSH activation.

In conclusion, we synthesized three thiol-inducible 2,4-dinitrobenzenesulfonyl derivatives. Among them, compound **1** could be efficiently triggered by thiols and released the corresponding phenol derivative **4**, which directly produced QM and induced DNA cross-linking under physiological conditions. Its quick response to thiols makes compound **1** the preferred lead compound for developing novel quinone methide precursors as selective DNA cross-linking agents. To further increase the selectivity and stability of compound **1**, we will attempt to introduce a cancer targeting unit such as biotin and other benzylic leaving groups, such as dimethyl amine, in the trimethyl amine position in future studies.

Acknowledgments

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Conflicts of interest

There are no conflicts to declare.

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Supplementary Material

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2. The inducible reactions of **1**–**3** were immediately triggered by RSH.
3. The inducible activity of **1** was highly selective for RSH over other amino acids.
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