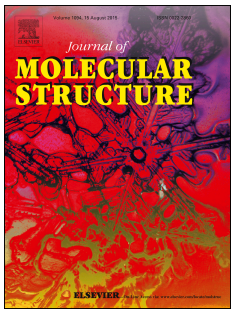

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Nidhi Nehra, Rahul Kaushik, Ghule Vikas D, Ram Kumar Tittal

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Credit Author Statement

Nidhi Nehra: Validation, Formal analysis, Investigation **Rahul Kaushik:** Formal analysis, Data Curation, Visualization **Ghule Vikas D.:** Software, Data Curation **Ram Kumar Tittal:** Conceptualization, Methodology, Resources, Data Curation, Visualization, Writing - Original Draft, Writing - Review & Editing, Supervision

Simpler Molecular Structure as Selective & Sensitive ESIPT-based Fluorescent Probe for Cysteine and Homocysteine Detection with DFT Studies

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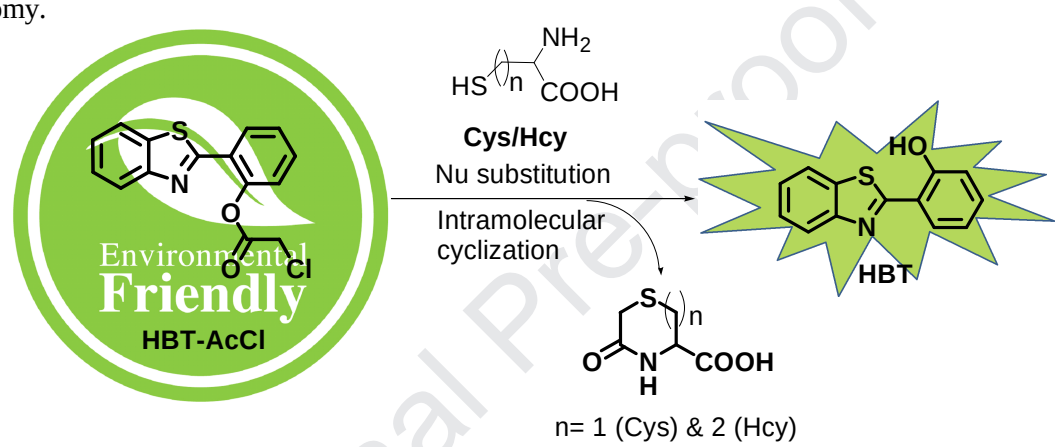
A new simpler, sensitive, selective turn-on fluorescent probe chloro-acetic acid 2-benzothiazol-2-yl-phenyl ester (HBT-AcCl) was developed from the negative research results of some other scheme. The probe selectively detected Cys & Hcy from other amino acids. The presented probe considered as a green probe that conserves complexity, reduces use of extra chloroacetate and has improved %atom economy.



Simpler Molecular Structure as Selective & Sensitive ESIPT-based Fluorescent Probe for Cysteine and Homocysteine Detection with DFT Studies

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Abstract: The undesired research results of triazole synthesis were successfully utilized as a simpler, sensitive and selective turn-on fluorescent probe **2** namely chloro-acetic acid 2-benzothiazol-2-yl-phenyl ester (HBT-AcCl) which was synthesized by simple chloroacylation of 2-hydroxyphenyl benzothiazole (HBT). Only one simple chloroacetate, a trigger unit as the reaction site of probe **2** makes it more simple, sensitive and selective for Cys and Hcy over other Amino Acids. The presented probe HBT-AcCl conserves simple structure, reduces the use of extra chloroacetate and improves %atom economy as compare to similar reported probes. Paper strip tests were carried out to explore the potential application for naked-eye detection of Cys under UV lamp. The selectivity of the HBT-AcCl probe for Cys and Hcy is supported by the results of absorbance and emission spectroscopy experiments and theoretical DFT calculations.

Keywords: HBT-AcCl Probe; Cysteine; Homo-Cysteine; Fluorescent probe; DFT calculations.

