*SQU Journal for Science*, 2018, 23(2), 85-94 DOI: http://dx.doi.org/10.24200/squjs.vol23iss2pp85-94
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# Synthesis of a Flavone Based Fluorescent Probe Bearing a Nitroolefin Moiety for Selective Detection of Cysteine

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**ABSTRACT:** In this paper, we report the synthesis, characterization and investigation of the optical properties of a new fluorescent probe, 4′-nitroolefinylflavone (4′-NOF) **1**, based on a flavone skeleton and bearing a nitroolefin moiety. Upon the addition of cysteine, a remarkable fluorescence enhancement (200 fold) was observed for probe **1** accompanied with a slight blue shift from 457 nm to 459 nm. The prepared probe displays high selectivity and sensitivity towards cysteine over other amino acids. The Michael addition of cysteine to probe **1** was confirmed by $^{1}$H-NMR spectroscopy. The detection limit of probe **1** towards cysteine was found to be 1.63 μM, a lower concentration than the normal human cysteine level, 30-200 μM.

**Keywords:** Fluorescent probe; Cysteine; Organic synthesis; Nitroolefin; UV/Vis absorption.

**التحضير الكيميائي لكاشف ضوئي فلافوني يحتوي على مجموعة نيتروأولوفين للكشف الإنتقائي للحمض الأميني سيستين**

**صالح البوصافي، نادية الهمامي، فخر الدين سليمان و بينا فيرجاس**

**الملخص:** إن تصميم كاشف ضوئي للتحليل الإنتقائي للحمض الأميني سيستين مع وجود احماض أمينية أخرى أصبح مجالا بحثيا بارزا. في هذا البحث تم تحضير كاشف ضوئي فلافوني جديد (4′-NOF) يحتوي على مجموعة النيترو اوليفين ودراسة خصائصه الضوئية. لقد وجد أن مع إضافة حمض السيستين للكاشف يزداد الإنبعاث الفسفوري 200 مرة مع انحراف ازرق خفيف من 457 نانومتر الى 459 نانو متر. أظهر الكاشف الجديد قدرة انتقائية ممتازه لحمض السيستين بوجود غيره من الأحماض الأمينية. تم التأكد من ارتباط السيستين بالكاشف الضوئي عن طريق جهاز الرنين المغناطيسي. يستطيع هذا المركب الكشف عن حمض السيستين عند تركيز قليل جدا يصل الى 1.63 mM وهو أقل من التركيز الطبيعي للسيستين في الجسم (30-200 mM).

**الكلمات المفتاحية:** كاشف ضوئي، سيستين، التحضير العضوي، نيتروأولوفين، امتصاص فوق البنفسجي.

## 1. Introduction

Cysteine (Cys) is an essential sulphur amino acid in living cells and plays various important roles in physiological processes [1]. In this regard, Cys acts as an intramolecular linker in protein and enzyme tertiary structures *via* the formation of disulfide bridges, acts as an antioxidant against damage from the reactive oxygen species [2], participates in post-translational modification [3], and works as redox sensor in cell signalling pathways [4]. It is also associated with metal binding [5], detoxification [6] and metabolism [7]. Numerous investigations have linked deficiency of Cys to many diseases such as liver damage, hair depigmentation, slow growth in children and skin lesions [8]. High levels of Cys can cause neurotoxicity, and cardiovascular and Alzheimer's diseases [9-11]. In order to verify some of these diseases and to understand the involvement of Cys in biological environments, an extensive amount of research has been directed towards finding a selective analytical technique to detect and measure trace amounts of it in bio-fluid samples under physiological conditions. Historically, methods like gas chromatography-mass spectrometry [12], HPLC [13], UV-visible analysis [14], and electrochemical assay [15] have been utilized for this purpose. Nevertheless, these analytical methods suffer from serious drawbacks such as complicated instrumentation, tedious sample preparation procedures, high analysis cost, low sensitivity and poor selectivity.

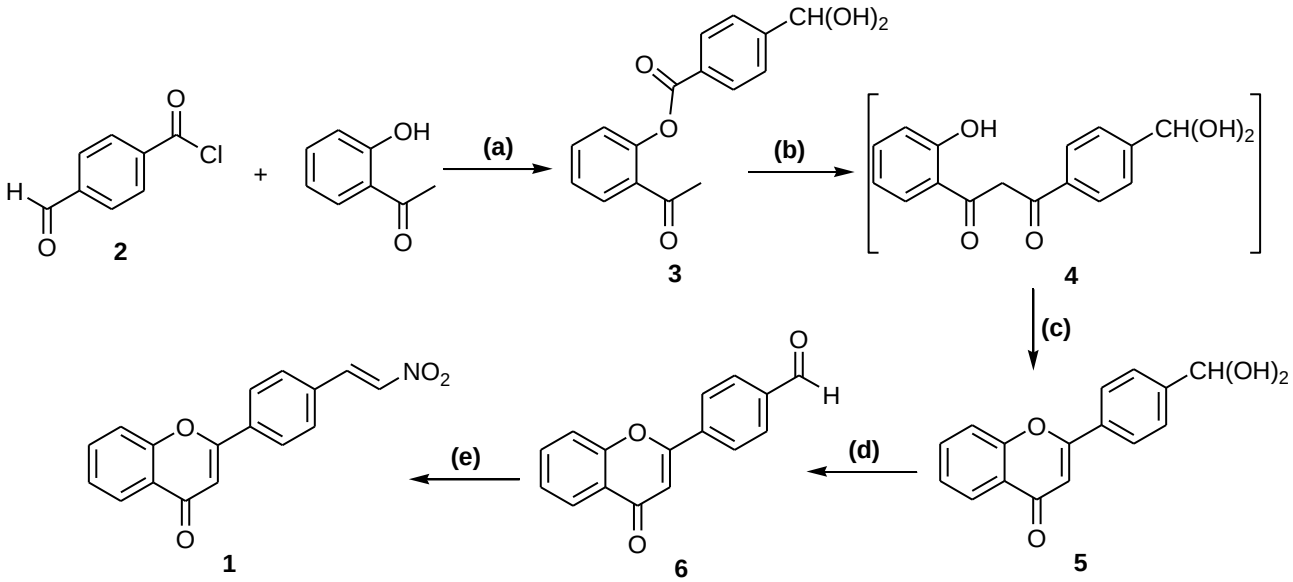
Recently, an analytical tool based on an organic fluorescent molecule that can selectively interact with Cys and induce a fluorescence signal has become widely accepted as an efficient analytical method due to its high sensitivity, selectivity, low cost and versatility [16]. So far, a number of varieties of fluorescence-based sensors for Cys and other bio-thiols have been developed [17].

