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Benzothiazole-Derived Sulfones and Sulfoxides as Reactive Templates for Biothiols and Sulfane Sulfurs

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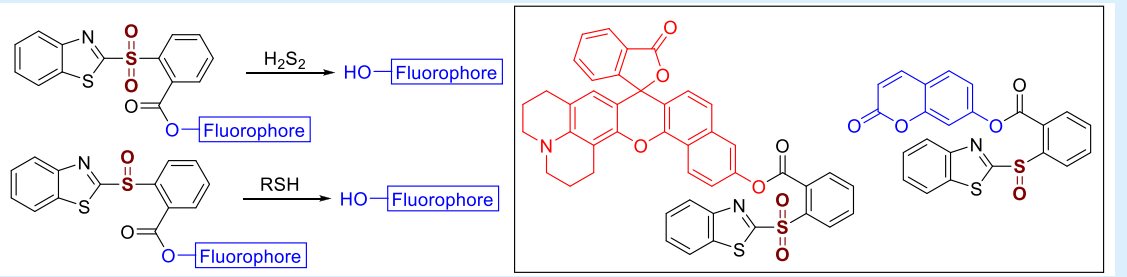
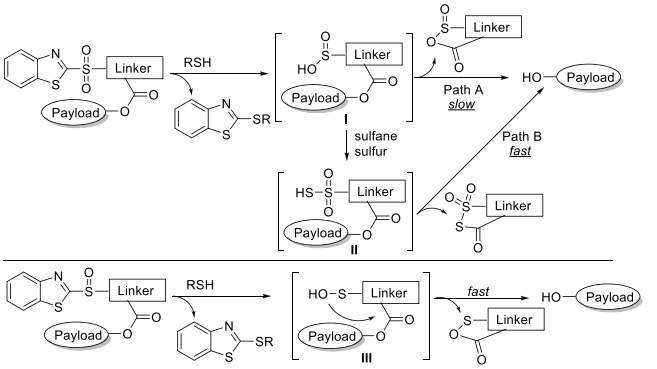


ABSTRACT: In this work we studied the reactions of benzothiazole sulfones and sulfoxides toward reactive sulfur species. The reaction of thiols with benzothiazole sulfones produces sulfinic acids (RSO_2H), which can further react with sulfane sulfurs to form thiosulfonic acids (RSO_2SH). This was used to design fluorescent sensors for hydrogen polysulfides. The reaction of thiols with benzothiazole sulfoxides produces sulfenic acids (RSOH), which can undergo fast intramolecular cyclization and be used to design thiol-triggered fluorescent sensors.

Reactive sulfur species (RSS) are sulfur-containing molecules that show regulatory effects in biological systems. Sulfane sulfurs and biothiols are key members of RSS. Sulfane sulfurs include polysulfides (RS_nR, $n > 1$), persulfides (RSSH), and hydrogen polysulfides (H_2S_n, $n > 1$). The importance of sulfane sulfurs has been recently recognized with the establishment of hydrogen sulfide (H_2S) as the third gasotransmitter after nitric oxide (NO) and carbon monoxide (CO).[1,2] Biothiols are vital in maintaining cellular redox homeostasis. Dysregulation of biothiols is linked to many diseases such as Alzheimer's disease, cancer, cardiovascular disorders, and AIDS.[3,4] It is therefore important to study the unique reactivity of biothiols and sulfane sulfurs and develop specific chemical tools for understanding their functions. To date, a large number of chemical tools, in particular for biothiols, has been reported. The most well-known thiol-selective reagents include iodoacetamide (IAM), *N*-ethylmaleimide (NEM), methylmethanethiosulfonate (MMTS), etc. While these reagents have been widely used in thiol-blocking, their specificity is sometimes questionable. For example, studies have found cross reactivity between IAM/NEM and other cellular thiol modification products such as sulfenic acids (RSOH).[5] To address this issue, our lab has developed sulfonylbenzothiazole derivatives as more selective thiol-labeling reagents.[5,6] These compounds have high reactivities toward biothiols, while remaining inert to other nucleophilic species including RSOH. More recently, we found that the sulfinic acid generated from the reaction between thiols and sulfonylbenzothiazoles was nucleophilic enough to undergo an intramolecular cyclization with a tethered ester group (Scheme 1, path A). This was used to develop a thiol-triggered template to release fluorophore and drug molecules.[7]