Introduction

The bio-thiols such as Cysteine (Cys), Homocysteine (Hcy), and Glutathione (GSH) play a vital role in the regulation of various physiological processes in biological systems.[1-3] Their most important role observed in cellular functions and reversible redox reactions.[4-6] The variation in the oxidation state of the sulfur made these bio-thiols important for biological applications such as antioxidant activity, detoxification, apoptosis, and signal transduction.[7] The proper physiological functioning of any living body requires an optimum level of bio-thiols. Any abnormal change in the concentration bio-thiols especially Cys and Hcy may cause severe damage or loss in the normal metabolism and physiological functioning of biological systems.[8-10] Alcoholic cirrhosis, neurological disorders, cardiovascular disorders, diabetes mellitus, and stroke are some of the common damages.[11-13] The most reduced bio-thiols Cys is an active

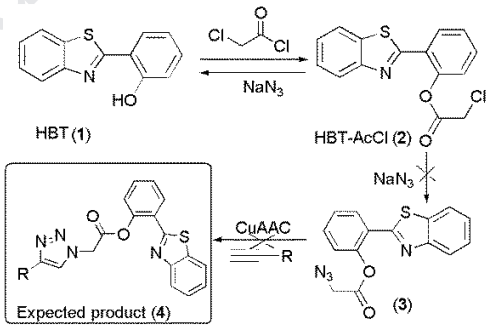
site in many proteins and their motifs. Therefore, the concentration of Cys should be high enough to support the normal functioning of various biological processes. Low levels of Cys may cause slower growth, liver damage, skin lesions, depigmentation of hairs, loss of muscle/fat, edema, lethargy, and weakness.[7, 14, 15] The level of Hcy is controlled by the trans-sulfuration and methionine conserving pathway in living systems. Its overproduction results in cardiovascular disease, dementia, neural tube infection, and Alzheimer's disease.[16-22] Therefore, the determination of Cys and Hcy concentration is very important for the diagnosis of the causative disease in living systems during biochemical investigations.[23]

During the last decade, various techniques were reported for the detection of bio-thiols and other amino acids, such as potentiometry, electrochemical analysis, high-performance liquid chromatography (HPLC), capillary electrophoresis, and fluorescent spectroscopy.[24-29] These techniques possess one or other drawbacks such as complicated sample preparation, complex instrumentation and non-suitable conditions for biological systems. The UV-Vis absorption and fluorescence spectroscopy are more reliable techniques for the detection of amino acids containing thiols such as Cys, Hcy, and GSH.[30-35] The fluorescent probes are sensitive, simple, fast action, cheap, non-intrusive, and biocompatible to the system.[1, 36-40] However, various probes still show some shortcomings such as poor efficiency, complicated structure, complicated synthetic procedures or sometimes even require the addition of cetyl trimethyl ammonium buffer (CTAB) to the test samples to boost the signal changes.[41-45] Therefore, modification of existing probes or development of a more advanced, sensitive and simple method for Cys and Hcy detection from other amino acids always remain thrust area in analytical chemistry. Simpler dyes such as 2-hydroxyphenyl benzothiazole (HBT) showed excited-state intramolecular proton transfer (ESIPT) mechanism and were used as fluorescent probes.

Recently, some fluorescent probes were reported to selectively detect the Cys, Hcy, and GSH among various amino acids in the solution media based on nucleophilic substitution followed by cyclization reaction mechanism.[1, 36, 46-48] The designing of any fluorescent probe require a fluorophores/chromophores unit like 2-(2-hydroxyphenyl) quinazolin-4(3H)-one (HPQ),[49, 50] Benzothiazole methyl quinolone (MBQ),[1] coumarin,[32, 51-54] benzothiazole,[55, 56] naphthalimide,[57] cyanine,[58, 59] other complex structure fluorophores,[35, 60-66] and a trigger unit like acrylate,[67-71] chloroacetate,[36] bromoacetate,[36] chloropropionate,[36, 47] bromopropionate,[47] etc. It was observed that the probes reported so far either constitute

complex structures of fluorophore units or trigger units or both. There is unnecessary wastage of atom economy, complexity in structures, not minimizing the excess and high diversity of probe are some of the important shortcomings in the existing probes that disobey the principles of green chemistry as proposed by Paul Anastas.[72, 73] Therefore, it is worthwhile to design and synthesize some more simpler fluorescent probe.

While working on the synthesis of biologically significant 1,2,3-triazole-HBT linked hybrid molecules containing some pre-existing biologically important motifs as shown in **Scheme 1**. We have easily synthesized chloro-acetic acid 2-benzothiazol-2-yl-phenyl ester (HBT-AcCl) **2** by simple chloroacylation of 2-benzothiazol-2-yl-phenol (HBT) **1** by chloroacylchloride using weak and safer base K_2CO_3. The structure of the new molecule (HBT-AcCl) **2** was successfully characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and ESI MS data. However, the corresponding azide (HBT-AcN_3) **3** was not formed and only HBT was recovered from the column chromatography of the reaction product. It revealed that the chloroacyl unit might have cleaved off from HBT-AcCl **2** to set HBT free. Although our efforts to synthesize HBT-AcN_3 were unsuccessful, close observation of physical changes during the progress of reactions revealed that HBT gave a fluorescent appearance in solution media via a well-known ESIPT mechanism. The literature survey on such compounds and their derivatives revealed that similar molecules were used as fluorescent probes for the detection of Cys, Hcy or GSH over other amino acids.



