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1,5-Benzodiazepin-2-ones: Investigation of a Family of Photoluminescent Materials

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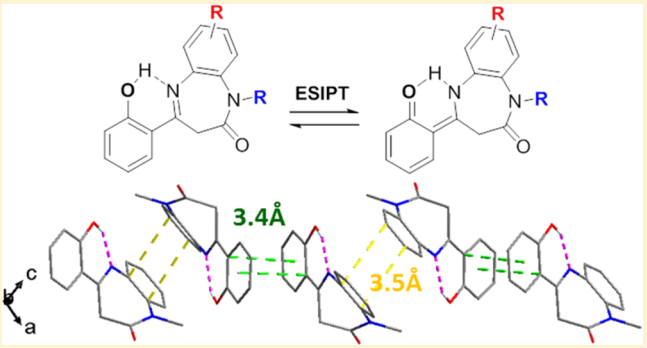
Supporting Information

ABSTRACT: Photoluminescent materials, that are now ubiquitous in our everyday life, have particularly attracted the attention of the scientific community these past few years due to potential important applications such as in bioimaging, sensing, or optoelectronics. In this context, relatively few different families of molecules have been reported to exhibit fluorescence in the aggregated or solid-state through the excited-state intramolecular proton transfer (ESIPT) photochemical process. The preparation and subsequent determination of photochemical properties of an underexplored family of 1,5-benzodiazepin-2-one derivatives are reported. From these data and X-ray diffraction analysis study, it emerged that photoluminescence (in the range 520−655 nm) was mostly attributed to ESIPT. The photoluminescent potential of 1,5-benzodiazepin-2-ones, their facile access, and functionalization were demonstrated through the preparation of two fluorogenic probes for the selective detection of biothiols.

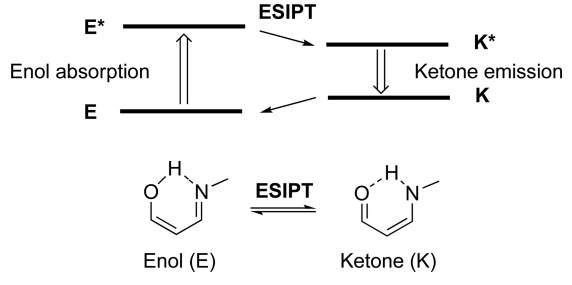


INTRODUCTION

While conventional organic fluorescent dyes exhibit strong fluorescence specifically in diluted solution, a growing number of photoluminescent materials reported in the literature are, on the other hand, emissive only in their aggregated or solid-state.[1] This singular photophysical phenomenon has been leveraged in high-tech applications of general interest including organic light-emitting diodes (OLEDs), organic photovoltaics (OPV), organic solid-state lasers, and in the design of chemo- and biosensors with useful applications in environmental and biomedical sciences.[2] Furthermore, the aggregation-induced emission (AIE) process provides a useful complementary strategy to the use of conventional hydrophobic organic fluorescent dyes, which tend to form nonemissive aggregates in physiological conditions, a phenomenon also referred to as aggregation-caused quenching (ACQ).[3]

A lot of efforts have been devoted to rationalize the unusual luminescent behavior arising from the restriction of intramolecular motions of dye aggregates or solid-state luminescent materials, and different explanations have been suggested according to the dye structure, including: intramolecular charge transfer (ICT), restricted intramolecular rotations (RIR), restricted intramolecular vibrations (RIV), twisted intramolecular charge-transfer (TICT), and excited-state intramolecular proton transfer (ESIPT). In the ESIPT photochemical process, the dye conformation in the aggregated or solid-state is locked by the formation of an intramolecular H-bonding between a proton donor (i.e., hydroxyl or amino group) and a proton acceptor (i.e., cabonyl or an imino group), and undergoes a radiative enol (E)−keto (K) tautomerization through a four-step E-E*-K*-K process, upon photoexcitation (Scheme 1).[4]

Scheme 1. ESIPT Principle



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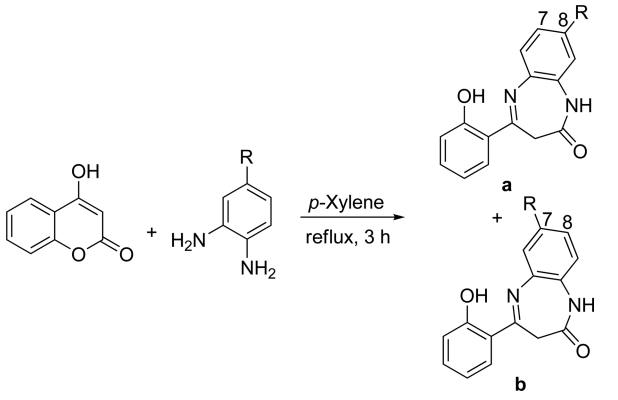
The significant structural difference between the excited species (E*) and the emissive species (K*) gives rise to large Stokes' shifts, which advantageously decreases the spectral overlap between the dye's absorption and emission peaks.[5] Different dyes emitting in the aggregate or solid-state using ESIPT have been reported, among others, hydroxyphenyl benzothiazole, hydroxyphenyl imidazopyridine, or hydroxyphenyl benzazoles dyes.[6] Besides, in the course of studies on the biological properties and synthetic applications of 4-(2-hydroxyphenyl)-1,3-dihydro-1,5-benzodiazepin-2-ones, it has been observed that this class of compounds shows bright fluorescence in the visible region in particular in the solid state. Surprisingly, to the best of our knowledge, photophysical properties of such derivatives have never been thoroughly investigated yet.[7]

Reported herein are the photophysical properties of a novel class of luminescent materials based on the 2-hydroxyphenyl-1,5-benzodiazepin-2-one scaffold. Substituent effects on the fluorescence properties have been investigated in order to determine factors that govern the fluorescence emission, and more generally to understand the mechanism of photoluminescence. Finally, the 1,5-benzodiazepin-2-one platform has been used to design two fluorogenic probes for the selective detection of biothiols.

