Organic & Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2021, **19**, 6527

Investigation of thiolysis of 4-substituted SBD derivatives and rational design of a GSH-selective fluorescent probe†

Chao Yang,[a] Xiaoqiang Tu,[b] Xiuru Ji,[c] Haishun Ye,[b] Shan Li,[b] Lu Sun,[c] Long Yi *[b] and Zhen Xi*[a]

Received 9th June 2021,
Accepted 6th July 2021

DOI: 10.1039/d1ob01114f

rsc.li/obc

In order to evaluate 7-sulfonamide benzoxadiazole (SBD) derivatives for the development of fluorescent probes, herein we investigated the thiolysis reactivity and selectivity of a series of SBD compounds with different atoms (N/O/S/Se) at the 4-position. Both SBD-amine and SBD-ether are stable toward biothiols in buffer (pH 7.4), while SBD-selenoether can react efficiently with biothiols GSH/Hcy, Cys, and H_2S to produce SBD-SG/S-Hcy, SBD-NH-Cys, and SBD-SH, respectively, with three different sets of spectral signals. Therefore, the SBD-selenoether compounds should be useful platforms for the differentiation of these biothiols. Though SBD-alkylthioether shows much lower reactivity than SBD-selenoether, SBD-arylthioether is a tunable motif and structural modifications at the aryl moiety enable the rate of thiol-mediated thiolysis to be modified. To this end, an ER-targeted GSH-selective fluorescent probe **7** was rationally designed *via* thiolysis of SBD-arylthioether. Compared with control probe SBD-Cl, probe **7** exhibits improved GSH selectivity and better biocompatibility. In total, this study highlights that the modification at the 4-position of SBD is an efficient strategy for the development of new fluorescent probes with tunable reactivity and selectivity.

Introduction

Biothiols, including glutathione (GSH), cysteine (Cys), homocysteine (Hcy), and hydrogen sulfide (H_2S), are potent nucleophiles and play diverse and important roles in living biosystems. GSH is the most abundant intracellular biothiol, and normally, the endogenous level of GSH (~1–10 mM) is much higher than that of Cys (<200 μM), Hcy (<15 μM), and H_2S (<1 μM).[1] GSH provides protection against oxidative stress through its reversible oxidation to GSSG.[2] A decrease in GSH levels can be related to different diseases, including neurodegenerative diseases, liver damage, schizophrenia, and cancers.[3] However, the concentration of GSH in some tumors is found to be much higher than that in normal tissues.[4] Therefore, chemical tools that enable the *in situ* detection and differentiation of GSH have potential applications in the treatment of various disease states. To this end, significant progress has been made in the development of fluorescent probes for selective detection of GSH among other biothiols.[5–16]

Many of the reported GSH-selective probes are based on the initial reactions toward all sulfhydryl nucleophiles and then, the resultant products undergo an additional intermolecular interaction or reaction (*e.g.*, the Smiles rearrangement for Cys/Hcy but not for GSH), producing different photophysical properties for the differentiation of GSH from Cys and Hcy.[6–11] Another relatively minor group of probes with relatively low reactivity toward sulfhydryl nucleophiles can selectively detect GSH over Cys/Hcy due to higher concentration of endogenous GSH,[12–16] which is more preferable if one only needs to detect GSH without probe-consuming from other thiols. For example, Yin and coworkers developed the thiolysis of sulfonamide-containing naphthalimides for selectively tracking mitochondrial GSH.[13] The Wang group and the Yoon group employed reversible thiol-Michael addition for quantitatively monitoring cellular GSH.[14] Li, Zang, and coworkers developed naphthalimide–sulfoxide based probes for revealing the functions of GSH in chemotherapy resistance and in developing neurons.[15] Many of these GSH fluorescent probes have been successfully used

[a]*State Key Laboratory of Elemento-Organic Chemistry and Department of Chemical Biology, College of Chemistry, National Pesticide Engineering Research Center, Collaborative Innovation Center of Chemical Science and Engineering, Nankai University, Tianjin, 300071, China. E-mail: zhenxi@nankai.edu.cn*
[b]*State Key Laboratory of Organic-Inorganic Composites and Beijing Key Lab of Bioprocess, Beijing University of Chemical Technology (BUCT), Beijing 100029, China. E-mail: yilong@mail.buct.edu.cn*
[c]*Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics (Theranostics), School of Pharmacy, Tianjin Medical University, Tianjin 300070, China*

† Electronic supplementary information (ESI) available: Experimental details and additional figures. See DOI: 10.1039/d1ob01114f