Scheme 1. A proposed plan for synthesizing 1,2,3-triazole-HBT linked hybrid molecules.

Experimental section

Materials and Instrument

Salicylaldehyde was supplied by Loba Chemie Pvt. Ltd., 2-amino thiophenol and solvents of analytical grade were purchased from Avra Chemicals Pvt. Ltd. India. Amino Acid kit was

purchased from SD Fine chemicals. The ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Advanced 400 MHz spectrophotometer. ESI-mass spectrometry was performed on a WATERS TOF-Q. Thermo Fischer Scientific Evolution instrument was used for absorbance studies and Agilent spectrophotometer was used for fluorescence studies.

Synthesis of 2-hydroxyphenyl benzothiazole (HBT) 1.

The HBT as shown in **Scheme 2** was synthesized using a reported method with little modification.[74] For the synthesis of 2-hydroxyphenyl benzothiazole (HBT), 2-amino thiophenol (1.25 g, 10 mmol) and salicylaldehyde (1.22 mL, 10 mmol) were mixed in DMF (10 mL). Then, the iodine (50 mol%) was added. The resulting mixture was heated and stirred at 100˚C until the reaction was completed. After completion of the reaction as monitored by TLC, the reaction mixture was cooled to room temperature and a solution of sodium thiosulfate (10 mol%) was added dropwise into the reaction mixture until the color of iodine was disappeared. The precipitate obtained was filtrated and washed with water. After vacuum drying, the crude products were purified by silica gel (60-120 mesh) column chromatography using hexane: ethylacetate (9:1) to obtain the pure HBT as white crystalline solid in 75% Yield. Its melting point is 130-132˚C. ^{1}H NMR (DMSO-d_6, 400 MHz): 11.64 (s, 1H), 8.16 (m, 2H), 8.05 (d, *J* = 8.06 Hz, 1H), 7.53 (m, 1H), 7.42 (m, 2H), 7.08 (dd, *J* = 7.33 Hz, 1H), 7.01 (m, 1H). ESI-MS (positive mode, calculated 227.04, obtained 228.00 [$C_{13}H_9NOS+H^+$].

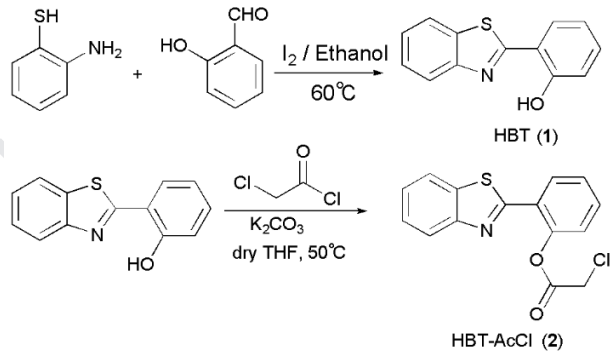
Synthesis of chloroacyl-2-hydroxy benzothiazole (HBT-AcCl) 2.

In a 50 mL round bottom flask, 2-(2-hydroxyphenyl)-benzo thiazole (227.14 mg, 1 mmol) was stirred for 30 minutes with anhydrous K_2CO_3 (691 mg, 5 mmol) in dry THF (20 mL). Then, chloroacetyl chloride (0.339 mL, 3 mmol) was added dropwise to the above mixture. The reaction mixture was allowed to react at room temperature for 30 minutes and then heated to 50˚C until the reaction was completed as monitored by TLC. After completion of the reaction in 12 hours, the solution was filtered to remove extra K_2CO_3. The filtrate was concentrated and dried by rotatory evaporator under reduced pressure wherein the solid product obtained was washed with dry hexane to give the pure off-white product in a 90% yield. The melting point of product **2** was 110-112˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.19-8.17 (dd, *J* = 7.89 Hz, 1H), 8.15-8.13 (d, *J* = 7.91 Hz, 1H), 8.07-8.05 (d, *J* = 8.12 Hz, 1H), 7.56-7.52 (t, *J* = 7.64 Hz, 1H), 7.46-7.39 (m, 2H), 7.13-7.11 (dd, *J* = 8.21Hz, 1H), 7.03-6.99 (t, *J* = 12 Hz, 1H), 4.27 (s, 2H). ^{13}C

NMR (DMSO-d_6, 101 MHz): 168.64, 166.50, 162.17, 152.70, 147.56, 134.59, 132.38, 130.15, 127.44, 126.84, 125.95, 124.11, 123.20, 122.25 and 41.62. ESI-MS (positive mode, calculated $[C_{15}H_{10}O_2NSCl]$ is 303.01 and obtained at 341.34 $[C_{15}H_{10}O_2NSCl+K]^+$.