RESULTS AND DISCUSSION

A series of 4-phenyl-1,3-dihydro-1,5-benzodiazepin-2-ones variously substituted either on the aromatic rings or on the amide moiety were prepared. First, modifications on the fused aromatic ring system were achieved according to known procedures, i.e., by reacting 4-hydroxycoumarin with unsubstituted or 4-substituted 1,2-phenylenediamines in refluxing *p*-xylene for 3 h (Table 1).[8,9] Whereas these transformations worked well, we observed, however, the formation of two regioisomers in each case, the predominant product being substituted at position 8 of the benzodiazepin-2-one for Me, Br, Cl, F, and at position 7 for NO_2. For compounds **3**–**6**, the mixture of regioisomers was readily separated by column chromatography on silica gel (Experimental Section), however, the reaction carried out with 4-methyl-*o*-phenylenediamine gave an inseparable mixture of benzodiazepin-2-one regioisomers **2a**/**b** in a 75% yield and an isomeric ratio of 1.56:1, which was used as such.

Table 1. Modifications on the Fused Aromatic Ring System



entry	R	cmpd	yield (%)[a]
1	H	**1**	85[a]
2	Me	**2a/b**	75[b]
3	Br	**3a/b**	58/15 (73)[c]
4	Cl	**4a/b**	72/18 (90)[c]
5	F	**5a/b**	65/18 (83)[c]
6	NO_2	**6a/b**	8/35 (43)[c]

[a]Isolated yield. [b]Isolated as an inseparable mixture of regioisomers. [c]Combined yield.

Next, benzodiazepin-2-ones **7** and **8** with modifications on the phenyl ring have been prepared in 90% and 84% yield, respectively, from benzoylacetates (Scheme 2).[10,11] Finally, the *N*-methylation of the amide function of the derivative **1** afforded the analogue **9** in good 90% yield.

With these compounds in hand, their photophysical properties were determined, and summarized in Table 2.

First, our study was focused on the unfunctionalized 4-(2-hydroxyphenyl)-1,3-dihydro-1,5-benzodiazepin-2-one **1** in order to unambiguously determine the mechanism of photoluminescence of this family of molecules. In solution, the benzodiazepin-2-one displayed a fluorescence emission (Φ_F = 0.05, entry 1) only in aqueous media such as 0.1 M PBS pH 7.4, in which **1** gave a slightly cloudy solution. The benzodiazepin-2-one was more emissive in the solid state, exhibiting an encouraging quantum yield of 0.26 and a large Stokes shift (~175 nm), which is in the range of those generally observed with ESIPT molecules. The absorption and emission spectra of **1** were measured, as depicted in Figure 1, in various solvents ranging from apolar aprotic to polar protic in order to investigate the AIE hypothesis. In solvents in which **1** was well dispersed, such as EtOAc, THF, DCM, MeCN, or EtOH, no significant modifications of the absorbance profile of **1** were noticed, the positions and intensities of absorption bands being almost the same. In contrast, in water and PBS, a flattening of the absorption bands was observed, probably due to the fact that **1** forms aggregates in these solvents.

The fluorescence emission spectra of the benzodiazepin-2-one **1** were determined at 330 nm excitation (Figure 1b). The spectra obtained in all organic solvents showed no fluorescence emission, fluorescence was observed only in aqueous media, which is consistent with the AIE hypothesis. Next, the absorption and emission spectra of **1** were recorded in THF/water mixtures with various water fractions (from 1:99 to 99:1, v/v) in order to further confirm the AIE effect (Figure 2a,b). As the water fraction increased, the intensity of the absorption bands around 340 nm decreased, in particular for a THF/water solution having a ratio of 1:99, probably due to the formation of insoluble aggregates. Besides, no photoluminescence was observed upon an excitation at 330 nm in mixtures rich in THF, for which **1** is well dispersed. On the other hand, in THF/water mixtures with high water contents (i.e., >70%) the benzodiazepin-2-one **1** became markedly more emissive (Figure 2c), which is still in line with the AIE mechanism. The conformational rigidity of **1** imposed in the solid-state favors the formation of a radiative 6-membered ring H-bonding system responsible for the ESIPT effect. In contrast, in the solution state, intramolecular motions (such as rotations of the phenol ring of **1** around the C–C bond) are not restricted anymore in the excited-state, favoring, thus, a nonradiative de-excitation process.

Crystals for X-ray analysis were obtained by slow evaporation of a DMF solution of **1**. The crystal structure of **1** has been determined from single crystal X-ray diffraction. Interestingly, it appears that compound **1** establishes strong intra- and intermolecular O–H···N hydrogen bonds in the crystalline state at a

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Scheme 2. Modifications on the Phenyl Ring System or Amide Function

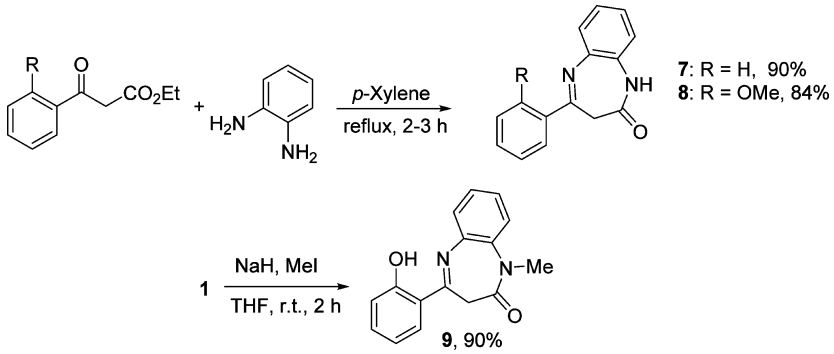

Table 2. Photophysical Data for Compounds 1–9

entry	cmpd	solution/solid	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Φ_F
1	**1**	CH_3CN	346	[a]	[a]	[b]
		EtOH	346	[a]	[a]	[b]
		PBS	352	529	177	0.05[c]
		solid	[d]	543	[d]	0.26[e]
2	**2a/b**	PBS	360	522	162	0.16[c]
		solid	[d]	538	[d]	0.30[e]
3	**3a**	PBS	360	531	171	0.06[c]
		solid	[d]	546	[d]	0.34[e]
4	**3b**	PBS	350	523	163	0.05[c]
		solid	[d]	535	[d]	~0.02[e]
5	**4a**	PBS	360	523	163	0.08[c]
		solid	[d]	520	[d]	0.14[e]
6	**4b**	PBS	370	531	161	0.06[c]
		solid	[d]	533	[d]	0.10[e]
7	**5a**	PBS	360	523	163	0.09[c]
		solid	[d]	527	[d]	0.18[e]
8	**5b**	PBS	347	536	189	0.04[c]
		solid	[d]	562	[d]	0.14[e]
9	**6a**	PBS	358	[a]	[a]	[b]
		solid	[d]	570	[d]	<0.01[e]
10	**6b**	PBS	370	[a]	[a]	[b]
		solid	[d]	655	[d]	~0.01[e]
11	**7**	PBS	310	[a]	[a]	[b]
		solid	[a]	[a]	[a]	[b]
12	**8**	PBS	307	[a]	[a]	[b]
		solid	[a]	[a]	[a]	[b]
13	**9**	PBS	335	[a]	[a]	[b]
		solid	[d]	540	[d]	0.35[e]

