Tetrahedron Letters 59 (2018) 2232–2237

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A dual-response naphthofluorescein-based fluorescent probe for multiple-channel imaging of cysteine/homocysteine in living cells

Xiuxiu Yue [a,b,d], Wenxiu Li [c,d], Wenqiang Chen [a,b,*], Liangliang Zhang [c,*], Guofang Li [a,b], Jiarong Sheng [a,b,*]

[a] College of Chemistry and Materials Science, Guangxi Teachers Education University, 530001 Nanning, Guangxi, PR China
[b] Guangxi Key Laboratory of Natural Polymer Chemistry and Physics (Guangxi Teachers Education University), Nanning 530001, PR China
[c] State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources of Education Ministry, Guangxi Normal University, 541004 Guilin, Guangxi, PR China

ARTICLE INFO

Article history:
Received 24 January 2018
Revised 2 April 2018
Accepted 10 April 2018
Available online 22 April 2018

Keywords:
Fluorescent probe
Naphthofluorescein-based
Cysteine (Cys)
Homocysteine (Hcy)

ABSTRACT

A naphthofluorescein-based fluorescent probe with two independent reaction sites (nitro-2,1,3-benzoxadiazole and acrylate moiety) was developed. Integrating these two reaction sites into a single molecule not only can guarantee the selective detection of Cys/Hcy in an elegant fashion, but also can enable Cys/Hcy detection in a multiple-channel responsive manner.

Introduction

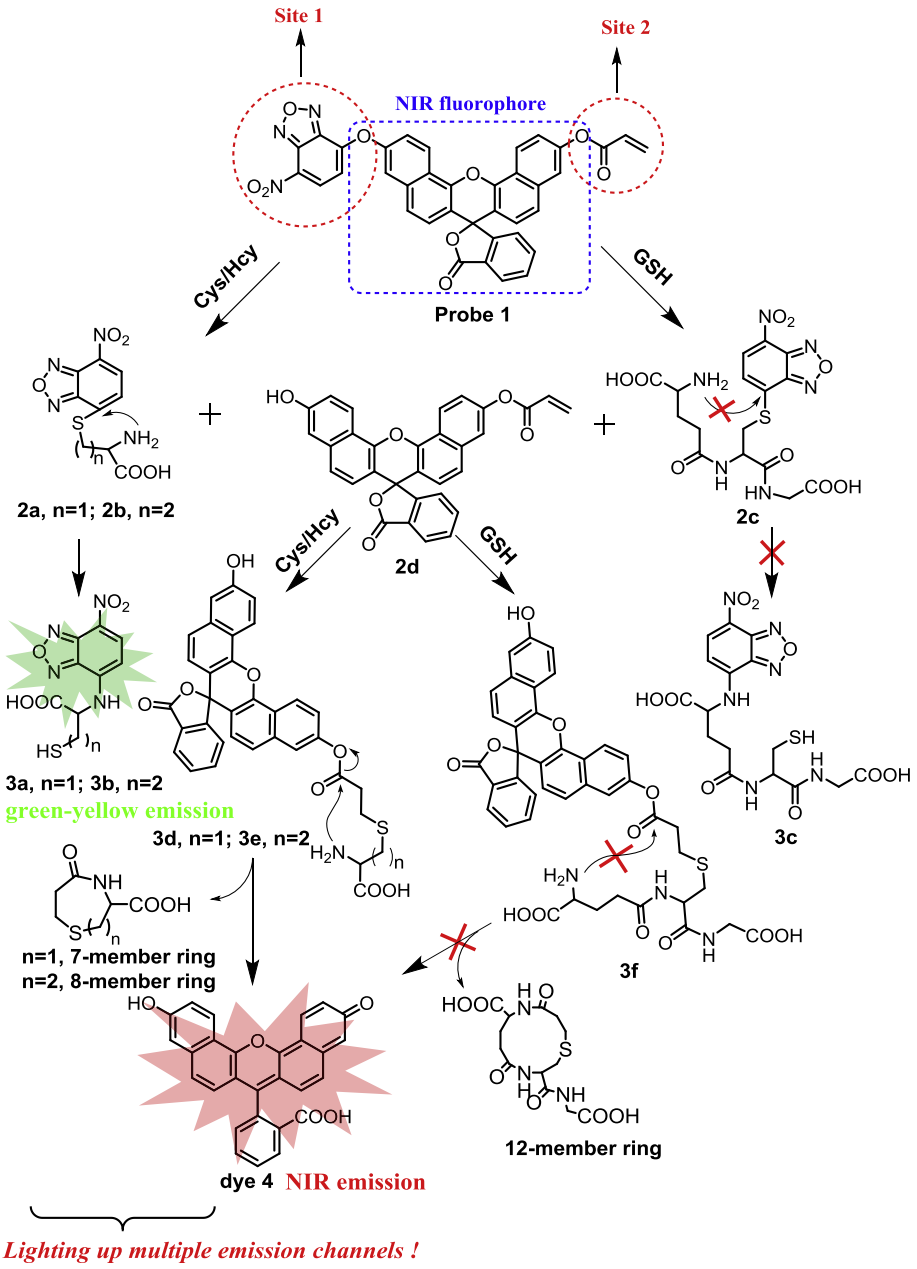
Cysteine (Cys) and homocysteine (Hcy) play crucial roles in numerous physiological processes[1] and are closely related to a variety of health disorders including Alzheimer's disease,[2] cardiovascular disease,[3] osteoporosis,[4] skin lesions,[5] liver damage and edema.[6] As a consequence, the assessment of abnormal levels of Cys/Hcy in living systems may aid early diagnosis of some diseases. Given its advantages of excellent sensitivity and high spatiotemporal resolution, fluorescent probes now have been recognized as the effective molecular tools that can help to detect or visualize biologically active molecules in living cells or tissues.[7] However, although these probes can highly selectively distinguish these biothiols from other amino acids, most of them cannot distinguish Cys/Hcy/GSH from each other due to the similar structures and reactivity of these biothiols. In fact, the discrimination between them has been a focal point and also a tough challenge for researchers, albeit some advances have been obtained.