The action of these probes is centered on various sensing mechanisms such as metal complex-displace coordination [18], Michael addition [19], formation of thiazolidine [20], cleavage of the sulfonate ester [21] and formation of iminium ions [22]. Fluorescent probes that work on 1,4-addition of Cys to a Michael acceptor have attracted great attention because of their high reactivity and sensitivity towards thiols [23]. The high reactivity of these probes towards Cys is driven by the strong nucleophilicity of the –SH group when it reacts with an electron-poor Michael acceptor fragment. Their fluorescent sensitivity is attributed to the inhibition of the intramolecular charge transfer mechanism (ICT) that occurs in the chromophore fragment after breaking the double bond that linked the signaling unit to the Michael acceptor [24]. Various α,β-unsaturated Michael acceptors have been exploited in designing effective fluorescent probes including ketone [25], nitrile [26], ester [27], nitroolefine [28] and pyrazolone [29]. The nitro group, with its powerful electron-withdrawing ability, has proven to be an effective Michael acceptor moiety to enhance the conjugate addition of cysteine to a fluorescent probe [30]. In addition, different molecular frameworks have been used as fluorescence reporters, including acrylate [31], naphthalimide [32], pyrene [33], fluorescein [34], coumarin [35] and cyanine [36].

Flavonoids are widespread plant products responsible for the beautiful colors of leafs and flowers [37]. Most flavonoid compounds exhibit fluorescence if they are excited with visible or UV light due to the presence of rigid, flat rings with delocalized π electrons [38]. Some of these compounds have been used as nutrition supplements because of their health benefits, such as antioxidant [39], UV-B protection [40], and anticancer [41] properties. In analytical chemistry, flavonol (3-hydroxyflavone) has been extensively used to detect various metal ions in aqueous samples as its fluorescence is vastly boosted by coordinating with metal ions [42]. Recently, synthetic diethylamino substituted flavonol has been utilized as a turn-on fluorescent probe to analyze thiols [43]. In this paper, we present a fluorescent turn-on probe for monitoring levels of cysteine in biomedical samples. The probe contains a nitroolefin side-chain that quenches the fluorescence of the flavonoid backbone of the probe by photoinduced electron transfer (PET). However, thiol molecules react with the probe by breaking the double bond of the nitroolefin and as a result, the fluorescence intensity of the probe is significantly enhanced.

## 2. Experimental

In this work, 4′-nitroolefinylflavone (4′-NOF) **1** was synthesized based on a flavone skeleton and equipped with a nitroolefin group as a Michael acceptor to selectively detect cysteine. The synthesis of probe **1** is presented in Scheme 1.




**Scheme 1**. Synthesis of probe **1**, (a) Pyridine, r.t., 25 min., aqueous HCl, yields 73%. (b) Pyridine, KOH, 50 $^0$C, 15 min. (c) AcOH, $H_2SO_4$, reflux, 1 h, yield 56.5%. (d) $H_2O$, AcOH, $H_2SO_4$, reflux, 3 h, yield 74%. (e) Nitromethane, ammonium acetate, AcOH, reflux, 5 h, yield 46%.

### 2.1 Reagents and apparatus

All the reagents and solvents used in this study were obtained from Sigma-Aldrich Chemical Company and used without further purification. The purity of the synthesized compounds was checked by TLC and analyses were carried out on 0.25 mm thick pre-coated silica plates (DC-Fertigplatten ADAMANT $UV_{254}$). Plates were visualized by a UVGL-58 Handheld 254/365 nm UV lamp. Column chromatography was performed using silica gel 60 Å (63–200 µm). Melting points were determined using GallenKamp-MPD350.BM2.5 melting point apparatus (UK). A rotary

evaporator was used to evaporate solvents. IR spectra were recorded on Agilent Technologies carry 630 FTIR (Malaysia). $^{1}$H NMR spectra were recorded using a 700 MHz Bruker Avance spectrometer. Chemical shifts (δ) for proton resonances were expressed in parts per million (ppm) relative to TMS as internal standard and coupling constants (*J*) in Hz. The following abbreviations were used: s, singlet; d, doublet; m, multiple and dd: doublet of doublet. $^{13}$CNMR spectra were recorded using a 700 MHz Bruker Avance spectrometer. Chemical shifts (δ) for carbon resonances were expressed in parts per million (ppm). Mass spectra were obtained using MALDI-TOF analysis which was performed using two matrices, namely HCCA and a graphite in ultraflex TOF/TOF instrument. A Shimadzu (model multispec-1501) UV–Vis spectrophotometer (Shimadzu, Japan) and a Perkin-Elmer (model LS 55) Fluorescence spectrometer (Perkin-Elmer, U.K) were used to collect absorption and fluorescence spectra, respectively.