[a]Not applicable. [b]Not fluorescent. [c]Measurement determined at 25 °C by a relative method using quinine sulfate as standard (ΦF = 0.52 in 0.05 M H_2SO_4) with excitation at 350 nm.[20] [d]Not determined. [e]Measurement determined at 25 °C by integrating sphere.

distance of 1.82 and 2.10 Å, respectively (Figure 3a). The corresponding dimers are stacked, their cohesion being ensured by $\pi-\pi$ interactions along *a*-axis, and van der Waals interactions along *b*- and *c*-axes (Figure 3b). The small torsion angle (3.7°) between the phenol and imine-ring system (i.e., $C_7-C_8-C_9-O_1$) is in accordance with the ESIPT phenomenon. On the other hand, an important torsion angle (74.0°) is observed within the 7-membered ring system ($N_1-C_7-C_{14}-C_{15}$) presumably due to the sp^3 geometry of the C_3 carbon atom. This results in a deviation of the backbone of the molecule from the planar structure, giving rise to a herringbone arrangement through the establishment of intermolecular hydrogen bonding interactions.

Next, the effect of structural modifications of **1** located either on the phenyl moiety, the fused ring system, or the N–H amide function on the photoluminescence properties was studied by recording the absorption and emission spectra of a range of analogues **2–9** (Table 2). It should be stressed that, as compound **1**, all these compounds were nonemissive in acetonitrile or ethanol, i.e., in solution state. As expected, the hydroxyl group on the phenyl ring proved crucial since the analogue **7** did not show any fluorescence emission neither in PBS nor in the solid-state upon an excitation at 350 nm (entry 11). Similarly, the replacement of the acidic hydrogen atom of the hydroxyl moiety by a methyl group in compound **8** suppressed completely the photoluminescence both in solution and solid phase environments (entry 12). This result suggests that luminescence is solely produced by the ESIPT mechanism, an electronic push–pull (methoxy–imine) process being by itself insufficient. Besides, the introduction of an electron donating and lipophilic methyl group on the phenyl moiety of the fused ring system has a beneficial effect on quantum yields, particularly in PBS (0.16, entry 2), which may be ascribed to an increase in hydrophobicity that favors aggregation. On the other hand, halogen atoms cause a substantial decrease in the fluorescence intensity except for the 8-bromo substituted compound **3a** whose quantum yields are similar to that of compound **1** (0.06 in PBS and 34% in the solid-state, entry 3). Substitution at the position 7 of the fused ring system by a fluorine atom causes a 19 nm bathochromic shift in the emission wavelength maxima in the solid state (entry 8). In addition, the strong electron withdrawing nitro group leads to almost the vanishing of the photoluminescence, but a remarkable (112 nm) bathochromic shift of the emission spectra in the far-red region (655 nm, entry 10). *N*-Methylation of the amide function of the 7-membered ring system increases the fluorescence in the solid state (0.35, entry 13), and interestingly, turns off the photoluminescence in PBS. Although this latter result may seem intriguing in view of the moderate quantum yield observed in PBS for its isomeric analogues **2a/b**, it is nevertheless consistent with the significant role played by intermolecular hydrogen bonding interactions in the crystalline state (c.f., Figure 3) and consequently in the AIE behavior.[12] Indeed, as can be seen from the X-ray diffraction of compound **9** (Figure 4), the presence of a methyl group on the nitrogen atom eliminates the intermolecular hydrogen bonding interactions observed for compound **1**, the internal hydrogen bond being preserved, ensuring the occurrence of ESIPT. Consequently, collectively these data highlight the importance of

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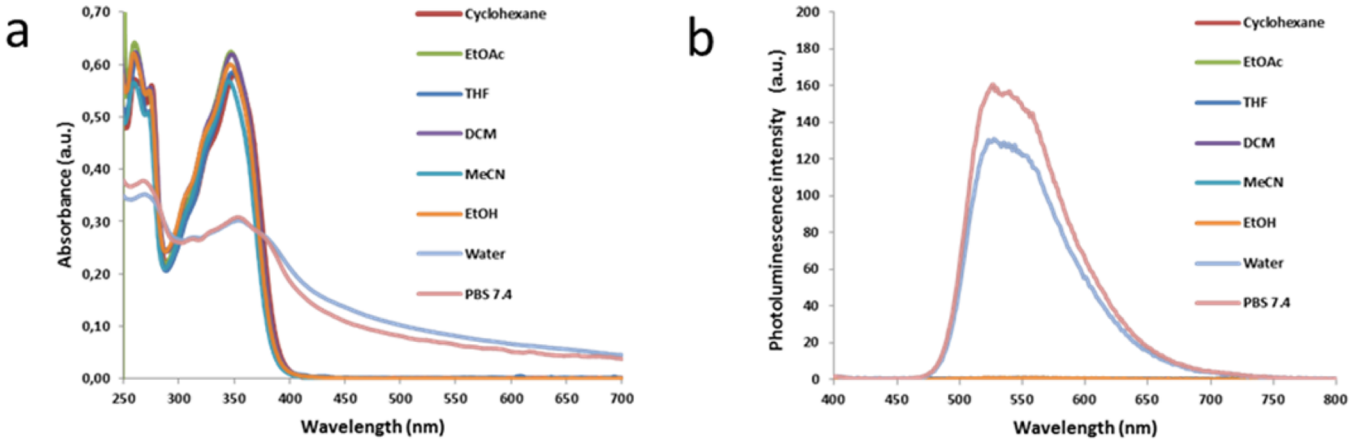

Figure 1. Absorption (a) and emission (b) spectra of **1** (c = 3.96, 10−5 mol/L) in various solvents at 25 °C upon an excitation at 330 nm.