Result and discussion:

Herein, we are presenting a new, simpler, sensitive and selective 2-hydroxyphenyl benzothiazole (HBT) **1** based fluorescent probe named chloro-acetic acid 2-benzothiazol-2-yl-phenyl ester (HBT-AcCl) **2**. The HBT as shown in **Scheme 2**, has been synthesized by a reported method with some modifications using 2-amino thiophenol and salicylaldehyde in presence of I_2 by overnight stirring at 100˚C in DMF solvent.[74] After several attempts for standardization of reaction condition with different combination of solvents and base as detailed in **table 1**., The so formed HBT have been easily transformed to its chloroacyl protected HBT i.e. chloro-acetic acid 2-benzothiazol-2-yl-phenyl ester (Probe **2**) by simple chloroacylation with chloroacyl chloride in presence of weak base K_2CO_3 in dry THF solvent at 50˚C. The pure product was easily obtained after filtration, concentration and fractional crystallization of the crude reaction product with dry hexane in 90% yield.



Scheme 2: Synthesis of probe **2** by chloroacyl chloride/K_2CO_3.

Table 1 Synthesis of (HBT-AcCl) **2** by chloroacylation of HBT under different conditions.

S. No.	Comp.(mmol) HBT:ClAcCl	Reaction Conditions				**2** Yield[a] (%)
		Base	Solvent	Temp (°C)	Time (h)	
1	1:1	K_2CO_3; 1 eq	Me_2CO	R.T.	24	40
2	1:1	K_2CO_3; 5 eq	Me_2CO	R.T.	24	42
3	1:3	K_2CO_3; 5 eq	Me_2CO	reflux	18	69
4	1:1	Et_3N; 3 eq	Me_2CO	R.T.	24	41
5	1:3	Et_3N; 5 eq	Me_2CO	reflux	18	52

6	1:1	K_2CO_3; 1 eq	DCM	R.T.	24	45
7	1:3	K_2CO_3; 5 eq	DCM	reflux	18	68
8	1:1	Et_3N; 3 eq	DCM	R.T.	24	52
9	1:3	Et_3N; 3 eq	DCM	reflux	18	77
10	1:1	K_2CO_3; 1 eq	THF	R.T.	24	47
11	1:1	K_2CO_3; 5 eq	THF	R.T.	24	50
12	1:3	K_2CO_3; 5 eq	THF	50	12	90
13	1:1	Et_3N; 3 eq	THF	R.T.	24	48
14	1:3	Et_3N; 3 eq	THF	50	18	65
15	1:1	NaOH;0.5M	H_2O	R.T	12	[b]
16	1:3	NaOH;0.5M	H_2O	50	12	[b]

[a]Yield refers to the pure compound after fractional crystallization.
[b]No reaction, HBT recovered unreacted.

As far as the green chemistry of the presented probe **2** is concerned, we have compared the %atom economy of some of the related probes which have been reported as a simpler and selective probe for Cys detection from other amino acids. The %atom economy of our presented probe **2** (see SI, comparison of %atom economy, Page no. S-4) found comparatively better (53.49%) than other similar reported probes under comparison i.e. probe BPBC (38.09%) and HBT-Bromopropionate (47.02%). Hence, our present probe shows a better %atom economy. Also, the presented probe **2** conserve simplicity and use less stoichiometry of acetyl chloride and Cys during acylation and fluorescence study, respectively.

In absorbance spectroscopy, the UV-Vis spectrum of probe **2** showed absorbance peak at about 300 nm which could be assigned to HBT moiety in DMSO: PBS buffer ((9:1), 10 mM, pH 7.2).[9, 47] In order to check the time-dependent reaction mechanism between probe **2** and Cys, we have performed time-dependent studies of probe **2** (2×10^{-5} M) with Cys (5×10^{-5} M, 2.5 equiv) in DMSO: PBS buffer (9:1, pH=7.2, 10 mM) using both absorbance spectroscopy and emission spectroscopy (**Figure S1-2**). In the absorbance spectrum, probe **2** was mixed with the Cys solution and the absorbance readings were taken with a time interval of 2 minutes. A similar experiment was carried out in emission spectroscopy using probe **2** (2×10^{-5} M) with Cys (5×10^{-5} M, 2.5 equiv) and in both cases, it was found that about 10 minutes were required for the completion of cyclization. This showed that the sensing of Cys using probe **2** is a time-dependent process. All the spectra were recorded after 10-15 min of incubation time.