### 2.2 Preparation of probe 1

#### 2.2.1 Synthesis of 2-acetylphenyl(4-dihydroxymethyl)benzoate 3

A mixture of 2'-hydroxyacetophenone (2.72 g, 20.0 mmol) and 4-formylbenzoyl chloride **2** (3.36, 20.0 mmol) in pyridine (5.0 mL) was stirred at room temperature for 25 min. After cooling, the reaction mixture was poured into a mixture of 60 mL aqueous HCl (3%) and 30 g crushed ice. The product was extracted with dichloromethane (3×20 mL) and the organic layers were combined and dried over sodium sulfate, filtered and concentrated under a vacuum to give a reddish brown crude liquid which was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (8:2) for elution to give compound **3** as a white powder (4.13 g, 73%). mp: 81.1-82.0 °C. IR (Neat) $\upsilon_{max}$ in cm$^{-1}$: 3032 (C-H), 1601 and 1415 (C=C), 1723 (C=O, ester), 1684 (C=O, ketone), 1281 and 1242 (C-O); $^{1}$H NMR (700 MHz, $CDCl_3$) δ in ppm: 2.17 (s, 2H), 2.55 (s, 3H), 6.77 (s, 1H), 7.24 (m, 1H), 7.40 (m, 1H), 7.6 (m, 1H), 7.74 (d, 2H, *J*=8.1 Hz), 7.89 (m, 1H), 8.26 (d, 2H, *J*=8.1 Hz); $^{13}$C NMR (176.0 MHz, $CDCl_3$) δ in ppm: 29.62 ($CH_3$), 70.84 (CH), 124.04 (CH), 126.50 (CH), 126.69 (CHx2), 130.78 (CH), 130.83 (-C), 130.98 (CHx2), 131.03 (C), 133.70 (CH), 145.37 (C), 149.26 (C), 164.47 (-C=O), 197.58 (C=O). MALDI-TOF *m/z*: calcd for $C_{16}H_{14}O_5$ 286.28; found 286.29.

#### 2.2.2 Synthesis of 4´-dihydroxymethylflavone 5

A mixture of 2-acetylphenyl(4-dihydroxymethyl)benzoate **3** (4.0 g, 14.0 mmol) and pyridine (8 mL) in a 50 ml RBF was warmed in a hot water bath at 50 °C until compound **3** dissolved completely. Finely powdered potassium hydroxide (0.6 g, 10.7 mmol) was added and the reaction mixture was stirred for 15 minutes at 50 °C. After cooling, the mixture was poured into a 100 mL beaker containing aqueous acetic acid solution (10%, 10 mL). The yellow solid intermediate **4** was collected using Buchner filtration, and then dissolved in a mixture of acetic acid (8 mL) and concentrated sulfuric acid (0.25 mL). The reaction mixture was heated under reflux for 1 hour. The cooled mixture was poured into ice and stirred with a glass rod. The solid product was collected by suction filtration, washed with water and re-crystallized from ethanol to give compound **5** as yellow crystals (2.26 g, 56.5%). Mp: 130.7-131.54 °C. IR (Neat) $\upsilon_{max}$ in cm$^{-1}$: 3039 (C-H), 1642 (C=O), 1462 (C=C), 1280 and 1242 (C-O); $^{1}$H NMR (700 MHz, $CDCl_3$) δ in ppm: 6.76 ( s, 1H), 6.81 ( s, 1H), 7.41 ( m, 1H), 7.55 ( m, 1H), 7.69 ( m, 1H), 7.72 (d, 2H, *J*= 8.4 Hz ), 7.94 (d, 2H, *J*=8.4 ), 8.21 (dd, 1H, *J*=1.3 , J= 8.0 Hz ); $^{13}$C NMR (176.0 MHz, $CDCl_3$) δ in ppm: 70.69 (CH), 108.29 (CH), 118.18 (CH), 123.99 (C), 125.52 (C), 126.81 (CH), 126.82 (CHx2), 126.98 (CH), 133.28 (C-H), 134.08 (CHx2), 143.31 (C), 156.26 (C), 162.16 (C), 178.33 (C). MALDI-TOF *m/z*: calcd for $C_{16}H_{12}O_4$ 268.26; found 268.19.