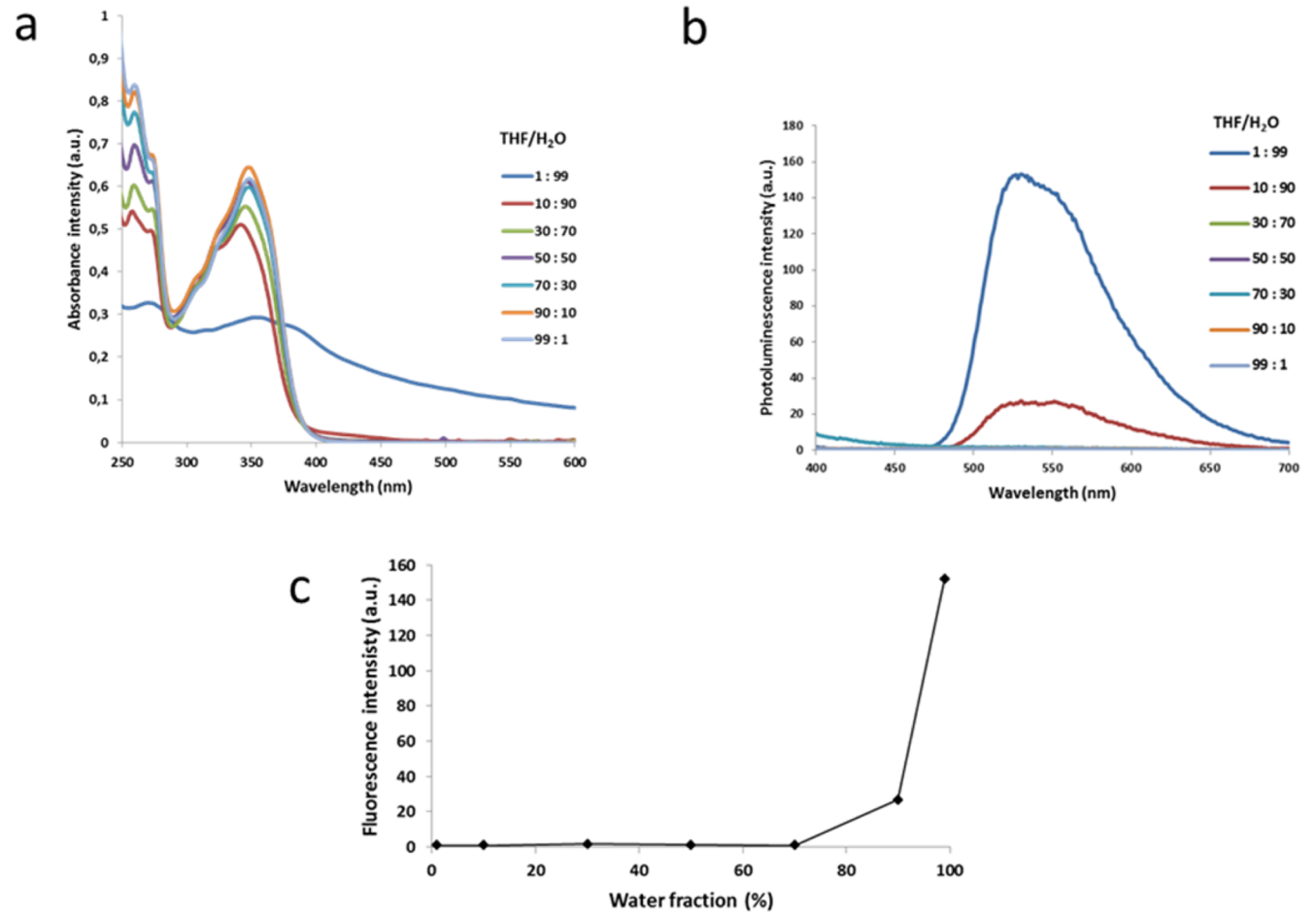

Figure 2. Absorption (a) and emission (b) spectra of **1** at 25 °C at various THF/water ratios. (c) Effect of water fraction on the fluorescence intensity of **1** determined at 529 nm upon an excitation at 330 nm at 25 °C.

strong intermolecular hydrogen bonding interactions to enable AIE behavior.

To examine the potential of benzodiazepin-2-one derivatives as effective precursors of profluorescent probes, the design of solid-state ESIPT fluorescent probes for the selective detection of biothiols directly from TLC plates was next investigated.[13] Due to the biological significance of thiols, the development of small organic fluorogenic probes for their rapid and specific detection in various aqueous matrices has become an intensive field of research in the few last years. To this end, several thiol-specific reactions have been used to design these probes including among others, reduction reactions, aromatic nucleophilic substitutions, Michael additions, and disulfide exchanges.[14] In this context, the 2,4-dinitrobenzenesulfonyl (DNBS) group proved to efficiently quench the fluorescence through a photoinduced electron transfer (PeT) process when attached to a fluorescent molecule.[15] Advantageously, this group is then easily removed in the presence of biothiols through an S_NAr process, restoring thus the fluorescence of the parent compound (Scheme 3).

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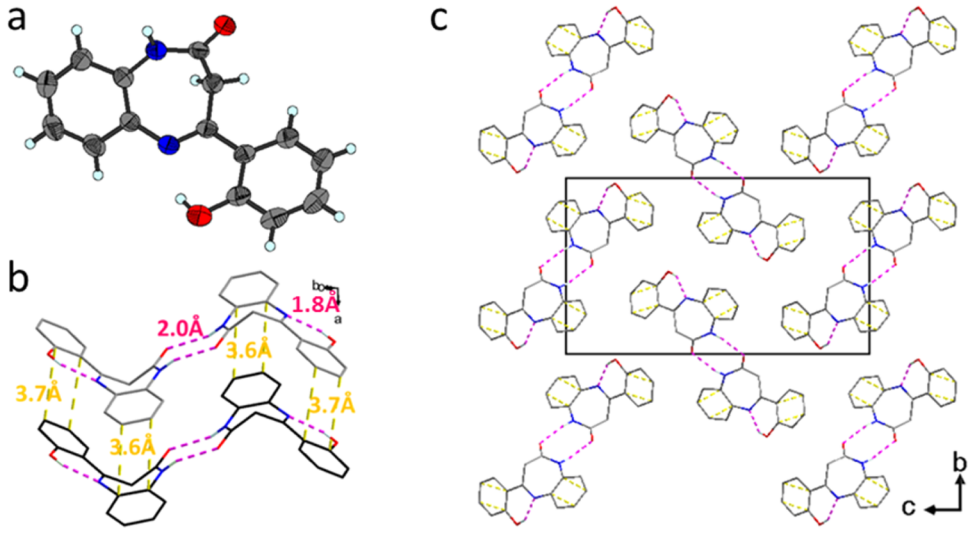


Figure 3. Crystal structure of compound **1**: (a) thermal ellipsoidal representation (50% of probability); (b) representation of dimers, the cohesion being ensured by hydrogen bond (dashed pink lines) and $\pi-\pi$ interactions (dashed yellow lines); (c) projection along *a*-axis, the cohesion being ensured by van der Waals interactions.