Scheme 1. Designs of Benzothiazole Sulfone and Sulfoxide-Based RSS-Reactive Templates



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However, this cyclization was found to be a slow process under physiological conditions. It required hours for the reaction to reach completion. Since sulfinic acid is known to react with sulfane sulfurs rapidly,[8] we envisioned the presence of sulfane sulfur could convert sulfinic acid (RSO_2H) to thiosulfonic acid (RSO_2SH). This might expedite the cyclization process (Scheme 1, path B) and serve as a unique way to recognize certain sulfane sulfurs (e.g., H_2S_n). Herein, we report the study of benzothiazole derived sulfones as a sulfane sulfur/H_2S_n-reactive template based on this mechanism. Furthermore, we also explored the thiol-triggered cyclization on benzothiazole sulfoxide derivatives. We found that benzothiazole sulfoxides can selectively and readily react with biothiols. The resulted SOH intermediates could rapidly undergo intramolecular cyclization with the tethered esters to release the −OH containing payloads.

Our first step was to test if the sulfinic acid intermediate **I** (Scheme 1) could be converted to thiosulfonic acid **II** by sulfane sulfurs and subsequently trigger the cyclization. A model compound **1** (Figure 1) was prepared and treated with

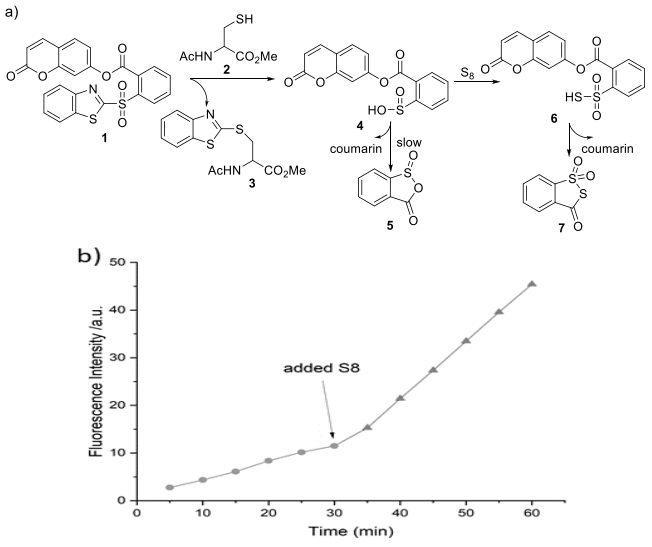

Figure 1. (a) Proposed mechanism of sulfane sulfur-promoted cyclization. (b) Fluorescence enhancement of **1** (10 μM) with Cys (100 μM) and S_8 (100 μM). Reactions were carried out for 30 min at 25 °C in PBS buffer (10 mM, pH 7.4). Excitation/emission: 350/454 nm.

cysteine (Cys). This reaction was carried in PBS buffer (10 mM, pH 7.4) with 10 μM of **1** and 100 μM of Cys. The change of fluorescence (resulting from the release of coumarin) was monitored as the indicator of cyclization. As shown in Figure 1b, the reaction between **1** and Cys under pH 7.4 led to a slow and weak fluorescence increase. However, when S_8, a representative sulfane sulfur species, was subsequently added, a much faster increase of fluorescence was noted. We also tested this process under pH 6.0 and pH 8.0. Very slow fluorescence "turn-on" was noted under pH 6.0 while much faster "turn-on" was observed under pH 8.0 (Figure S9). We analyzed the reaction by mass spectrometry. When **1** was treated with *N*-acetylcysteine methyl ester **2**, the formation of −SH blocking product **3** and sulfinic acid **4** was confirmed, indicating that thiol attack of sulfonylbenzothiazole initiated the reaction. After the addition of S_8, the thiosulfonate intermediate **6** and the cyclization product **7** were also confirmed by mass spectrometry. These results demonstrated that sulfane sulfur did promote faster cyclization of the sulfinic acid intermediate following the proposed mechanism.