#### 2.2.3 Synthesis of 4´-formylflavone 6

4´-Dihydroxymethylflavone **5** (2.04 g, 7.60 mmol) was dissolved in a mixture of acetic acid (86 ml), water (30.4 ml) and sulfuric acid (6.08 ml). The mixture was refluxed for 3 h and the reaction was monitored using TLC. The cooled mixture was poured into ice/water, filtered and dried under a vacuum. The crude product was recrystallized from ethanol to give compound **6** as an orange powder (1.62 g, 74%). Mp: 172.4-172.5 °C. IR (Neat) $\upsilon_{max}$ in cm$^{-1}$: 3072 (C-H), 1698 (C=O: aldehyde), 1630 (C=O: ketone), 1460 (C=C), 1282 (C-O); $^{1}$H NMR (700 MHz, $CDCl_3$) δ in ppm: 6.71 (s, 1H), 7.43 ( m, 1H), 7.58 (dd, 1H, *J*=8 Hz, *J*=0.5 Hz), 7.72 ( m, 1H ), 8.01 ( d, 2H, *J*=7.0 Hz), 8.08 (2H, d, *J*=7.0 Hz), 8.21(dd, 1H, *J*=8.0 Hz, *J*=1.6 Hz), 10.09 (s, 1H); $^{13}$C NMR (176.0 MHz, $CDCl_3$) δ in ppm: 109.19 (CH), 118.24 (CH), 124.42 (C), 125.69 (CH), 125.88 (CH), 126.96 (CHx2), 130.23 (CHx2), 134.29 (CH), 137.15 (C), 138.17 (C), 156.30 (C), 161.76 (C), 178.32 (C), 191.40 (C). MALDI-TOF *m/z*: calcd for $C_{16}H_{10}O_3$ 250.25; found 250.21.

#### 2.2.4 Synthesis of 4´-nitroolefinylflavone (4´-NOF) 1

A mixture of 4´-formylflavone **6** (0.5 g, 2.00 mmol), nitromethane (0.24 g, 4 mmol), ammonium acetate (0.37 g, 4.8 mmol) and acetic acid (8 mL) was heated under reflux at 100 °C for 5 hours. After cooling, the reaction mixture was poured into 100 mL of water and the product was extracted by ethyl acetate (3×30 ml), dried over $Na_2SO_4$, filtered, and evaporated under reduced pressure. The resulting solid was recrystallized from ethanol to give compound **1** as an orange powder (0.23 g, 46%). Mp: 231.5-234.8 °C. IR (Neat) $\upsilon_{max}$ in cm$^{-1}$: 3106.41 (C-H), 1570.08 (C=C), 1628.26 (C=O), 1499.3, 1331.6 (N-O); $^{1}$H NMR (700 MHz, DMSO) δ in ppm: 8.3 (d, 1H, *J*=13 Hz), 8.19 (d, 2H, J=7 Hz), 8.16 (s, 1H), 8.02 (d, 2H, *J*=8 Hz), 7.84 (m, 1H), 7.80 (d, 1H, *J* = 8 Hz), 7.50 (m, 1H), 7.16 (s, 1H). $^{13}$C NMR

(176.0 MHz, $CDCl_3$) δ in ppm: 191.5 (C=O), 177.18 (C), 161.5 (C), 155.7 (C), 139.44(CH), 137.91 (C), 136.29(CH), 134.49 (CH), 130.34 (CH), 129.92 (2CH), 127 (2CH), 125.70 (C), 124.8 (CH), 123.4 (CH), 118.64 (CH). MALDI-TOF *m/z*: calcd for $C_{17}H_{11}NO_4$ 293.27; found 293.16.

### 2.2.5 UV-visible and fluorescence spectra measurements

Stock solutions (8 mmol/L) of amino acids including cysteine (Cys), alanine (Ala), phenylalanine (Phe), serine (Ser), tyrosine (Tyr), aspartic acid (Asp), arginine (Arg), histidine (His) and lysine (Lys), glutathione (GSH) and N-acetylcysteine in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (10 mmol/L, pH 7.5) were prepared. A stock solution of probe **1** (5 mmol/L) was also prepared in acetonitrile (ACN). For UV-vis spectra measurements, 10 μL of probe stock solution was mixed with 30 μL of each amino acid, diluting the mixture to 5 mL with HEPES/ ACN (3:2, *v/v*). For fluorescence spectrum measurement, (1 mmol/L) of working stock solution of probe was prepared by placing 1 ml of the probe stock solution into a 5 ml volumetric flask. All fluorescence measurements were done using a 330 nm excitation wavelength with the excitation and emission slit widths of 10 nm. Fluorescence spectra were measured 15 min after the addition of the analyte. The fluorescence titration experiments were performed using a probe **1** concentration of 0.5 mM in $CH_3CN$-HEPES (10 mmol/L, pH=7.5, 2:3, v/v) with Cys concentration range of 0−1.2 mmol/L at room temperature.

### 2.2.6 Detection limit

The detection limit was calculated based on the fluorescence titration. In the titration experiment, (0.5 mmol/L) probe gave a good linear relationship between the fluorescence intensity and the Cys concentration from 0 to1 mmol/L ($R^2$= 0.9985). The detection limit was then calculated with the equation: detection limit = 3σ/m, where σ is the standard deviation of blank measurements and m is the slope between intensity versus cysteine concentration. The emission intensity of **5** without Cys was measured ten times and the standard deviation of blank measurements was determined.

## 3. Results and Discussion

### 3.1 Selectivity and competition studies

### 3.1.1 UV-visible absorption spectra

First, the UV-visible absorptions of probe **1** in the absence and presence of L-cysteine (Cys) and non-thiol amino acids (L-alanine (Ala), L-phenylalanine (Phe), L-serine (Ser), L-tyrosine (Tyr), L-aspartic acid (Asp), L-arginine (Arg), L-histidine (His) and L-lysine (Lys) in 10 mmol/L HEPES buffer/$CH_3CN$ (4:1, V:V) were measured. As shown in Figure 1, probe **1** displays a maximum absorption at 340 nm, while in the presence of Cys the absorption maximum was blue-shifted to 300 nm with obvious enhancement in the absorption intensity. On the other hand, non-thiol amino acids showed almost no effect in the absorption of the probe at 340 nm. In addition, amino acids with highly polar side-chains (Tyr, Asp, Arg, His and Lys) enhanced the absorption intensity of probe **1** at 266 nm. Such effects can be attributed to the hydrogen bonds and dipole-dipole forces between the probe and these acidic and basic amino acids.