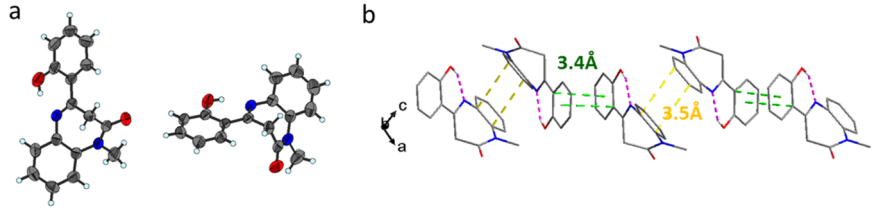
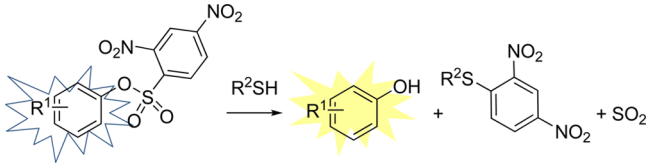


Figure 4. Crystal structure of compound 9: (a) thermal ellipsoidal representation (50% of probability) and (b) a packing view along *b*-axis.

Scheme 3. Principle of the DNBS-Based Fluorogenic Probes for Biothiols Detection

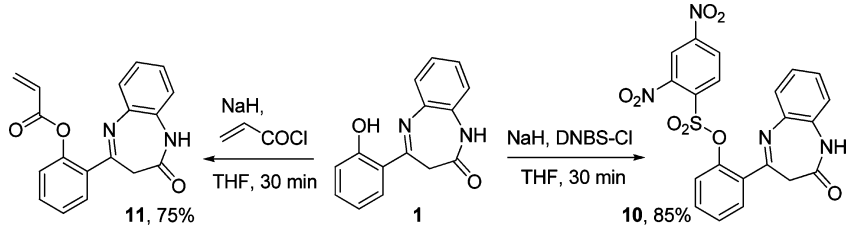


Then, the design of a solid-state fluorescent probe for the detection of total biothiols directly from TLC plates was first investigated by conjugating the green emissive compound **1** with 2,4-DNBS group (Scheme 4). The desired probe **10** was obtained in 85% yield. The regioselective *O*-sulfonylation of the transformation was confirmed by both ^{1}H NMR analysis that showed the complete disappearance of the proton peak at 14 ppm corresponding to the phenol group of **1**, and ^{1}H−^{13}C HMBC NMR experiment that unambiguously exhibited a correlation between the N−H proton and the C3 carbon atom (c.f., Supporting Information).

With the probe in hand, its ability to selectivity detect thiol-containing amino acids over other biologically relevant species directly on TLC plates was investigated by dropping a ∼10 mM solution of each corresponding amino acid (2 × 9 μL) in PBS 7.4 on the probe spots (5 μL of a ∼2 mM solution in THF). The eluted TLC (EtOAC/cyclohexane 1:1) was next visualized under visible and UV light (365 nm), and as one might expect, the fluorescence emission was observed in the presence of free thiol-containing biological species (i.e., cysteine, glutathione GSH, and bovine serum albumin (BSA)) only. In sharp contrast, no fluorescent spots were detected for all other *O*- and *N*-nucleophilic species tested, showing the compatibility of the DNBS approach with the 1,5-benzodiazepin-2-one scaffold (Figure 5a and b).

Following these encouraging results, the masking of the fluorophore with acrylate was also envisioned in order to specifically detect cysteine over other biological species

Scheme 4. Preparation of Probes 10 and 11 for Fluorescence Sensor Applications



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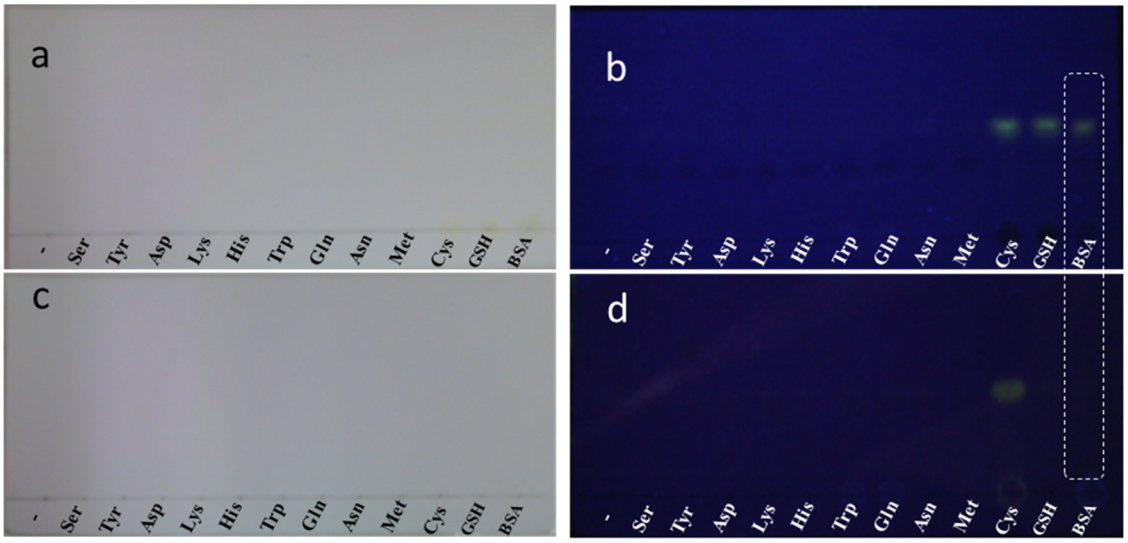

Figure 5. Photographs of TLC plates taken under visible light (a) or under 365 nm UV illumination (b) showing the detection of total biothiols in the presence of the probe **10**. Photographs of TLC plates taken under visible light (c) or under 365 nm UV illumination (d) showing the detection of cysteine in the presence of the probe **11**.