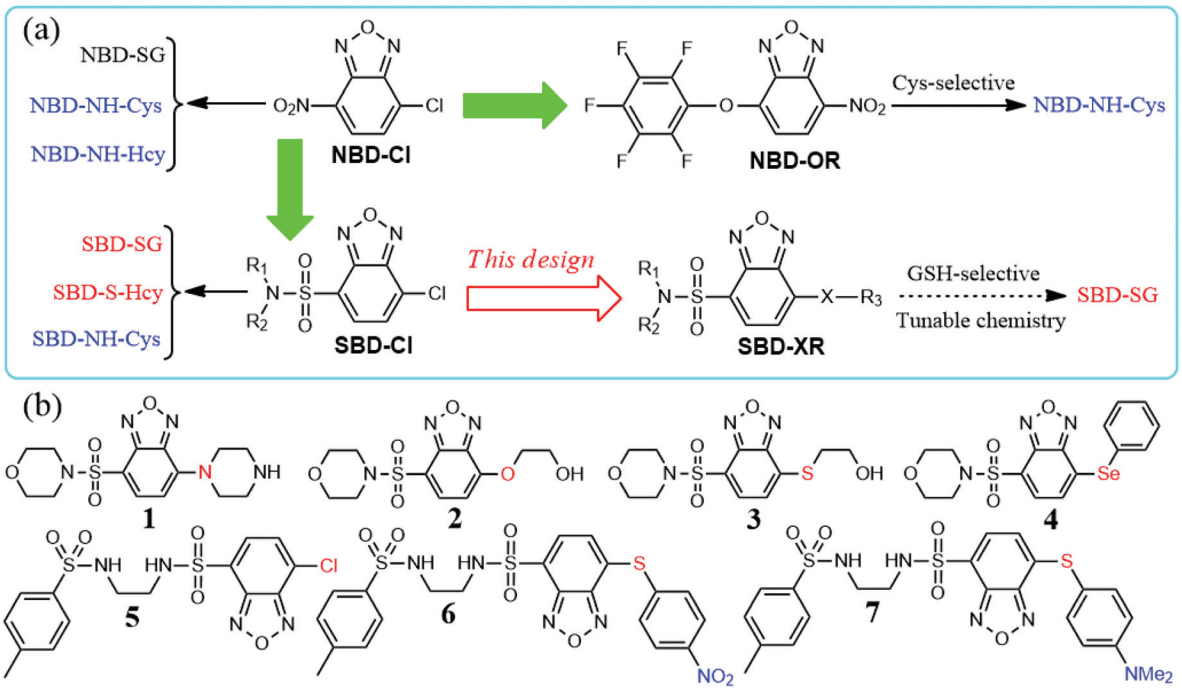
for predicting disease evolution, differentiation of cancer cells, and tumor imaging.[5–16] Building from these broad bioapplications, the development of GSH-selective probes is still in its infancy.

We have been interested in compounds with a nitrobenzodioxzole (NBD) skeleton for the detection and scavenging of biothiols (including H_2S) for several years.[5f,17,18] For example, we as well as others found that NBD derivatives show high reactivity toward biothiols and amines, resulting in distinct colorimetric and fluorescence changes.[5f] Pluth and coworkers revealed that after the initial S_NAr reaction between Cys/Hcy and the NBD electrophile, the resultant NBD-thioether undergoes an intermolecular Smiles rearrangement to generate fluorescent NBD-NHR compounds.[19a,b] The strong emission of NBD-NHR derives from intramolecular charge transfer (ICT) transitions, where the amino group and the nitro group are the ICT donor and acceptor, respectively. GSH only generates a non-fluorescent thioether product (NBD-SG). To this end, Ma and coworkers combined NBD-ether with resorufin to differentiate between GSH and Cys based on the formation of NBD-NH-Cys.[19c] In addition, Kim and coworkers compared the thiolysis reactivity and selectivity of several NBD ethers with different aromatic substituents at the 4-position and developed a Cys-selective probe (Scheme 1a).[20] Yoon, Yin, and coworkers reported that the differentiation of Cys over Hcy/GSH at pH 6.0 can be achieved by an NBD derivative with sulfur replacement of oxygen at the oxazole group.[21] To further obtain different reactivities and selectivities with distinct photophysical properties, we note that the expansion of NBD-based probes with sulfonamide substituents at the 7-position is an additional area of likely investigation.[5f] For example, SBD-Cl derivatives have been developed for the differentiation of Cys and Hcy/GSH, and unlike NBD-SG, the SBD thioethers are fluorescent with a different emission wavelength from that of SBD amines (Scheme 1a).[22] In addition, SBD contains two sites for the facile development of multi-functional probes.[23] Therefore, SBD should be a useful dye platform for the development of new fluorescent probes with tunable properties.

On the other hand, chemical modifications at the 4-position of SBD are considered to impact the reactivity and selectivity of the probes, but such studies are understudied in detail. Herein, we prepared a series of SBD derivatives (Scheme 1b) and studied their thiolysis reactions in aqueous buffer (pH 7.4). We demonstrate that the thiolysis reactivity of SBD derivatives increases with 4-substituted atoms varing from N, O, S to Se. SBD selenoether can react with all biothiols efficiently and can be a useful receptor motif for sensing and differentiating biothiols. Moreover, SBD arylthioethers are found to be tunable motifs and structural modifications at the aryl group lead to a new GSH-selective receptor for the development of improved fluorescent probes.

Results and discussion

One major challenge in the development of GSH probes is the discovery of a chemical reaction to effectively separate the reac-



Scheme 1 (a) NBD-Cl can react with biothiols to give fluorescent products NBD-NH-Cys/Hcy and non-fluorescent NBD-SG; NBD-OR can selectively react with Cys to give NBD-NH-Cys; SBD-Cl can react with biothiols to give two kinds of fluorescent products SBD-NH-Cys and SBD-S-G/Hcy. In this design, we hope to tune the thiolysis reactivities of SBD-XR derivatives for the selective detection of GSH. (b) Chemical structures of SBD compounds **1–7**.

tivity of GSH and other biothiols. To address this challenge, we intend to investigate the chemistry of SBD derivatives. Probes **1–4** contain an SBD fluorophore with a water-soluble group and different atoms at the 4-position, while probes **5–7** contain an endoplasmic reticulum (ER)-targeting group and a thiol recognition group or Cl atom as a control (Scheme 1).[24] These probes can be facilely prepared from commercially available reagents. The structures of these probes were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy and HRMS (see the ESI† for details).