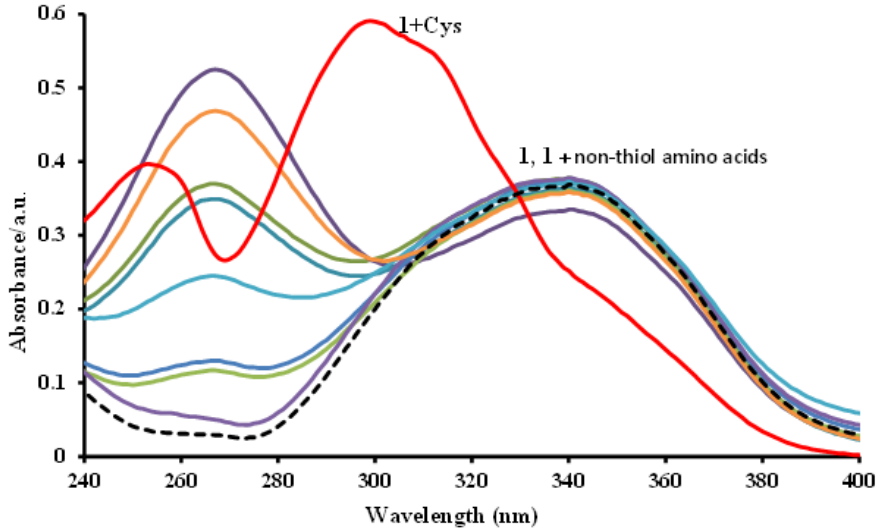



**Figure 1.** UV/Vis absorption spectra of **1** (0.01 mmol/L) (dashed line) in $CH_3CN$-HEPES (10 mmol/L, pH 7.5) in the presence of 0.05 mmol/L analytes including: Ala, Phe, Ser, Cys, Tyr, Asp, Arg, His and Lys. The spectra were acquired 60 min after Cys addition.

Under the same conditions, the UV-visible absorptions of **1** when added to other thiol compounds such as N-acetyl-L-cysteine and glutathione (GSH) were examined (Figure 2). N-Acetyl-L-cysteine showed absorption maxima at 311 nm with lower intensity compared to P**1**-Cys, while GSH caused a maximum absorption at 259 nm with a shoulder at 320 nm.

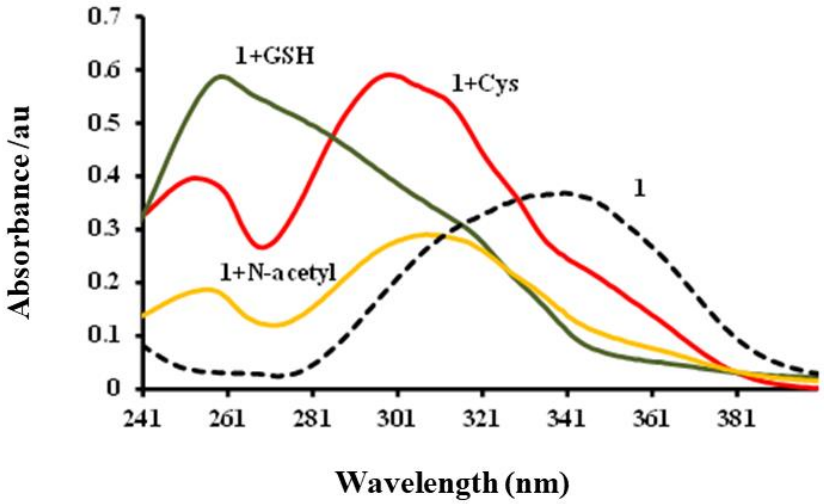



**Figure 2.** UV/Vis absorption spectra of **1** (0.01 mmol/L) in $CH_3CN$-HEPES (10 mM, pH 7.5) in the presence of 0.05 mmol/L analytes including GSH, Cys and N-acetyl-L-cysteine.

### 3.1.2 The Fluorescence spectra

Next, we explored the sensing response of probe **1** (0.01 mmol/L) towards Cys and other non-thiol amino acids by fluorescence spectroscopy. As displayed in Fig. 3, probe **1** alone in 10 mmol/L HEPES buffer/$CH_3CN$ (4:1, V/V) at pH 7.5 exhibits weak fluorescence at 457 nm. The feeble fluorescence of 1 is related to the presence of a nitroolefin moiety that acts as an electron acceptor for the photoinduced electron transfer process. The presence of non-thiol amino acids (Ala, Phe, Ser, Tyr, Asp, Arg, His and Lys) induced a negligible fluorescence enhancement. However, in the presence of Cys there was a significant boost in the fluorescence intensity of 200 fold observed at 459 nm with a slight blue shift of 2 nm. These results indicate that the presence of non-thiol amino acids has no effect on the ability of probe **1** to detect Cys. We can say that probe **1** is an excellent fluorescent sensor to detect Cys in biomedical samples without interference from other amino acids.