Pioneered by Strongin et al., the selective detection of Cys/Hcy over GSH was realized for the first time by taking advantage of the unique cyclization of Cys/Hcy with aldehydes.[8] From then on, a large numbers of fluorescent probes for Cys/Hcy were constructed based on the further extension of this strategy.[9] However, most of the reported probes for Cys/Hcy emit in the visible range. It is well known that fluorescent probes that emit in near-infrared (NIR) regions are more suitable for biological imaging applications due to minimum photodamage to biological samples, deep tissue penetration and negligible interference from background.[7b] Unfortunately, NIR fluorescent probes for Cys/Hcy are rare, to the best of our knowledge, only two NIR fluorescent probes for Cys/Hcy have been reported.[10] Moreover, almost all of the reported fluorescent probes for Cys/Hcy exhibit fluorescence signal variations only in one channel, the variations in sample environment, probe distribution and the instrument factors may be problematic for utilization in quantitative measurements. In fact, to eliminate the false positive that arise from environmental factors, the development of small molecule fluorescent probe that can sense Cys/Hcy in multiple channels is highly valuable[11] but even more challenging. Herein, we have developed a multiple-channel responsive fluorescent probe for Cys/Hcy by incorporation of two potential reaction sites into a NIR naphthofluorescein dye. The probe not only can distinguish Cys/Hcy from GSH but also display obvious turn-on fluorescence signals in green, yellow and NIR emission channels, thereby holding great potential in biological applications.

The design rational is depicted in Scheme 1 and illustrated as follows. Naphthofluorescein (dye **4**) was prudentially selected as

* Corresponding authors at: College of Chemistry and Materials Science, Guangxi Teachers Education University, 530001 Nanning, Guangxi, PR China (W. Chen and J. Sheng).
E-mail address: chenwq@csu.edu.cn (W. Chen).
[d] These two authors contribute equally in this work.

https://doi.org/10.1016/j.tetlet.2018.04.017
0040-4039/



Scheme 1. The chemical structure of Probe **1** and the proposed sensing mechanism of Probe **1** to Cys/Hcy over GSH.

the fluorophore based on the following considerations: (1) naphthofluorescein is a bright NIR emitter;[12] (2) like the fluorescein, the photophysical properties of naphthofluorescein can be easily tuned through the protection/de-protection of its hydroxyl group;[13] (3) naphthofluorescein contains two hydroxyl groups which is well suited for integrating two independent reaction sites into a single fluorescent probe. Next, 7-nitro-2,1,3-benzoxadiazole (NBD) was chosen as the first reaction site (Site 1) because it functions not only as a leaving group in the reaction of thiol-mediated S_NAr substitution, but also as an effective fluorescence quencher to ensure a low background emission of the probe. We speculated that the 4-aryloxy-7-nitro-2,1,3-benzoxadiazoles (NBD-OAr) moiety could initially be cleaved by Cys (or Hcy) to produce thioNBD **2a** (or **2**b), and the subsequent intramolecular Smiles rearrangement involving 5 (or 6) membered ring would ultimately lead to strongly emissive aminoNBD **3a** (or **3b**). However, in the case of GSH, non-fluorescent thioNBD **2c** instead of aminoNDB **3**c was expected to be the desired product due to the kinetically disfavored formation of macrocyclic transition state.[14] As a result, only Cys/Hcy could trigger significant fluorescence enhancement in the visible region due to the formation of highly emissive aminoNBD **3a**/**3b**. Now, let us turn our attention to the fluorescence signal in the near-infrared region. If the NBD moiety is the only reaction site of this probe, it appears to be difficult to discriminate Cys/Hcy from GSH in the NIR region due to the similar reactivity of Cys, Hcy and GSH, thereby necessitating an additional regulatory factor, here is an acrylate moiety (Site 2), a classical Michael receptor. We anticipate that Cys, Hcy and GSH would react with unsaturated double bond in acrylate moiety to produce the corresponding thioethers through Michael addition reaction. In the case of Cys/Hcy, the corresponding thioethers **3d**/**3e** could rapidly undergo a further intermolecular cyclization[15] to release the NIR fluorophore dye **4**, thereby triggering significant fluorescence enhancement in NIR range. As for GSH, the initially produced thioether **3f** could