With these probes in hand, we first performed the time-dependent HPLC of the probes in the presence of 10 mM GSH in PBS buffer (pH 7.4, containing 30% DMSO). As shown in Fig. 1, probes **1** and **2** were stable toward 10 mM GSH within 5 hours, and this stability lasted for at least 72 hours (Fig. S1†), while probe **3** exhibited a certain thiolysis reactivity over time. A small amount of **3** could still be detected in HPLC traces after 5 h of incubation with GSH, suggesting that the thiol-exchange thiolysis may be reversible. The thiolysis rate of **4** is much faster than that of **3**, since more than 95% of **4** was consumed within 3 min of reaction with 1 mM GSH. Time-dependent UV-Vis spectra of **1–4** in the presence of GSH (Fig. S2†) supported the results from HPLC analysis. Therefore, the thiolysis reactivities of **1–4** increase with substituted atoms varing from N, O, and S to Se (Fig. 1e).

Compared with these SBD probes, the stronger electron-withdrawing nitro group in NBD increases the electrophilicity of the carbon at the 4-position, resulting in more efficient thiolysis of NBD probes. For example, the H_2S-specific thiolysis of the NBD-piperazinyl moiety discovered by our group has been widely employed as a fast receptor for the development of H_2S probes,[5f,18] but probe **1** cannot react with H_2S under similar conditions (Fig. S3†). The reaction of probe **3** and 10 mM GSH could not reach completion after 1 h, but the thiolysis rate of NBD thioether with GSH could reach 80.7 M^{-1} s^{-1}.[17d] It was noted that the reaction of **4** and GSH could lead to a significant fluorescence increase at 520 nm, which was employed to determine the pseudo-first-order rate, k_{obs}. The linear fitting between k_{obs} and GSH concentrations gives the reaction rate (k_2) of 5.16 M^{-1} s^{-1} for SBD selenoether (Fig. S4†), which is also much lower than that of NBD thioether. In total, SBD probes show much lower reactivities than NBD probe analogs.

Now that probe **4** can react with GSH efficiently, we further examined the absorbance and fluorescence spectra of **4** toward different biothiols (Fig. 2). Probe **4** showed quite weak fluorescence due to the fluorescence-quenching ability of the heavy atom Se. Upon the thiolysis reaction with GSH or Hcy, a large fluorescence turn-on at 520 nm was observed because of the formation of fluorescent SBD-SR.[22] However, Cys triggered fluorescence at 580 nm, which is because the resultant SBD-thioether can further undergo an intermolecular Smiles rearrangement to generate SBD-NHR. These sensing mechanisms of SBD selenoether are similar to those of SBD-Cl probes.[22a] Though H_2S cannot trigger fluorescence turn-on of **4**, a ratiometric UV-Vis absorption spectrum was observed for the time-dependent reaction of **4** and H_2S (Fig. 2b). Such a ratiometric response is based on H_2S-mediated cleavage of the selenoether bond, which is similar to that of an NBD selenoether probe.[25] However, the stronger electron-withdrawing nitro group increases the maximum absorbance peak from 490 nm (SBD-SH) to 530 nm (NBD-SH). The above results suggest that SBD selenoethers may be used as a new robust tool to differentiate H_2S, Cys, and GSH/Hcy simultaneously *via* the different photochromic and fluorescence properties in the future.

Because the SBD alkylthioether **3** is slow reactive towards GSH, we further considered SBD arylthioethers as tunable motifs because structural modifications at the aryl group should enable the rate of thiol-mediated thiolysis to be modified. To this end, we designed **6** and **7** with strong electron-

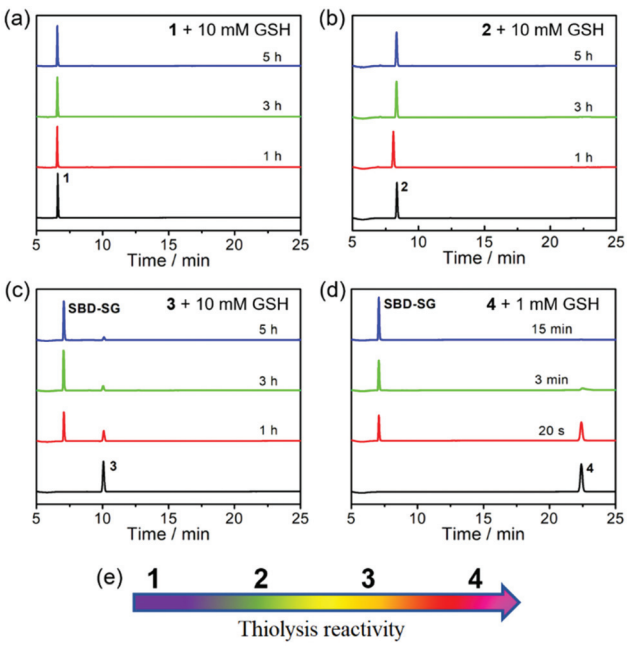


Fig. 1 (a–d) Time-dependent HPLC traces for probes **1–4** (200 μM) in the presence of GSH in PBS buffer (pH 7.4, containing 30% DMSO), respectively. (e) Illustration of the relative reactivities of these probes with GSH.