We have tested our probe **2** (2×10^{-5} M) with different amino acids i.e. Cys, Hcy, Ala, Arg, Asp, GSH, His, Gly, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Val (5×10^{-5} M, 2.5 equiv), as predicted in presence of Cys (5×10^{-5} M, 2.5 equiv), the absorbance peak at 300 nm gets decreased and a new absorbance peak was generated at about 330 nm with an isosbestic point at

about 315 nm (**Figure 1**). However, other amino acids (5×10^{-5} M, 2.5 equiv each) showed no change or less significant change in the absorbance spectra except Hcy (5×10^{-5} M, 2.5 equiv).

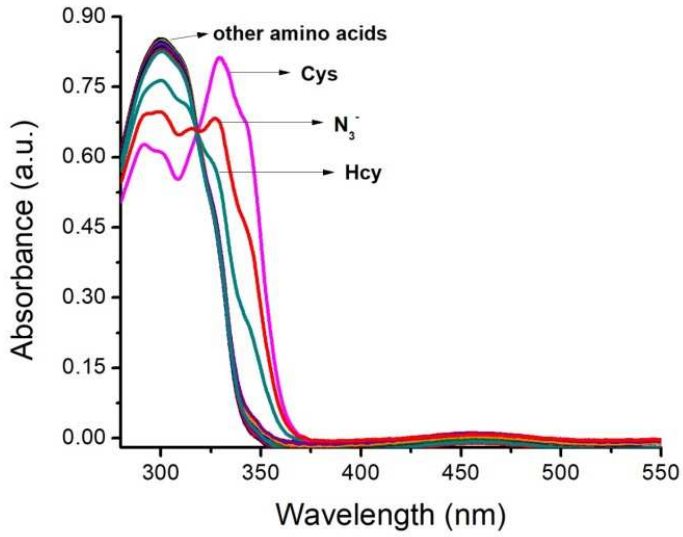


Figure 1: Absorbance selective studies of probe **2** (2×10^{-5} M) with different Amino Acids (5×10^{-5} M, 2.5 equiv) in DMSO: PBS buffer 9:1, pH=7.2, 10 mmol.

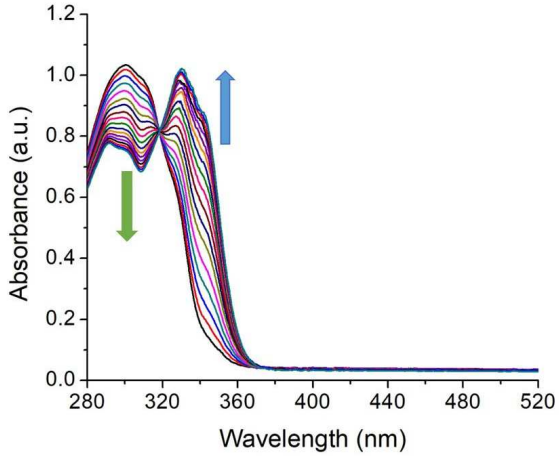


Figure 2: Systematic absorbance titration of probe **2** (2.5×10^{-5} M) with Cys (0-6.25×10^{-5} M, 0-2.5 equiv) in DMSO: PBS buffer 9:1, pH=7.2, 10 mmol.

The change in absorbance peak of both Cys and Hcy could be due to the removal of the chloroacetyl group through the cyclization process. Further, we have conducted systematic absorbance titration of probe **2** (2.5×10^{-5} M) with different concentrations of Cys (0-6.25×10^{-5}

M, 0-2.5 equiv) (**Figure 2**). With the successive rise in the concentration of Cys the change in absorbance was observed with a decrease at 300 nm and an increment at 330 nm.