(including peptide thiols, such as GSH).[16] Indeed, while a thiol ether linkage is formed upon the conjugate addition of thiols to acrylates, the fluorescent molecule is released only upon the second step of intramolecular ester aminolysis. Accordingly, the acrylate-based probe **11** synthesized in 75% yield from **1** was subjected to a similar TLC experiment to that performed previously with the probe **10** (Figure 5c,d). The fluorescence emission was observed only in the presence of cysteine, GSH, and BSA producing no luminescent response, which is in accordance with the mechanism of action of such reported acrylate-based fluorogenic probes designed for the detection of cysteine. The approximate detection limit for probe **10** and **11** toward cysteine was determined to be in the range of 2−20 μM (c.f., Supporting Information). Interestingly, the combination of TLC results obtained with probes **10** and **11** would also provide valuable information regarding the presence (or not) of peptide thiols in the biological medium investigated. This was illustrated with BSA whose TLC results suggested the presence of peptide thiols, but not of free cysteine (dashed white rectangle, Figure 5b,d).[17]

■ CONCLUSION

We have reported the preparation of a family of 1,5-benzodiazepin-2-one analogues, whose promising photophysical properties were hitherto underexplored. Fluorescence spectroscopic results and X-ray diffraction analyses showed that their photoluminescence was mostly ensured by ESIPT in the aggregated and solid-state, favored by strong intermolecular interactions. Interestingly, it appeared that the dimeric intermolecular NH···O hydrogen bonding was not essential for the fluorescence in the solid-state.

■ EXPERIMENTAL SECTION

All solvents were dried following standard procedures: toluene and methylene chloride (DCM) were obtained from a solvent drying system. THF: distillation over Na°/benzophenone. Commercially available reagents were used without further purification, unless otherwise stated. All reactions involving air- and moisture-sensitive reagents were performed under argon using syringe-septum cap technique. Column chromatography purifications were performed on silica gel (40−63 μm). Thin-layer chromatography (TLC) analyses were carried out on F-254 aluminum sheets. The spots were visualized through illumination with a UV lamp (λ = 254 nm) and/or staining with $KMnO_4$. Phosphate buffered saline PBS (0.1 M, pH 7.4) was prepared using water purified with a Milli-Q system (purified to 18.2 MΩ.cm).

Instruments and Methods. IR spectra were recorded with a universal ATR sampling accessory. ^{1}H and ^{13}C NMR spectra (C13APT or C13CPD experiments) were recorded on a 300 MHz spectrometer. Chemical shifts are expressed in parts per million (ppm) from the residual nondeuterated solvent signal: DMSO-d_6 (δ_H = 2.50, δ_C = 39.52).[18] Multiplicities are described as s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), etc., t (triplet), dt (doublet of triplets), td (triplet of doublets), m (multiplet), and bs (broad singlet). Coupling constants, J values, are reported in Hz. High-resolution mass spectra (HRMS) were obtained using an orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer equipped with an electrospray source and in the positive and negative modes (ESI ±). Low-resolution mass spectra (LRMS) were obtained with a mass spectrometer equipped with an ESI source. UV−visible spectra were obtained using a rectangular quartz cell (Open Top, 10 × 10 mm, 3.5 mL). Regarding the solvent effect on the absorption and emission spectra of compound **1** (Figure 1), 30 μL of a stock solution (1 mg/mL) of **1** in DMSO was diluted in 2970 μL of the corresponding solvent, except for the cyclohexane that required, for miscibility reasons, the preparation of a stock solution (1 mg/mL) of **1** in THF. Solid-state fluorescence quantum yields were determined using an integrating sphere instrument according to a reported method.[19] Fluorescence spectroscopic studies in solution were performed with a semimicro quartz fluorescence cell (light patch base thickness: 10 × 4 mm, chamber volume: 1400 μL), with excitation and emission slit widths of 5 nm. Solvents for spectroscopy were spectroscopic grade. Fluorescence quantum yields in 0.1 M PBS, pH 7.4 were measured at 25 °C by a relative method using quinine sulfate (Φ_F = 52% in 0.05 M H_2SO_4)[20] as a standard. The following equation was used to determine the relative fluorescence quantum yield in solution:

$$\Phi_F(x) = (A_S/A_X)(F_X/F_S)(n_X/n_S)^2\Phi_F(s)$$

where A is the absorbance (in the range of 0.01−0.1 A.U.), F is the area under the emission curve, n is the refractive index of the solvents (at 25 °C) used in measurements, and the subscripts s and x represent standard and unknown, respectively. The following refractive index values were used: 1.333 for aq. H_2SO_4, and water, 1.337 for PBS.

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General Procedure for the Synthesis of Compounds 1, 2a/b−6a/b, 7. A solution of 4-hydroxycoumarin (1.62 g, 10 mmol) and the appropriate 1,2-phenylenediamine derivative (10 mmol, 1 equiv) in *p*-xylene (50 mL) or acetic acid/ethanol (40 mL, 1:1, v/v) was refluxed for 3 h. Then, the reaction mixture was cooled to RT, was filtered, and the precipitate was washed with methanol several times. Silica gel column chromatography of the precipitate was performed to separate the two regioisomers.

Compounds **1**[8] and **7**[11] were prepared according to an experimental procedure reported in the literature.

*Mixture of 4-(2-Hydroxyphenyl)-8-methyl-1,5-benzodiazepin-2-one and 4-(2-Hydroxyphenyl)-7-methyl-1,5-benzodiazepin-2-one **2a/b**.* It was isolated by chromatography on silica gel as a yellow solid and as an inseparable mixture of regioisomers of **2a** and **2b** in a 1.56:1 ratio (2 g, 75% yield).[8,9] mp = 299−301 °C. IR (ATR): ν_{max}: 3150 (NH), 3100 (OH) 1670 (CN), 1585 (CO). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.35 (s, 5 H), 3.59 (s, 3.2 H), 7.11−7.31(m, 6.6H), 7.42−7.64 (m, 3.3 H), 8.5−8.08 (m, 1.6H), 10.6 (s, 1H), 10.67 (s, 0.64H), 14.15 (s, 1H), 14.16 (s, 0.6H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 20.2, 20.6, 38.5, 117.6, 117.6, 118.1, 118.2, 118.9, 122, 125.5, 127.1, 127.3, 128.1, 128.8, 129.6, 129.6, 131, 133.7, 133.9, 133.9, 134, 136, 136.9, 161.4, 161.5, 162.3, 1162.8, 165.8 ppm.