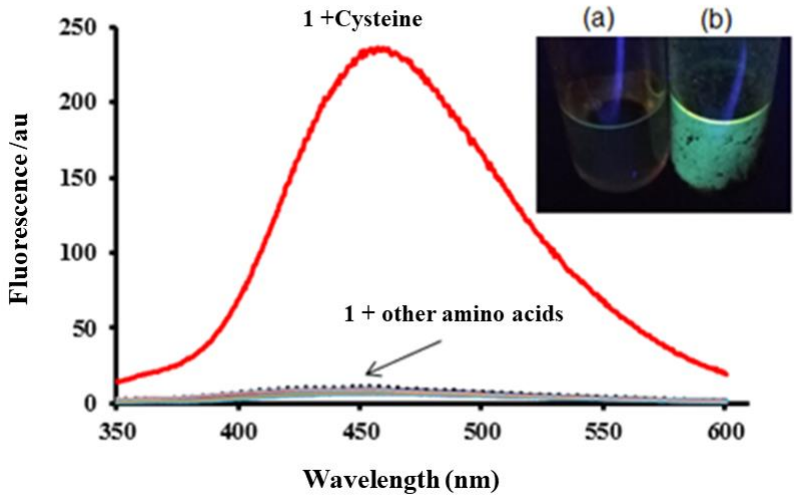



**Figure 3.** Fluorescence spectra of **1** (0.01 mmol/L) with various analytes (0.05 mmol/L) including: Ala, Phe, Ser, Cys, Tyr, Asp, Arg, His and Lys in $CH_3CN$-HEPES (10 mmol/L, pH 7.5) ($\lambda_{ex}$ = 470 nm, slit: 10 nm/10 nm). Inset photo is of **1** in the absence (a) and presence (b) of Cys.

Furthermore, under the same conditions, we investigated the fluorescence behavior of probe **1** in response to other thiol compounds such as GSH and N-acetyl-L-cysteine. As shown in Figure 4, both GSH and N-acetyl-L-cysteine caused almost no fluorescence enhancement when mixed with probe **1,** which means there is no interference from thiol compounds with Cys detection.

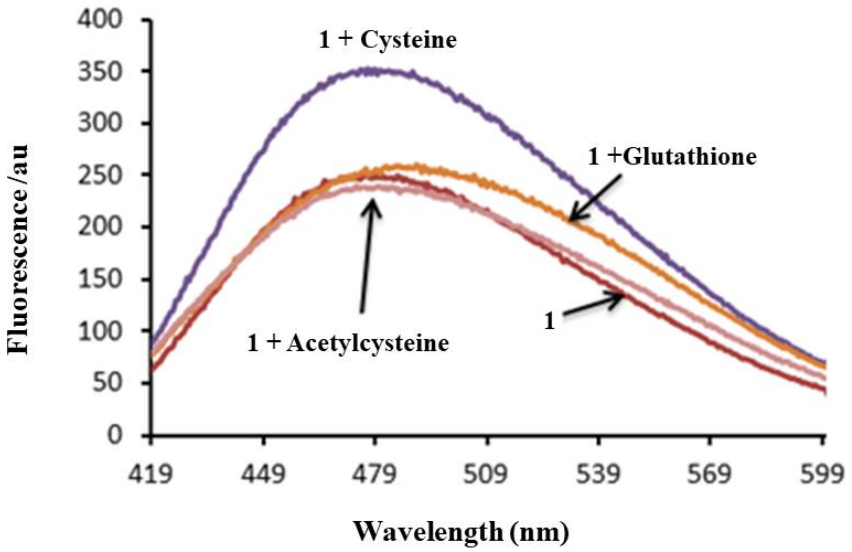



**Figure 4.** Fluorescence spectra of **1** (0.1 mmol/L) and (0.5 mmol/L) of each thiol in $CH_3CN$-HEPES (10 mmol/L, pH 7.5, 2:3 v:v), $\lambda_{ex}$ = 470 nm.

### 3.2 The pH effect

The fluorescence of probe **1** towards Cys was also explored in the pH range from 3.0 to 8.0 (Figure 5). When Cys (0.08 mmol/L) was added to probe **1** (0.01 mmol/L), the fluorescence was almost constant in the pH range from 3.0 to 7.0, but increased rapidly when the pH reached 7.5 and then decreased back at pH 8.0. Interestingly, there was a slight blue shift in the maximum wavelengths at lower pH values, which may be attributed to the protonation of the oxygen atom of the carbonyl group leading to ring cleaving. These results support the application of this fluorescent probe in physiological conditions.