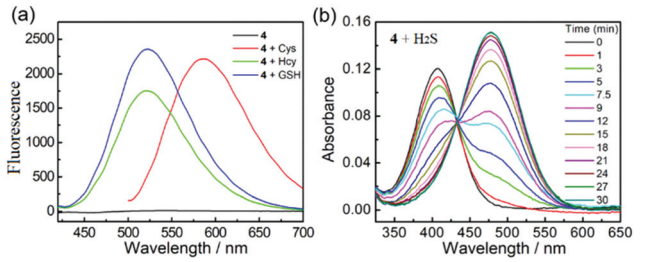


Fig. 2 (a) Fluorescence spectra of **4** (10 μM) and its reaction with 1 mM biothiols for 1 h. Excitation = 400 nm for **4**, **4** + Hcy, and **4** + GSH; excitation = 440 nm for **4** + Cys. (b) Time-dependent UV-vis spectra of **4** (10 μM) and H_2S (0.25 mM).

withdrawing and strong electron-donating groups at the aryl moiety, respectively. SBD-Cl **5** was used as a control probe. As shown in Fig. 3, probes **5** and **7** showed quite weak fluorescence within 1 h in PBS buffer, while probe **6** exhibited increasing fluorescence over time due to its water instability. The strong electron-withdrawing nitro group increases the electrophilicity of the carbon at the 4-position of SBD, resulting in the possible hydrolysis of probe **6**. Subsequently, we only employed probe **7** for the following studies.

We further examined the fluorescence spectra of **7** in the presence of different thiols. Because the GSH and Cys levels normally remain in the range of millimolar and micromolar concentrations, respectively, we tested the reaction between the probe and 2 mM GSH or 100 μM Cys/Hcy. The results showed that probe **7** displayed a selective response to GSH and led to a strong enhancement of emission at 520 nm, while only a slight fluorescence response was detected when it was incubated with Cys/Hcy (Fig. 4a and S5†). We further investigated the time-dependent emission changes of the probe with biothiols in PBS buffer. Compared with SBD-Cl **5**, probe **7** worked better in the selective detection of GSH (Fig. 4b and S6†). In addition, we can easily distinguish GSH from other biothiols under a 365 nm UV lamp by the naked eye since Hcy and Cys do not show green fluorescence even after overnight reaction (Fig. S7†). All these results imply that probe **7** is GSH-selective, with higher selectivity than that of probe **5**.

Moreover, the reaction kinetics for probes **5** and **7** were monitored by obtaining emission data at 520 nm. The k_{obs} was determined by fitting the intensity data with single exponential function (Fig. S8 and S9†). The linear fitting between k_{obs} and GSH concentrations gives a k_2 of 0.29 and 0.03 M^{-1} s^{-1} for **5** and **7**, respectively (Fig. 5), both of which are lower than that of **4** (5.16 M^{-1} s^{-1}). Comparing the chemical structures of these probes, we surmise that 4-(dimethylamino)benzenethioether at the 4-position decreases the reactivity of the electrophilic SBD in **7**, providing a better GSH-selective receptor. Therefore, probe **7** was chosen and employed for the selective detection of GSH in the following studies.

Next, we conducted the concentration titration of GSH toward probe **7** for the investigation of its sensitivity (Fig. S10†). The fluorescence intensity at 520 nm was linearly related to the concentration of GSH in the 0.1–1.0 mM range. The detection limit of probe **7** for GSH was calculated to be 2.1 μM, indicating that probe **7** has high sensitivity for GSH. To reveal the sensing mechanism, we further analysed the reaction of probe **7** with biothiols by HPLC and HRMS (Fig. S11 and S12†). Time-course HPLC traces indicated that probe **7** reacted with GSH more efficiently than with Hcy and Cys. The product (**7-GSH**) was further identified by HRMS (Fig. S11c†). The studies were consistent with the fluorescence tests showing that **7** selectively reacted with GSH.

The high selectivity of probe **7** to GSH is an important parameter to examine its biological applicability. To this end, the fluorescence response of probe **7** to diverse interfering species including various amino acids and thiols was further studied. As shown in Fig. 6, only the samples containing GSH showed an obvious fluorescence enhancement, suggesting that probe **7** could selectively detect GSH over amino acids and other biothiols. Furthermore, we carried out competition selectivity studies *via* the addition of GSH and other analytes at the same time. All the samples showed a significant fluorescence enhancement, which means that other biologically relevant species show no obvious interference with GSH detection. The results suggested that probe **7** was highly selective to GSH.