H_2S_n belong to the sulfane sulfur family and are known to be the key regulators in redox biology. Some biological activities originally attributed to H_2S are now believed to be mediated by H_2S_n. For example, H_2S_n can facilitate translocation of Nrf2,[9] activate TRPA1,[10] and regulate PTEN.[11] Also, much effort has been focused toward the development of H_2S_n specific sensors.[12−14] H_2S_n possess a unique dual reactivity, as they can serve as both nucleophile and electrophile in reactions. Thus, we expected **1** could rapidly sense H_2S_n because H_2S_n can serve as an RSH-like nucleophile to react with **1** and also serve as a S_8-like electrophile for the resulting sulfinic acid to promote the cyclization. We then tested the response of **1** toward Na_2S_2. As expected, **1** showed fast and strong fluorescence increases when treated with Na_2S_2 (Figure 2). For comparison, treatment of **1** with GSH or Cys showed only marginal levels of fluorescence.

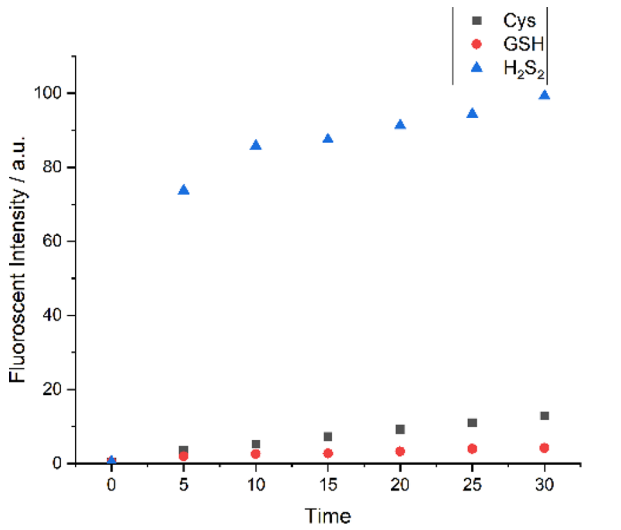

Figure 2. Fluorescence responses of **1** (10 μM) to cysteine, GSH, and Na_2S_2 (100 μM). Tests were carried out for 30 min at 25 °C in PBS buffer (10 mM, pH 7.4). Excitation/emission: 350/454 nm.

Having demonstrated that compound **1** was a selective fluorescent sensor for H_2S_n, we next prepared and tested two more sensors (**8** and **9**, Figure 3) employing *O*-methylfluorescein and Fl4, a near-infrared fluorophore developed by our group,[15] as the fluorophores. Similar to **1**, their fluorescence was turned on by H_2S_n in ∼20 min while they showed little response to Cys and GSH under the same conditions. Among the three sensors, **9** was selected for further evaluations because of its good response to Na_2S_2 and longer emission wavelength (640 nm). **1** and **8** were also tested, and their data can be found in the Supporting Information (Figures S1 and S2).

The selectivity of **9** was studied by measuring its responses toward a series of representative sulfur species (Na_2S, Na_2SO_3, $Na_2S_2O_3$), amino acids (arginine, alanine, lysine, histidine, etc.), and oxidants (NaClO, $NaNO_2$). As shown in Figure 4a, those species did not cause significant fluorescence, demonstrating the specificity of **9**. We also tested the detection limit of **9** by treating it with different concentrations of Na_2S_2 (1−100 μM) (Figure 4b). The fluorescent intensities were linearly

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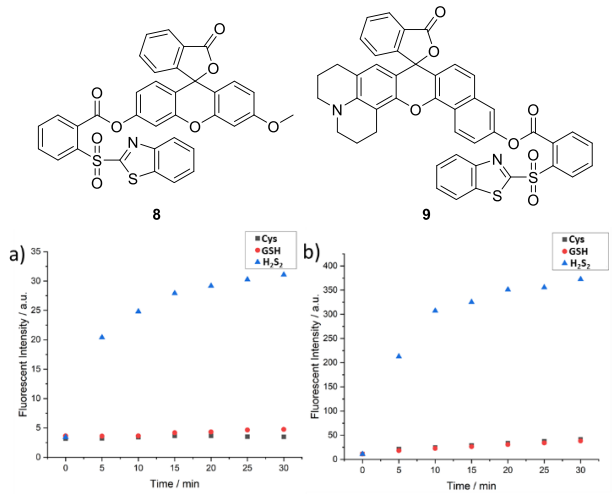


Figure 3. Fluorescence responses of **8** (a) and **9** (b) (10 μM) to different RSS (100 μM). Tests were carried out for 30 min at 25 °C in PBS buffer (10 mM, pH 7.4). Excitation/emission: **8** 476/516 nm, **9** 572/640 nm.