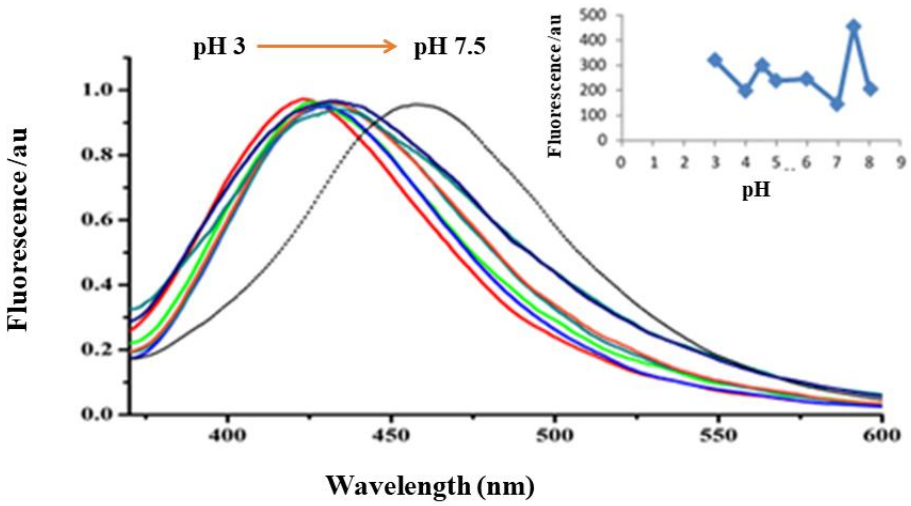



**Figure 5.** Effect of pH on the fluorescence intensity of **1** (0.01 mmol/L) in the presence of Cys (0.08 mmol/L) in $CH_3CN$-HEPES (10 mmol/L, 4:1*v/v*) upon exciting at 330 nm. Ex slit was set as 5 nm. Em slit was set at 10 nm. Inset: the linear correlation of the reaction against different pH values (3-8).

### 3.3 Sensitivity studies

A series of concentration-dependent fluorescence experiments was performed in order to assess the detection sensitivity of probe **1** at pH 7.5 in 10 mmol/L HEPES buffer/ $CH_3CN$ (3:2, v/v) towards Cys. As displayed in Figure 6, when the concentration of Cys was allowed to increase from 0 to 1.2 mmol/L, the fluorescence intensity of the solution of probe **1** (0.5 mmol/L) gradually increased. In the titration experiment, probe **1** gave a linear relationship between the fluorescence intensity and Cys concentration (Figure 7), from which the detection limit for Cys was calculated to be 1.63 μmol/L, which is lower than the normal human intracellular cysteine concentration (30-200 μmol/L. This result further proved that probe **1** is highly sensitive to Cys.

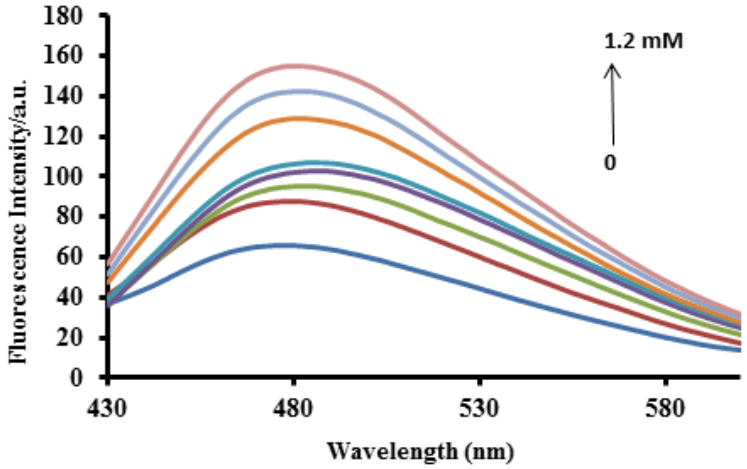



**Figure 6.** Fluorescence spectra of **1** (0.5 mmol/L) in $CH_3CN$-HEPES (10 mmol/L, pH=7.5, 2:3, v/v) with Cys (0−1.2 mmol/L). Excitation at 330 nm. Ex slit and Em slit were both set at 10 nm.

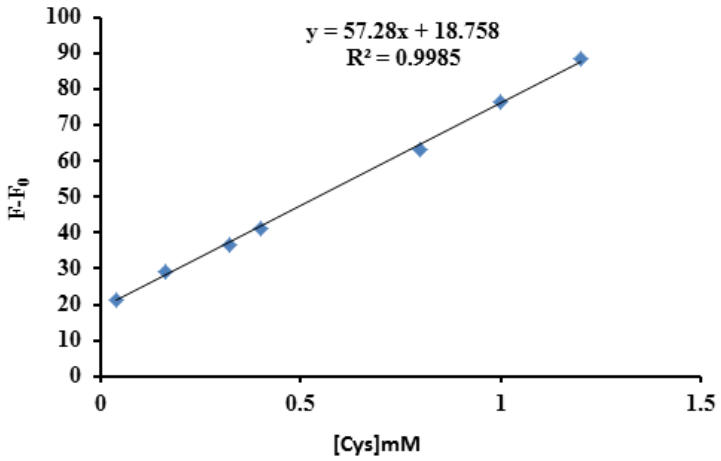
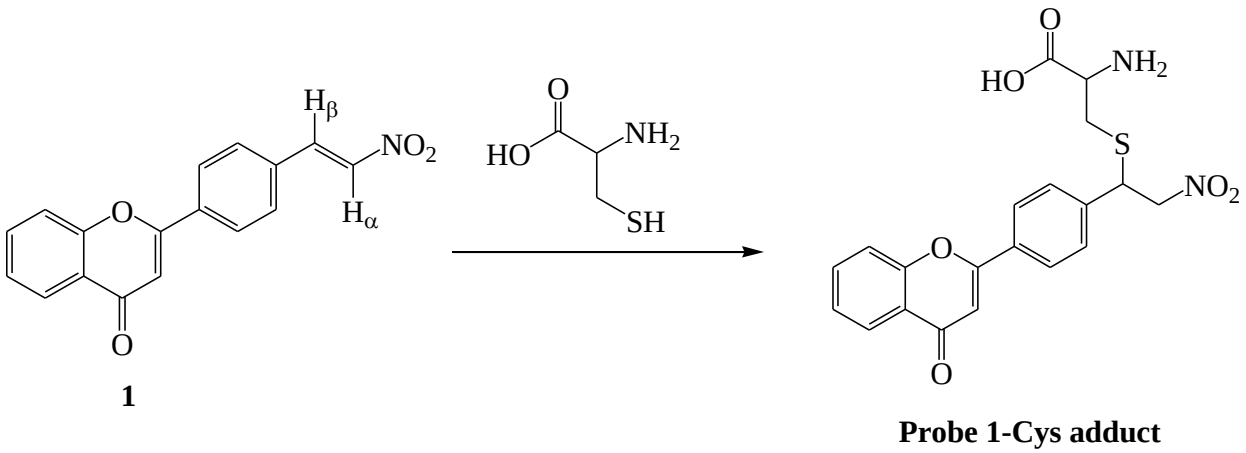