Encouraged by the good performances of probe **7**, we further evaluated the biological applications of probe **7**. To

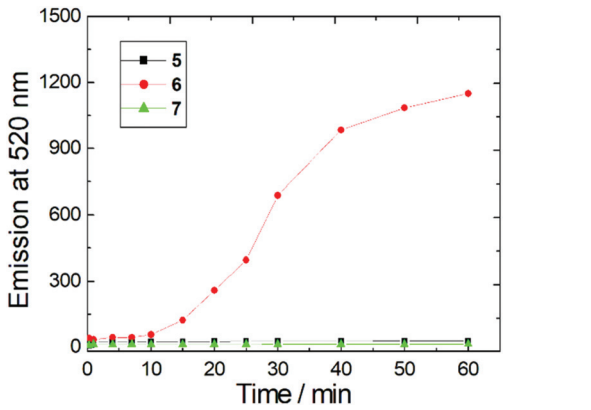


Fig. 3 Time-dependent emission intensities at 520 nm of the probes **5**–**7** (10 μM) in PBS buffer (pH 7.4).

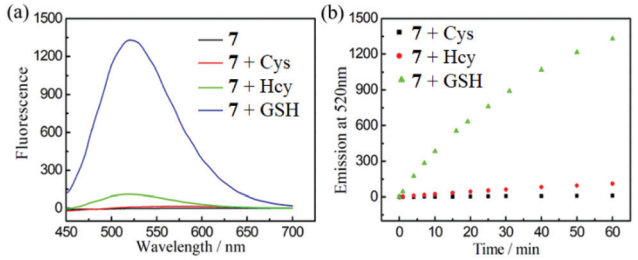


Fig. 4 (a) Fluorescence spectra of 10 μM probe **7** and its reaction with Cys (100 μM), Hcy (100 μM) or GSH (2 mM) for 1 h. (b) Time-dependent emission intensities at 520 nm of 10 μM **7** toward these biothiols.

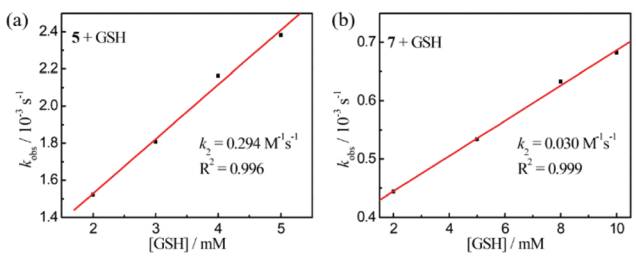


Fig. 5 The linear relationships between the concentration of GSH and k_{obs} values to produce the reaction rate k_2 values for probes **5** (a) and **7** (b).

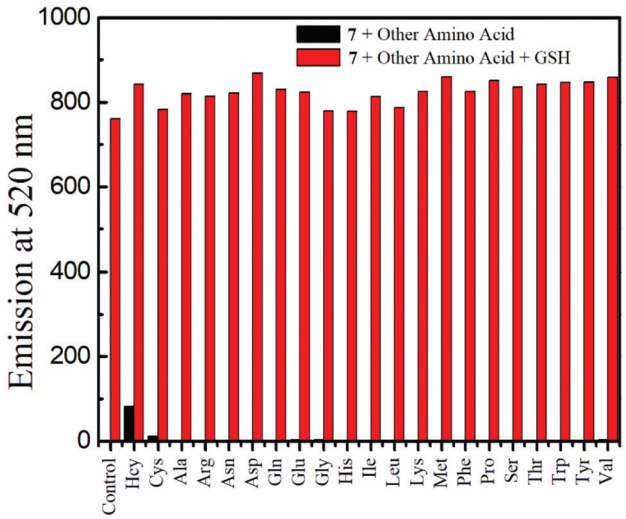


Fig. 6 Emission response at 520 nm of probe **7** (10 μM) toward various biologically relevant species (100 μM) with or without GSH (1 mM) in PBS buffer (pH 7.4).

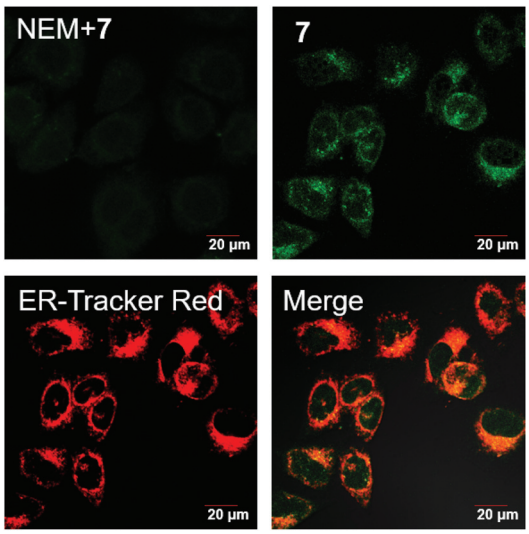


Fig. 8 Fluorescence images of intracellular GSH with probe **7** and ER-Tracker Red. Scale bar, 20 μm.

start with, the cytotoxicity of probes **5** and **7** was evaluated *via* a standard MTT assay with non-cancerous FHC (human fetal normal colonic mucosa) cells (Fig. 7). The result indicated that compared to probe **5**, probe **7** had no obvious inhibitory effect on cell growth after 24 hours of incubation. Even if the concentration of probe **7** reached 75 μM, the cell viability was still higher than 90%. However, probe **5** displayed obvious cytotoxicity to cells when its concentration was greater than 25 μM. These results implied that probe **7** was more biocompatible for live cell imaging than probe **5**.