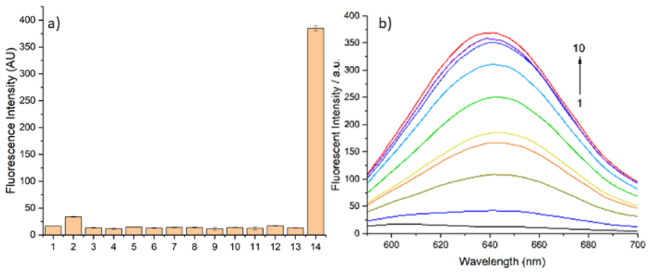


Figure 4. a) Fluorescence responses of **9** (10 μM) toward various species (100 μM): (1) Probe alone; (2) Na_2S; (3) arginine; (4) alanine; (5) Na_2SO_3; (6) $Na_2S_2O_3$; (7) lysine; (8) histidine; (9) tryptophan; (10) serine; (11) tyrosine; (12) NaClO; (13) $NaNO_2$; (14) Na_2S_2. (b) Fluorescence spectra of **9** (10 μM) to different concentrations of Na_2S_2 (1, 4, 8, 12, 15, 20, 40, 60, 80, 100 μM for curve 1−10). All reactions were carried out for 30 min at 25 °C in PBS buffer (10 mM, pH 7.4).

related to the concentrations in the range of 1−20 μM (Figure S3). The detection limit was determined to be 6.3 nM.

Finally, we wondered if **9** could be used in visualizing H_2S_n in cells, so cell imaging was performed with HeLa cells. Briefly, HeLa cells were incubated with **9** (10 μM) for 30 min and then washed with PBS to remove excess sensor. No significant fluorescence was observed in these cells (Figure 5a). However, if the cells were also treated with 100 μM Na_2S_2 for 30 min, strong fluorescence was observed (Figure 5b). Moreover, cells pretreated with **9** also gave notable fluorescence after incubating with 100 μM cysteine polysulfides (Figure 5c). This suggested that **9** was unmasked by cellular thiols and could then sense sulfane sulfurs.

After benzothiazole sulfones were demonstrated as thiol (SH)-triggered sulfane sulfur-reactive platforms, we wondered if benzothiazole sulfoxides could show similar reactivity. DFT calculations were carried out to assess the electronic orbitals of the reaction between MeSH and benzothiazole sulfoxide **10a** vs the reaction between MeSH and benzothiazole sulfone **11a**. Results showed the HOMO−LUMO difference for MeSH/**10a** was 7.18 eV, slightly higher than that of MeSH/**11a** (6.75 eV, Figure S8). This small energy difference suggested that

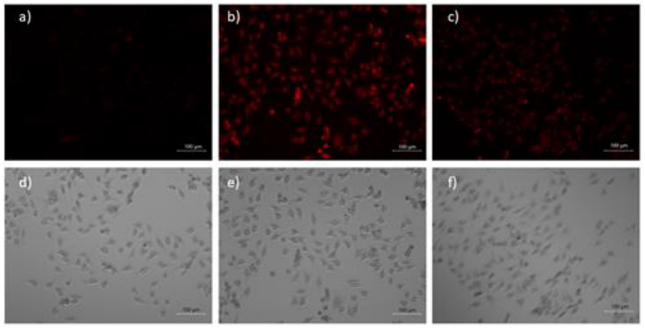
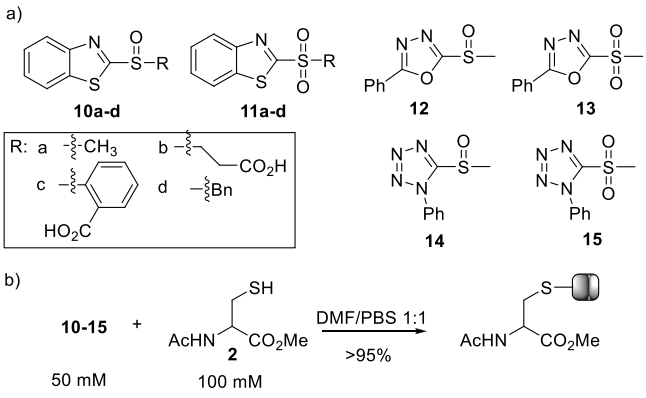