**Figure 7.** A plot of fluorescence intensity changes of 5 at 480 nm against concentration of Cys from 0 to 1.2 mM.

### 3.4 Reaction Mechanism and $^1$HNMR study

The reaction of Cys with probe **1** is a typical Michael addition reaction in which the –SH group in Cys acts as a Michael donor and the nitroolefin group in probe **1** acts as a Michael acceptor. The nucleophilic –SH group connects to the β-carbon atom of the α,β-unsaturated nitro moiety in probe **1** since it is the most electrophilic site due to the electron withdrawing effect of the nitro group (Scheme 2).




**Scheme 2.** Proposed sensing mechanism of probe **1** towards Cys.

This mode of addition can be confirmed by $^{1}$H-NMR. Figure 8 displays $^{1}$H-NMR spectra of probe **1** (Spectrum A) and of probe **1**-Cys adduct (Spectrum B). The most deshielded proton in probe **1** is H-β , which appears as a doublet at 8.33 ppm and couples with H-α , which is located at 8.17 ppm (Fig. 8, spectrum A). With the addition of 10 equiv Cys to a solution of probe **1** in DMSO-d6/$D_2O$ (2:1, V/V), the signals of H-α and H-β protons have disappeared (Figure 8, spectrum B). In addition, signals of other protons in rings A, B, and C have changed their positions.

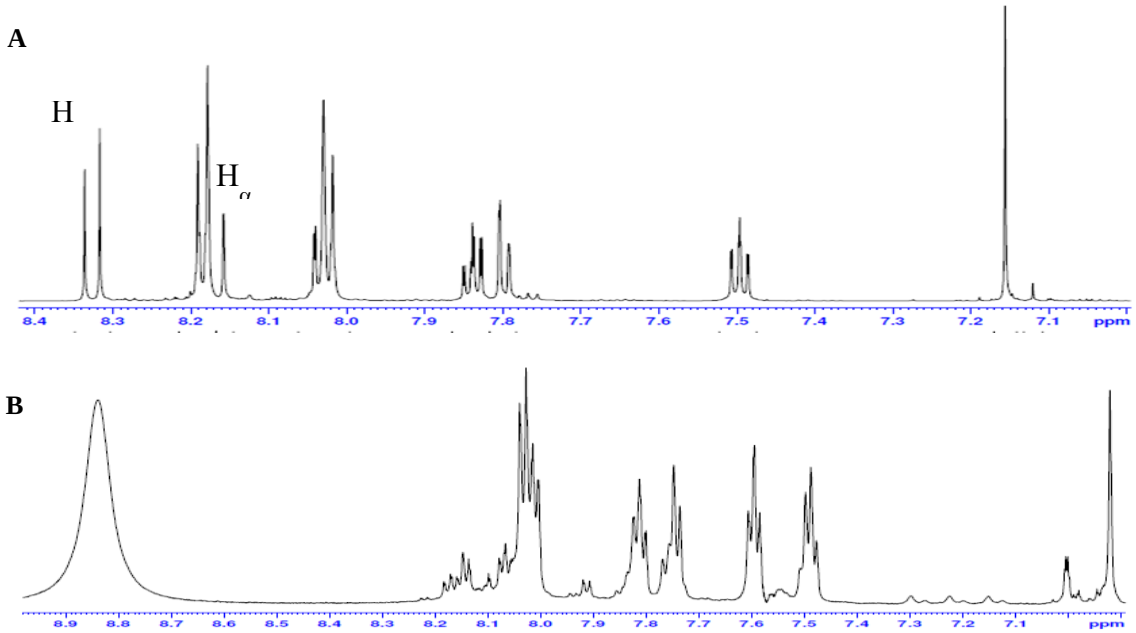



**Figure 8.** $^{1}$H-NMR spectra of probe **1** (A) and probe **1**-Cys adduct (B).

## 4. Conclusion

A blue-emitting fluorescent probe based on flavone structure was successfully synthesized for selective detection of Cys in the presence of thiol and non-thiol amino acids. The sensing mechanism of the probe was designed *via* Michael addition of Cys to a α, β-unsaturated nitro moiety. An increase in the fluorescence intensity accompanied with a bathochromic shift was observed when Cys was added to a solution of the probe. The proposed reaction mechanism between Cys and probe **1** was verified by $^{1}$H NMR spectroscopy. Finally, the prepared probe has demonstrated its ability to detect cysteine over other amino acids with a detection limit of 1.63 μmol $L^{-1}$ under the physiological environment.

## Acknowledgment

We acknowledge, with thanks, financial support from the Sultan Qaboos University and Ministry of Education.

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Received 3 July 2017
Accepted 21 February 2018