Encouraged by these positive results, we next used HeLa cells as the model biological system to evaluate the potential of probe **7** for the imaging of endogenous GSH in the ER. HeLa cells were co-incubated with probe **7** and ER-Tracker Red, while the control cells were pre-treated with the thiol blocking reagent *N*-ethylmaleimide (NEM, 1 mM) for 30 min, and then incubated with probe **7**. The cells were then examined using a confocal microscope. As shown in Fig. 8, the green fluorescence was hardly detected in the NEM-treated cells, while the fluorescence signal produced by the reaction of endogenous GSH and **7** was observed. In addition, the red fluorescence signal from ER-Tracker merged well with the green fluorescence of **7** in the cells, with a Pearson's correlation coefficient of 0.598. These preliminary data implied that probe **7** could be a promising tool for imaging of GSH in the ER.

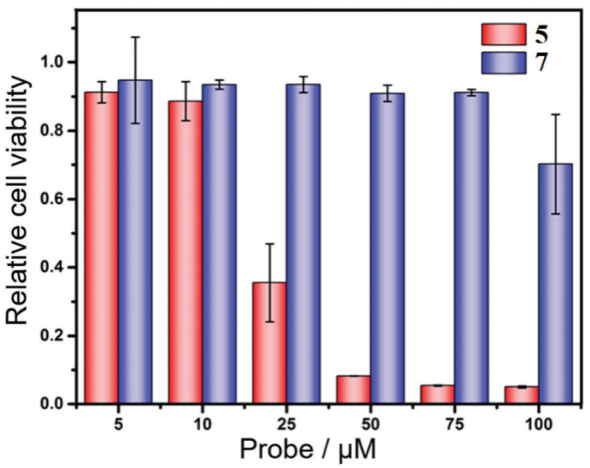


Fig. 7 Cell viability studies of probes **5** and **7** with FHC cells for 24 h of incubation by the MTT assay.

Conclusions

In summary, a series of SBD derivatives were synthesized and characterized for the evaluation of their thiolysis reactivity in PBS buffer (pH 7.4). Both SBD-amine and SBD-ether are stable toward biothiols in buffer, while SBD-selenoether can react with all biothiols with three different sets of spectral signals, suggesting that SBD-selenoether should be an efficient motif for differentiated detection of biothiols. In addition, SBD-arylthioethers are tunable motifs because structural modifications at the aryl group enable the rate of thiol-mediated thiolysis to be modified. For example, the reaction rates of probes **5** and **7** toward GSH are 0.29 and 0.03 M^{-1} s^{-1}, respectively. In addition, probe **7** displayed good selectivity and sensitivity to GSH, excellent biocompatibility, and ER-targeting ability for imaging of GSH in the ER in live cells. We propose that the expansion of BD-based probes with different substituents at the 4-position and/or electron-withdrawing groups at the 7-position is an efficient strategy to obtain different reactivities and selectivities and is an additional area of likely future investigation.[5f]

Conflicts of interest

The authors declare no competing financial interests.

Acknowledgements

We acknowledge the support of the National Key R&D Program of China (2017YFD0200501), NSFC (21877008, 21837001), and the Beijing Natural Science Foundation (2192038).

Notes and references

1 (*a*) M. Iciek, G. Chwatko, E. Lorenc-Koci, E. Bald and L. Wlodek, *Acta Biochim. Pol.*, 2004, **51**, 815–824; (*b*) M. R. Filipovic, J. Zivanovic, B. Alvarez and R. Banerjee, *Chem. Rev.*, 2018, **118**, 1253–1337.

2 (*a*) A. Ghanizadeh, S. Akhondzadeh, M. Hormozi, A. Makarem, M. Abotorabi-Zarchi and A. Firoozabadi, *Curr. Med. Chem.*, 2012, **19**, 4000–4005; (*b*) P. Maher, *Ageing Res. Rev.*, 2005, **4**, 288–314; (*c*) X.-X. Zhang, H. Qi, Y.-L. Liu, S.-Q. Yang, P. Li, Y. Qiao, P.-Y. Zhang, S.-H. Wen, H.-l. Piao and K.-L. Han, *Chem. Sci.*, 2020, **11**, 11205–11213.

3 (*a*) C. Gorrini, I.-S. Harris and T.-W. Mak, *Nat. Rev. Drug Discovery*, 2013, **12**, 931–947; (*b*) V.-M. Hudson, *Free Radicals Biol. Med.*, 2001, **30**, 1440–1461; (*c*) Y. Chen, H. Dong, D.-C. Thompson, H.-G. Shertzer, D.-W. Nebert and V. Vasiliou, *Food Chem. Toxicol.*, 2013, **60**, 38–44; (*d*) S. Saharan and P.-K. Mandal, *J. Alzheimer's Dis.*, 2014, **40**, 519–529; (*e*) J.-P. Jacobsen, R.-M. Rodriguiz, A. Mørk and W.-C. Wetsel, *Neuroscience*, 2005, **132**, 1055–1072.

4 (*a*) R.-A. Cairns, I.-S. Harris and T.-W. Mak, *Nat. Rev. Cancer*, 2011, **11**, 85–95; (*b*) T. Schnelldorfer, S. Gansauge, F. Gansauge, S. Schlosser, H.-G. Beger and A.-K. Nussler, *Cancer*, 2000, **89**, 1440–1447; (*c*) Y. Xiong, C. Xiao, Z. Li and X. Yang, *Chem. Soc. Rev.*, 2021, **50**, 6013–6041.