Further, HBT is known as a fluorescent active molecule and well known for its ESIPT behavior.[31] However, probe **2** showed weak fluorescence peak at about 470 nm with an excitation wavelength of 330 nm in DMSO: PBS buffer ((9:1), 10 mM, pH 7.2), which could be due to the electron-withdrawing nature of attached chloroacyl group. We expected that the Cys could remove the chloroacyl group from the HBT unit and revive the fluorescence via the ESIPT process. Therefore, we have checked probe **2** as a sensor for Cys using emission spectroscopy. At first, selectivity experiments of probe **2** (2×10^{-5} M) with different amino acids (5×10^{-5} M, 2.5 equiv) were carried out with an excitation wavelength of 330 nm. It was found that an increase in the fluorescence at 470 nm was observed in the presence of Cys (5×10^{-5} M, 2.5 equiv) (**Figure 3**). Other amino acids (2.5 equiv each) showed no change or very little change in the emission spectra. Similar to the results obtained in UV-Vis experiments, Hcy (5×10^{-5} M, 2.5 equiv) also showed significant change at 470 nm but it was less as compared to the Cys. The change in fluorescence with Cys could also be detected through naked eyes under UV light (**Figure 4**). In order to attain a clear picture, concentration-dependent studies of probe **2** with simpler bio-thiol Cys were conducted. Systematic titration of probe **2** (1.8×10^{-5} M) with some varying concentrations of Cys (0-4.5 $x10^{-5}$ M, 0-2.5 equiv) was carried out (**Figure 5**). The results showed that the conc. of Cys and fluorescence intensity is linearly proportional and the fluorescence signal of probe **2** was drastically increased at 470 nm. Also, the limit of detection (LOD) was calculated as 1.2×10^{-6} M using the change in fluorescence intensity (**Figure S1**). The LOD was calculated by using the earlier reported methods which use the slope of the straight line and putting it in the formula: LOD = 3σ/slope, where σ stands for the standard deviation determined by three reading of probe **2**.[75]

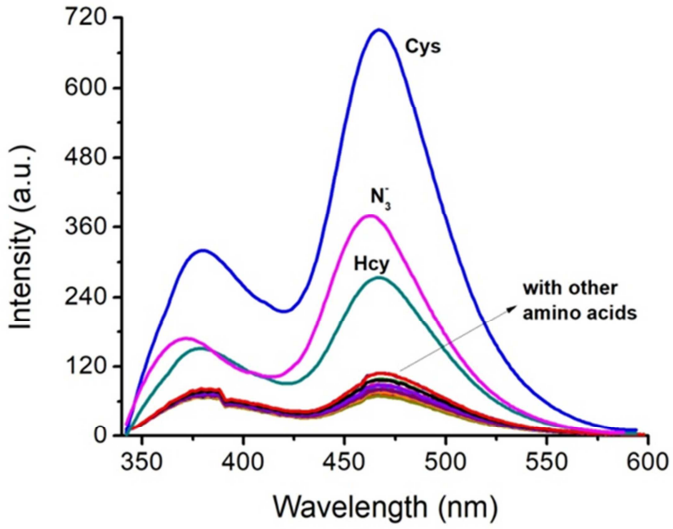


Figure 3: Fluorescence selective studies of probe **2** (2×10^{-5} M) with different amino acids (5×10^{-5} M, 2.5 equiv) in DMSO: PBS buffer 9:1, pH=7.2, 10 mmol, with λ_{exc} = 330 nm.

Figure 4: Naked eye detectable change in fluorescence probe **2** (1×10^{-4}M) with different A. A. (2.5×10^{-4}M, 2.5 equiv) in DMSO:PBS buffer 9:1, pH=7.2, 10 mmol under UV lamp.

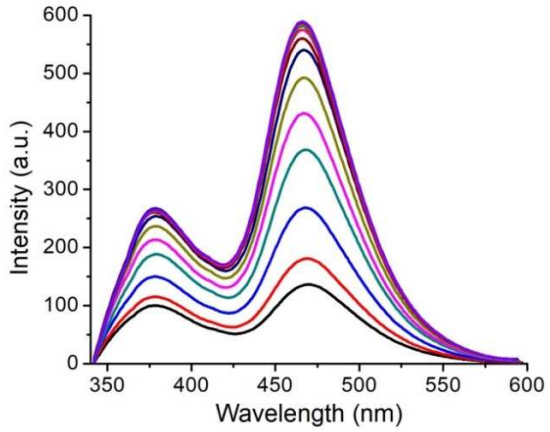


Figure 5: Fluorescence titration of probe **2** (1.8 X 10^{-5} M) with Cys (0-4.5 x 10^{-5} M, 0-2.5 equiv) in DMSO:PBS buffer 9:1, pH=7.2, 10 mmol with λ_{exc} = 330 nm. Slit width= 10 nm/ 5nm.