Figure 5. Fluorescent images of **9** in HeLa cells. Cells were treated with 10 μM **6** for 30 min, washed, and then subjected to different treatment for 30 min: (a) buffer; (b) Na_2S_2 100 μM; (c) cysteine polysulfide 100 μM. (d−f) Bright field images of (a), (b), and (c) respectively. Scale bars are 100 μm.

benzothiazole sulfoxides might also serve as −SH labeling agents. We then prepared and tested a series of benzothiazole sulfoxides (**10a−d**, Scheme 2). For comparison the corresponding sulfones (**11a−d**) were also tested. Since oxadiazole and tetrazole sulfones (**13**, **15**) react similarly as benzothiazole sulfones in thiol-blocking,[5] their sulfoxides (**12**, **14**) were also prepared and tested. These compounds were treated with a model RSH substrate **2** in a 1:1 DMF/PBS solution. In all cases the sulfoxide and sulfone compounds were rapidly consumed (within 1 h) and the desired RS-blocking products were obtained in almost quantitative yields. Moreover, we tested the selectivity of the sulfoxides. Compounds **10a** and **12** and **14** were treated with other amino acids such as lysine, serine, proline, etc. (Figure S4). We did not observe any reactions with these substrates. These results suggest that sulfoxides derived from benzothiazole, oxadiazole, and tetrazole are efficient thiol-blocking reagents and they can also be used to develop thiol-triggered prodrugs or sensors.

Scheme 2. (a) Structures of 10−15; (b) Reactions of 10−15 with Thiol 2



To further demonstrate the efficiency of these sulfoxides in blocking thiols under cellular environments, we carried out cell imaging with Cys-Fl4, a thiol-specific probe.[15] As shown in Figure 6, HeLa cells treated with Cys-Fl4 showed obvious red fluorescence, reflecting cellular free thiols. However, if the cells were pretreated with **10b**, the addition of Cys-Fl4 resulted in significantly decreased fluorescence. Clearly, **10b** was able to deplete cellular thiols.

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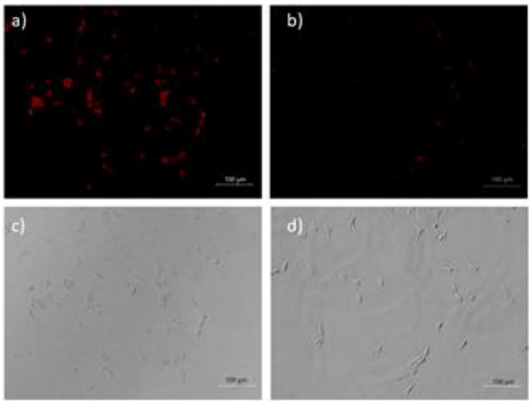
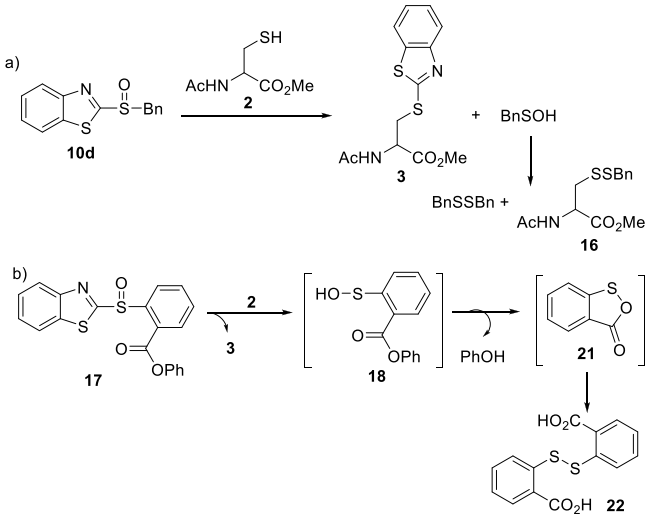