5 (*a*) X. Chen, Y. Zhou, X. Peng and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 2120–2135; (*b*) L.-Y. Niu, Y.-Z. Chen, H.-R. Zheng, L.-Z. Wu, C.-H. Tung and Q.-Z. Yang, *Chem. Soc. Rev.*, 2015, **44**, 6143–6160; (*c*) Y. Yue, F. Huo, F. Cheng, X. Zhu, T. Mafireyi, R. M. Strongin and C. Yin, *Chem. Soc. Rev.*, 2019, **48**, 4155–4177; (*d*) Y. Yue, F. Huo and C. Yin, *Chem. Sci.*, 2021, **12**, 1220–1226; (*e*) Y. Liu, Y. Yu, Q. Zhao, C. Tang, H. Zhang, Y. Qin, X. Feng and J. Zhang, *Coord. Chem. Rev.*, 2021, **427**, 213601–213623; (*f*) C. Jiang, H. Huang, X. Kang, L. Yang, Z. Xi, H. Sun, M. D. Pluth and L. Yi, *Chem. Soc. Rev.*, 2021, **50**, 7436–7495; (*g*) Y. M. Poronik, K. V. Vygranenko, D. Gryko and D. T. Gryko, *Chem. Soc. Rev.*, 2019, **48**, 5242–5265.

6 (*a*) J. Liu, Y.-Q. Sun, Y. Huo, H. Zhang, L. Wang, P. Zhang, D. Song, Y. Shi and W. Guo, *J. Am. Chem. Soc.*, 2014, **136**, 574–577; (*b*) M. Isik, R. Guliyev, S. Kolemen, Y. Altay, B. Senturk, T. Tekinay and E. U. Akkaya, *Org. Lett.*, 2014, **16**, 3260–3263; (*c*) L.-Y. Niu, Y.-S. Guan, Y.-Z. Chen, L.-Z. Wu, C.-H. Tung and Q.-Z. Yang, *J. Am. Chem. Soc.*, 2012, **134**, 18928–18931.

7 (*a*) H. Yan, F. Huo, Y. Yue, J. Chao and C. Yin, *J. Am. Chem. Soc.*, 2021, **143**, 318–325; (*b*) L. He, Q. Xu, Y. Liu, H. Wei, Y. Tang and W. Lin, *ACS Appl. Mater. Interfaces*, 2015, **7**, 12809–12813; (*c*) Z. Liu, W. Zhou, J. Li, H. Zhang, X. Dai, Y. Liu and Y. Liu, *Chem. Sci.*, 2020, **11**, 4791–4800.

8 (*a*) R. Li, H. Kassaye, Y. Pan, Y. Shen, W. Li, Y. Cheng, J. Guo, Y. Xu, H. Yin and Z. Yuan, *Biomater. Sci.*, 2020, **8**, 5994–6003; (*b*) X.-B. Wang, H.-J. Li, C. Liu, Y.-X. Hu, M.-C. Li and Y.-C. Wu, *Anal. Chem.*, 2021, **93**, 2244–2253.

9 S.-Y. Lim, K.-H. Hong, D.-I. Kim, H. Kwon and H.-J. Kim, *J. Am. Chem. Soc.*, 2014, **136**, 7018–7025.

10 (*a*) J. Ou-Yang, Y. Li, W.-L. Jiang, S.-Y. He, H.-W. Liu and C.-Y. Li, *Anal. Chem.*, 2019, **91**, 1056–1063; (*b*) H. Zhang, B. Ye, Y. Wang, W. Chen and X. Song, *Org. Biomol. Chem.*, 2019, **17**, 9631–9635.

11 (*a*) H. Zhang, R. Liu, J. Liu, L. Li, P. Wang, S.-Q. Yao, Z. Xu and H. Sun, *Chem. Sci.*, 2016, **7**, 256–260; (*b*) J. Li, Z.-E. Hu, X.-L. Yang, M.-Q. Zhang, Y.-H. Liu, N. Wang and X.-Q. Yu, *ACS Appl. Bio Mater.*, 2020, **3**, 7382–7387.

12 J. Yin, Y. Kwon, D. Kim, D. Lee, G. Kim, Y. Hu, J.-H. Ryu and J. Yoon, *J. Am. Chem. Soc.*, 2014, **136**, 5351–5358.

13 (*a*) Z. Xu, X. Huang, X. Han, D. Wu, B. Zhang, Y. Tan, M. Cao, S.-H. Liu, J. Yin and J. Yoon, *Chem*, 2018, **4**, 1609–1628; (*b*) M. Cao, H. Chen, D. Chen, Z. Xu, S.-H. Liu, X. Chen and J. Yin, *Chem. Commun.*, 2016, **52**, 721–724.

14 (*a*) Z. Liu, X. Zhou, Y. Miao, Y. Hu, N. Kwon, X. Wu and J. Yoon, *Angew. Chem., Int. Ed.*, 2017, **56**, 5812–5816; (*b*) X. Jiang, Y. Yu, J. Chen, M. Zhao, H. Chen, X. Song, A.-J. Matzuk, S.-L. Caroll, X. Tan, A. Sizovs, N. Cheng, M.-C. Wang and J. Wang, *ACS Chem. Biol.*, 2015, **10**, 864–874; (*c*) J. Chen, X. Jiang, S.-L. Carroll, J. Huang and J. Wang, *Org. Lett.*, 2015, **17**, 5978–5981; (*d*) J. Chen, X. Jiang, C. Zhang, K.-R. MacKenzie, F. Stossi, T. Palzkill, M.-C. Wang and J. Wang, *ACS Sens.*, 2017, **2**, 1257–1261.