For the sensing of biologically important molecules such as Cys, the sensing probe should have worked at a wide pH range. For this, we have performed the pH-dependent sensing experiment of Cys at pH range from 4-10 using emission spectroscopy. We have found that the probe **2** (2 × 10^{-5} M) with Cys (5 × 10^{-5} M, 2.5 equiv) showed enhancement in emission intensity at pH = 5.0, 6.0, 7.4, 8.0, 9.0, 10.0 and showed the potential to work over wide pH range 5-10 (**Figure 6**). Hence, probe **2** could work at all physiological pHs. This showed that probe **2** could be used for Cys detection in biological samples too. To establish the mechanism for Cys sensing, the ESI-MS spectrum of probe **2** (**Figure S12**) was recorded in the presence of Cys (2.5 equiv). The peak corresponds to probe **2** at m/z = 341.34 [Probe **2** + K]$^+$ was disappeared and a new peak of HBT at m/z = 228.00 was observed (**Figure S13**). Further, the peaks at 178.05 [C5H6NO3S + NH4$^+$], could be due to the cyclized product. To further have insight into the mechanism, the role of chloroacyl was checked. We have synthesized a new probe **2'** (HBT-Ac) by simple acylation of 2-(2-hydroxyphenyl)-benzo thiazole (HBT) named acetic acid 2-benzothiazol-2-yl-phenyl ester (HBT-Ac).[76] We have checked probe **2' (**HBT-Ac) with analytes (Cys, Hcy and N_3) and found that almost no emission enhancement was occurred. This proved that the formation of stable cyclized product played an important role in the sensing mechanism. In literature also, it is mentioned that the acetylated derivatives showed negligible change in the presence of Cys, Hcy and N_3.[77] This confirmed the removal of the chloroacyl group and the formation of a free HBT unit. Further, we have also carried out a simple TLC experiment for confirmation of the free HBT unit. For this, probe **2** (10 mg) was vigorously stirred with Cys for 5 minutes. Then, the spot of the resulting solution was monitored with the pure HBT on a TLC plate. It was observed

that the spot of the resulting reaction mixture of probe **2**+Cys was moved identically with the spot of pure HBT, which confirmed the presence of free HBT. These results supported the mechanism proposed earlier for the reaction of probe **2** with bio-thiols like Cys and Hcy. In a comparison study of Cys and Hcy, it was found that Hcy having one carbon more is almost similar to the Cys in chemical structure and biological functions. Due to the presence of the same functional groups, we have decided to compare the effect of Cys and Hcy (5×10^{-5} M, 2.5 equiv) on probe **2** (2×10^{-5} M).

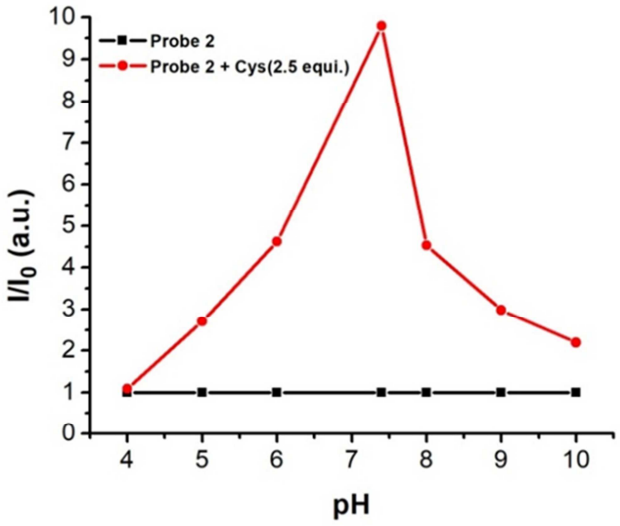
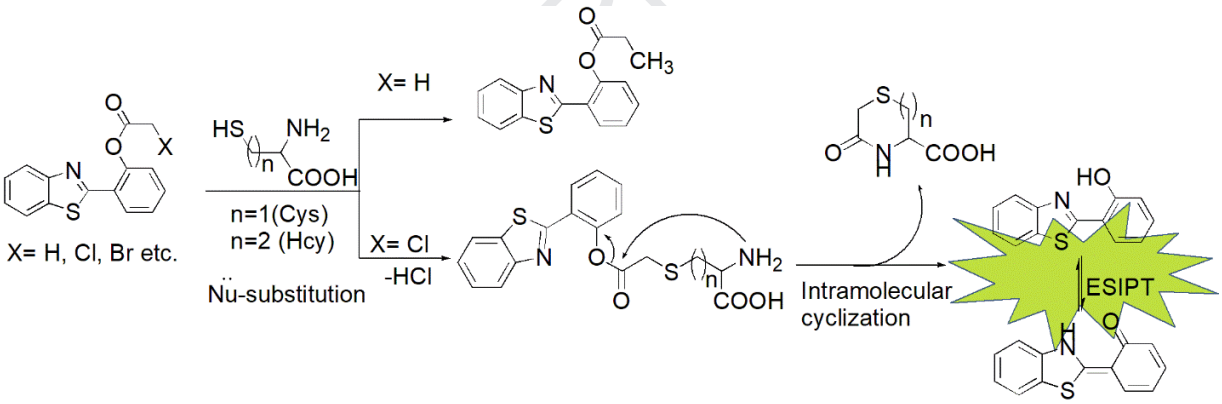