*8-Bromo-4-(2-hydroxyphenyl)-1,5-benzodiazepin-2-one **3a** and 7-Bromo-4-(2-hydroxyphenyl)-1,5-benzodiazepin-2-one **3b**.* The two regioisomers (**3a**/**b**) were separated by chromatography on silica gel (EtOAc/cyclohexane, 1:1, v/v) to give the compound **3a** (1.91 g, 58% yield), which has already been characterized,[9] and **3b** (0.495 g, 15% yield), both as yellow solids (**3b** eluted first). Compound **3b**: mp = 279−281 °C. IR (ATR): ν_{max}: 3333 (NH), 3205 (OH) 1673 (CN), 1600 (CO). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.66 (s, 2H), 6.97−7.02 (m, 2H), 7.42−7.5 (m, 4H), 7.92 (d, J = 7.2 Hz, 1H), 10.80 (s, 1H), 13.77 (s, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 38.8, 117.6, 118.2, 119, 119, 124.2, 127.2, 129.4, 129.8, 132.5, 134.1, 135.6, 161.3, 163.5, 165.9, ppm. HRMS (ESI+): calcd. for $C_{15}H_{12}BrN_2O_2$ [M+H]$^+$ 331.0082; found 331.0088.

*8-Chloro-4-(2-hydroxyphenyl)-1,5-benzodiazepin-2-one **4a** and 7-Chloro-4-(2-hydroxyphenyl)-1,5-benzodiazepin-2-one **4b**.* The two regioisomers (**4a**/**b**) were separated by chromatography on silica gel (EtOAc/cyclohexane, 1:1, v/v) to give the compound **4a** (2.05 g, 72% yield), which has already been characterized,[8] and **4b** (0.51 g, 18% yield), both as yellow solids (**4b** eluted first). Compound **4b**: mp = 269−271 °C. IR (ATR): ν_{max}: 3105 (NH), 2916 (OH), 1682(CN), 1611(CO). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.67 (s, 2H), 6.97−7.02 (m, 2H), 7.28−7.33 (m, 2H), 7.44−7.52 (m, 2H), 7.91 (d, J = 7.8 Hz, 1 H), 10.81 (s, 1H), 13.78 (s, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 38.7, 117.7, 118.2, 119, 121.3, 124.4, 129.2, 130.8, 132.3, 134.1, 135.3, 161.3, 163.4, 165.9 ppm. HRMS (ESI+): calcd. for $C_{15}H_{12}ClN_2O_2$ [M+H]$^+$· 287.0587; found 287.0593.

*8-Fluoro-4-(2-hydroxyphenyl)-1,5-benzodiazepin-2-one **5a** and 7-Fluoro-4-(2-hydroxyphenyl)-1,5-benzodiazepin-2-one **5b**.* The two regioisomers (**5a**/**b**) were separated by chromatography on silica gel (EtOAc/cyclohexane 1:1, v/v) to give the compound **5a** (1.75 g, 65% yield) and **5b** (0.54 g, 18% yield), which has already been characterized,[8] both as yellow solids (**5b** eluted first). Compound **5b**: mp = 289−281 °C. IR (ATR): ν_{max}: 3160 (NH), 3100 (OH) 1686 (CN), 1591 (CO).^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.66 (s, 2H), 6.97−7.06 (m, 3H), 7.12−7.19 (td, J = 8.1 Hz, 2.7 Hz, 1 H), 7.43−7.56(m, 2H), 7.91 (dd, J = 8.7 Hz, 1.5 Hz, 1 H), 10.85 (s, 1H), 13.90 (s, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 38.6, 107.8, 108.2, 111.8, 112.1, 117.6, 118.2, 119, 129.5, 139.6, 129.7, 132.4, 132.5, 133.2, 133.2, 133.9, 158.4, 161.3, 161.7, 162.7, 165.8 ppm. ^{19}F NMR (282 MHz, DMSO-d_6): δ= −114.70 ppm. HRMS (ESI+): calcd. for $C_{15}H_{12}FN_2O_2$ [M+H]$^+$ 271.0873; found 271.0883.

*4-(2-Hydroxyphenyl)-8-nitro-1,5-benzodiazepin-2-one **6a** and 4-(2-Hydroxyphenyl)-7-nitro-1,5-benzodiazepin-2-one **6b**.* The two regioisomers (**6a**/**b**) were separated by chromatography on silica gel (EtOAc/cyclohexane, 1:1, v/v) to give the title compounds **6b** (1.03g, 35% yield), which has already been characterized,[9] and **6a** (0,230 g, 8% yield) as yellow solids (**6b** eluted first). Compound **6a**: mp = 301−303 °C. IR (ATR): $\nu_{max:}$ 3105 (NH), 2969(OH), 1675(CN), 1620(CO). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.76 (s, 2 H), 7.03 (m, 2H), 7.41−7.53 (m, 2H), 7.94−7.96 (m, 1H), 8.17−8.20 (m, 1H), 8.34(s, 1H), 11.25 (s, 1H), 13.32 (s, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 39.3, 117.7, 118.2, 119.2, 121.5, 122.9, 123.3, 130.1, 134.5, 136.2, 136.8, 143.1, 161.1, 16.9, 165.9 ppm. HRMS (ESI+): calcd. for $C_{15}H_{12}N_3O_4$ [M+H]$^+$·298.0826; found 298.0824.

*4-(2-Methoxyphenyl)-1,5-benzodiazepin-2-one **8**.* To a stirred boiling solution of benzene-1,2-diamine (1.08 g, 0.1 mol) in *p*-xylene (10 mL), methyl 3-(2-methoxyphenyl)-3-oxopropanoate[21] was added (2.49 g, 0.12 mol) dropwise and refluxed for 2 h. The reaction mixture was left to stand at room temperature for 24 h. The precipitated solid was collected by filtration and recrystallized from ethanol to give the desired product (2.23 g, 84% yield) as white solid. mp = 198−200 °C. IR (ATR): ν_{max}: 3190(NH), 1680 (CN), 1611 (CO). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.39 (s, 2H), 3.39 (s, 3H), 6.99 (td, J = 7.5 Hz, 0.9 Hz, 1 H), 7.18(m, 4H), 7.33 (m, 1H), 7.43 (m, 1H), 10.38 (s, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 43.3, 55.5, 111.9, 120.3, 121.4, 123.8, 126.1, 127.9, 128.7, 129.9, 130.2, 131.7, 139.0, 157.6, 160.9, 167.2 ppm. HRMS (ESI+): calcd. for $C_{16}H_{15}N_2O_2$ [M+H]$^+$· 267.1134; found 267.1126.