Figure 6. Fluorescence images of biothiols in HeLa cells. Cells were subjected to (a) buffer and (b) 500 μM **10b** for 30 min, and then treated with Cys-Fl4 (10 μM) for 30 min. (c and d) Bright field images of (a) and (b). Scale bars are 100 μm.

The reaction between benzothiazole sulfoxides with RSH should provide sulfenic acids (RSOH) as the products. Sulfenic acids (RSOH) are known to be much more labile than sulfinic acids (RSO_2H). We then wondered if benzothiazole sulfoxides could also be used in the design of thiol-triggered cyclization templates. We first analyzed the products of thiol **2** reacting with **10d**. In addition to the −SH blocking product **3**, benzyl disulfide and benzyl-Cys disulfide were also identified (Scheme 3a), which indicated the high instability or reactivity of RSOH

Scheme 3. Reactions of Benzothiazole Sulfoxides with 2



in the presence of thiols. A model sulfoxide **17** was then tested in the possible thiol-triggered cyclization (Scheme 3b). When **17** was treated with **2** (rt, 1.5 h), we observed the formation of phenol and **3**. These results indicated that the sulfenic acid intermediate **18** was formed and it underwent a fast cyclization. However, instead of the cyclization adduct **21**, we only isolated 2,2′-dithiodibenzoic acid **22** as the final product. This can be explained by the high instability of **21**, as sulphenyl carboxylates are known to decompose rapidly.[16] **22** should be the decomposition product of **21**. We expected the cross reaction between **2** and **18** would occur, but we were unable to observe the formation of the desired disulfide by product analysis. This suggests that the cyclization was a fast process.

Finally, we tested the possibility of using the sulfoxide template to develop RSH-triggered sensors. A coumarin-conjugate **23** was prepared, and its fluorescence responses toward thiols were evaluated. As expected, **23** showed fast and high fluorescent signals to all thiols (Cys, GSH, Hcys) and H_2S_2 (Figure 7). It did not show much selectivity.

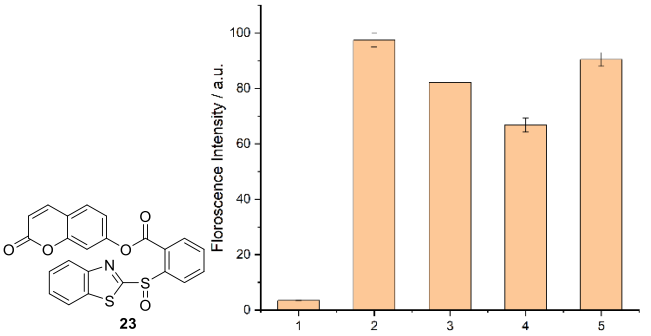


Figure 7. Fluorescence responses of **23** (10 μM) toward various species (100 μM): (1) **23** alone; (2) Cys; (3) GSH; (4) homocysteine; (5) H_2S_2. Reactions were carried out for 30 min at 25 °C in PBS buffer (10 mM, pH 7.4).

In conclusion, in this work we demonstrated that benzothiazole derived sulfones and sulfoxides are efficient thiol-blocking reagents. The reaction of RSH with benzothiazole sulfones produces sulfinic acid (RSO_2H) intermediates which can further react with sulfane sulfurs to form thiosulfonic acid (RSO_2SH). This can be used to design specific sensors for H_2S_n. On the other hand, the reaction of RSH with benzothiazole sulfoxides produces sulfenic acid (RSOH) intermediates, which can also undergo intramolecular cyclization with tethered ester groups. This reaction can be used to design general thiol-triggered sensors or prodrugs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.2c00734.

Experimental and characterization of each compound (PDF)

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Notes

The authors declare no competing financial interest.

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