15 (*a*) Y. Jiang, J. Cheng, C. Yang, Y. Hu, J. Li, Y. Han, Y. Zang and X. Li, *Chem. Sci.*, 2017, **8**, 8012–8018; (*b*) H. Zong, J. Peng, X.-R. Li, M. Liu, Y. Hu, J. Li, Y. Zang, X. Li and T.-D. James, *Chem. Commun.*, 2020, **56**, 515–518.

16 (*a*) M. She, Z. Wang, T. Luo, B. Yin, P. Liu, J. Liu, F. Chen, S. Zhang and J. Li, *Chem. Sci.*, 2018, **9**, 8065–8070; (*b*) Z. Li, W. Xiong, X. He, X. Qi, F. Ding and J. Shen, *Analyst*, 2020, **145**, 4239–4244; (*c*) H.-Z. Liu, W.-T. Song, S.-R. Zhang, K.-S. Chan, Z.-J. Guo and Z. Shen, *Chem. Sci.*, 2020, **11**, 8495–8501.

17 (*a*) L. Yi, H. Li, L. Sun, L. Liu, C. Zhang and Z. Xi, *Angew. Chem., Int. Ed.*, 2009, **48**, 4034–4037; (*b*) Z. Zhu, W. Liu, L. Cheng, Z. Li, Z. Xi and L. Yi, *Tetrahedron Lett.*, 2015, **56**, 3909–3912; (*c*) Y. Men, Z. Li, J. Zhang, Z. Tong, Z. Xi, X. Qiu and L. Yi, *Tetrahedron Lett.*, 2015, **56**, 5781–5786; (*d*) L. Sun, Y. Jiang, C. Zhang, X. Ji, D. Lv, Z. Xi and L. Yi, *New J. Chem.*, 2018, **42**, 15277–15283; (*e*) H. Huang, X. Ji, Y. Jiang, C. Zhang, X. Kang, J. Zhu, L. Sun and L. Yi, *Org. Biomol. Chem.*, 2020, **18**, 4004–4008.

18 (*a*) C. Wei, L. Wei, Z. Xi and L. Yi, *Tetrahedron Lett.*, 2013, **54**, 6937–6939; (*b*) C. Wei, Q. Zhu, W. Liu, W. Chen, Z. Xi and L. Yi, *Org. Biomol. Chem.*, 2014, **12**, 479–485; (*c*) K. Zhang, J. Zhang, Z. Xi, L.-Y. Li, X. X. Gu, Q.-Z. Zhang and L. Yi, *Chem. Sci.*, 2017, **8**, 2776–2781; (*d*) C. Zhang, Q.-Z. Zhang, K. Zhang, L.-Y. Li, M. D. Pluth, L. Yi and Z. Xi, *Chem. Sci.*, 2019, **10**, 1887–1900; (*e*) I. Ismail, Z. Chen, L. Sun, X. Ji, H. Ye, X. Kang, H. Huang, H. Song, S.-G. Bolton, Z. Xi, M. D. Pluth and L. Yi, *Chem. Sci.*, 2020, **11**, 7823–7828.

19 (*a*) L.-A. Montoya, T.-F. Pearce, R.-J. Hansen, L.-N. Zakharov and M.-D. Pluth, *J. Org. Chem.*, 2013, **78**, 6550–6557; (*b*) M.-D. Hammers and M.-D. Pluth, *Anal. Chem.*, 2014, **86**, 7135–7140; (*c*) X. Gao, X. Li, L. Li, J. Zhou and H. Ma, *Chem. Commun.*, 2015, **51**, 9388–9390.

20 J.-M. An, S. Kang, E. Huh, Y. Kim, D. Lee, H. Jo, J.-F. Joung, V.-J. Kim, J.-Y. Lee, Y.-S. Dho, Y. Jung, J.-K. Hur, C. Park, J. Jung, Y. Huh, J.-L. Ku, S. Kim, T. Chowdhury, S. Park, J.-S. Kang, M.-S. Oh, C.-K. Park and D. Kim, *Chem. Sci.*, 2020, **11**, 5658–5668.

21 D. Lee, G. Kim, J. Yin and J. Yoon, *Chem. Commun.*, 2015, **51**, 6518–6520.

22 (*a*) L. He, X. Yang, K. Xu and W. Lin, *Anal. Chem.*, 2017, **89**, 9567–9573; (*b*) J. Huang, Y. Chen, J. Qi, X. Zhou, L. Niu, Z. Yan, J. Wang and G. Zhao, *Spectrochim. Acta, Part A*, 2018, **201**, 105–111.

23 H. Xu, C. Zhu, Y. Chen, Y. Bai, Z. Han, S. Yao, Y. Jiao, H. Yuan, W. He and Z. Guo, *Chem. Sci.*, 2020, **11**, 11037–11041.

24 P. Gao, W. Pan, N. Li and B. Tang, *Chem. Sci.*, 2019, **10**, 6035–6071.

25 J. Bae, M.-G. Choi, J. Choi and S.-K. Chang, *Dyes Pigm.*, 2013, **99**, 748–752.