*4-(2-Hydroxyphenyl)-1-methyl-1,5-benzodiazepin-2-one **9**.* To a solution of 4-(2′-hydroxypheny1)-1,5-benzodiazepin-2-one **1** (1.01 g, 4 mmol) in anhydrous THF (50 mL) at 0 °C, was added NaH (60% in mineral oil, (0.176 g, 4.4 mmol, 1.1 equiv). The mixture was stirred for 10 to 15 min before the addition of methyl iodide (0.498 mL, 8 mmol, 2 equiv). The reaction mixture was maintained at room temperature for 2 h, before removing the solvent under reduced pressure. The crude material was purified by flash column chromatography on silica gel (cyclohexane/EtOAc from 100:0 to 80:20) and the desired product was obtained (0.95 g, 90% yield) as yellow solid. mp = 172−174 °C. IR (ATR): ν_{max}: 3100 (OH), 1661 (CN), 1593 (CO).^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.05 (d, J = 12 Hz, 1H), 3.32(s, 3H), 4.3(d, J = 12 Hz, 1H), 6.96−7.02(m, 2H), 7.33−7.49 (m, 4H), 7.56−7.59 (dd, J = 8.4 Hz, 1.5 Hz, 1H),7.92−7.95 (dd, J = 8.1 Hz, 1.5 Hz, 1H), 13.94 (s, 1H) ppm, ^{13}C NMR (75 MHz, DMSO-d_6): δ = 34.8, 37.9, 117.6, 117.8, 118.6, 119, 122.5, 125.3, 126.6, 127.2, 129.9, 134, 135.7, 137.7, 161.3, 164.8, 165.3. HRMS (ESI+): calcd. for $C_{16}H_{15}N_2O_2$ [M+H]$^+$·267.1134; found 267.1125.

*4-(Phenyl (2-(2,4-dinitrobenzenesulfonate))-1,5-benzodiazepin-2-one **10**.* To a solution of 4-(2′-hydroxypheny1)-1,5-benzodiazepin-2-one **1** (0.25g, 1 mmol) in anhydrous THF (20 mL) at 0 °C, was added NaH (60% in mineral oil, 0.044 g, 1.1 mmol, 1.1 equiv). The reaction mixture was stirred for 10 to 15 min before the addition of 2,4-dinitrobenzenesulfonyl chloride (0.292 g, 1.1 mmol, 1.1 equiv). The reaction mixture was maintained at 0 °C for 30 min, and THF is then removed under pressure, and purified by flash column chromatography on silica gel (cyclohexane/EtOAc from 100:0 to 80:20) to obtained the titled compound (0.41 g, 85% yield) as white solid. mp = 199−201 °C IR (ATR): ν_{max}: 2975(NH), 1672(CN), 1613(CO). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.92 (d, J = 12 Hz, 1H), 3.36(s, 2H), 7.11−7.31(m, 5H), 7.58−7.76 (m, 3H), 8.22 (d, J = 9 Hz, 1H), 8.48−8.52 (dd, J = 9 Hz, 3 Hz, 1H), 8.93(d, J = 3 Hz), 10.54 (s, 1H) ppm, ^{13}C NMR (75 MHz, DMSO-d_6): δ = 43.4, 120.9, 121.9, 123.5, 123.8, 126.7, 127.5, 127.6, 128.4, 129.6, 130.6, 131.9, 131.9, 132.9, 133, 138.5, 145.9, 147.6, 150.8, 156.4, 165.9. HRMS (ESI-): calcd. for HRMS (ESI-): calcd. for $C_{21}H_{14}N_4O_8SCl$ [M+Cl]$^-$ 517.0243; found 517.0229.

*4-(2-(Buta-1,3-dien-2-yloxy)phenyl)-1,5-benzodiazepin-2-one **11**.* To a solution of 4-(2′-hydroxypheny1)-1,5-benzodiazepin-2-one **1** (0.25g, 1 mmol) in anhydrous THF (20 mL) at 0 °C, was added NaH (60% in mineral oil, 0.044 g, 1.1 mmol, 1.1 equiv). The reaction mixture was stirred for 10 to 15 min before the addition of acryloyl chloride (0.09 mL, 1.1 mmol, 1.1 equiv). The mixture was stirred for 30 min, and THF was then removed under pressure, and purified by flash column chromatography on silica gel (cyclohexane/EtOAc from 100:0 to 80:20) to obtained the titled compound (0.228 g, 75% yield) as white solid. mp = 195−197 °C. IR (ATR): ν_{max}: 3050(NH), 1736 (CO), 1670(CN), 1614(CO). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.39(s, 2H), 6.22(dd, J = 12 Hz, J = 3 Hz, 1H), 6.45(dd, J = 15 Hz, J = 9 Hz, 1H), 6.55(dd, J = 15 Hz, J = 3 Hz, 1H), 7.44 (d, J = 9 Hz, 1H),

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7.45−7.99(m, 2H), 8.09−8.12(m, 2*H*), 8.17(d, *J* = 3 Hz, 1H), 10.08(s, 1H) ppm, ^{13}C NMR (75 MHz, DMSO-d_6): δ= 34.9, 42.2, 118.5, 119.3, 119.6, 126.8, 127.8, 131.8, 134.1, 134.9, 135, 137.3, 144.6, 145.1, 145.2, 159.8, 162.6, 165.7, 167.1 ppm. HRMS (ESI+): calcd. for $C_{18}H_{15}N_2O_3$ $[M+H]^+$ 307.1083; found 307.1075.

Procedure for the Detection of Biothiols on TLC Plates. Five microliters of a ∼2 mM solution of the corresponding fluorogenic probe (**10** or **11**) in THF was dropped on the TLC plate. Then, 9 μL (2 times at interval of 10 min) of a ∼10 mM solution of the corresponding amino acid (except BSA whose concentration was 50 μM) in PBS 7.4 was dropped on the probe spot. After 10 min, the spot was dried under air flow, and the TLC plate subsequently eluted in EtOAC/cyclohexane (1:1), and then visualized under visible and UV light at a wavelength of 365 nm.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00627.

^{1}H and ^{13}C NMR spectra and photophysical data for all new compounds (PDF)

X-ray crystallographic data for compound **1** (CIF)

X-ray crystallographic data for compound **9** (CIF)

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Notes

The authors declare no competing financial interest.

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