Figure 6: The pH-dependent change in emission intensity of the probe **2** (2×10^{-5} M) with Cys (5×10^{-5} M, 2.5 equiv) at pH = 4.0, 5.0, 6.0, 7.4, 8.0, 9.0, 10.0. The excitation was observed at 330 nm and emission wavelength at 470 nm.

In absorbance spectra, the change is more in the case of Cys as compared to Hcy at 300 nm and 330 nm. In emission spectra, the increase in emission intensity of probe **2** (2×10^{-5} M) with Cys (5×10^{-5} M, 2.5 equiv) is almost 8-times, whereas with Hcy (5×10^{-5} M, 2.5 equiv), the change is only 3-times at 470 nm. As we have proposed the cyclization reaction for sensing mechanism, we have further investigated the effect of the structural difference of one carbon atom in Cys and Hcy. For this, we have performed the time-dependent studies and found that the reaction completion time for Cys (5×10^{-5} M, 2.5 equiv) with probe **2** (2×10^{-5} M) was about 10 minutes. On the other hand, the reaction completion time for the same probe **2** (1×10^{-5} M) with Hcy (2.5×10^{-5} M, 2.5 equiv) was about 25 minutes. This change could be due to one carbon more in Hcy that could make the cyclization process a little slower as compared to Cys. Therefore, these results revealed that although probe **2** could detect both Cys and Hcy, the

change is more prominent and quick for Cys as compared to Hcy. The overall comparison of results showed that the probe **2** is better than the earlier reported HBT based probes for Cys and Hcy sensing through nucleophilic attack via a ESPIT mechanism as shown in **Table S2** (see SI file, page S-15-16) wherein our probe **2** showed long response time and short emission wavelength (below 500 nm).

Finally, from these observations and literature study, a plausible mechanism as shown in **Scheme 3** involving the nucleophilic attack of the free terminal amine of both Cys and Hcy onto the carbonyl carbon of HBT-AcCl was proposed. The attack of amine was followed by intramolecular cyclization and cleavage to set HBT free for ESIPT-based fluorescence and corresponding cyclic structures of Cys and Hcy cleaved as six and seven-member rings, respectively. The formation of seven-member ring 5-oxo-[1,4]thiazepane-3-carboxylic acid (n=2) from Hcy requires more time and is thermodynamically less stable as compared to the formation of six-member ring 5-oxo-thiomorpholine-3-carboxylic acid (n=1) from Cys.[78]



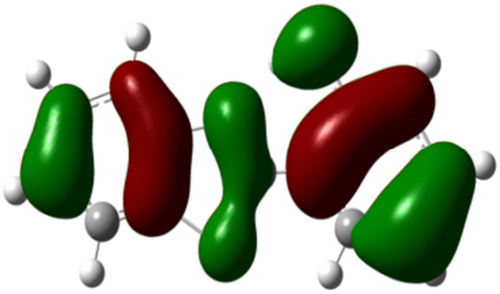
Scheme 3: A plausible mechanism of HBT-AcCl for Nu-attack and cyclization of Cys and Hcy, (Cys: n= 1 and Hcy: n=2).

The potential application of the presented simple fluorescent probe **2** was explored by using test paper strips. The paper strips were prepared by dipping the Whattmann filter paper in a 20 mL acetone solution containing 5 mg of probe **2**. The test strips were dipped for 2 hours, air-dried and used for further experiments. For an experiment, test strips containing probe **2** were dipped in different concentrations of Cys (10, 100, 1, 10 and 100 mmol) solutions prepared in PBS buffer for 10 minutes. Then, the test strips were removed, air-dried and were taken under UV lamp (**Figure S15**). The increase in the fluorescence intensity was observed as the concentration

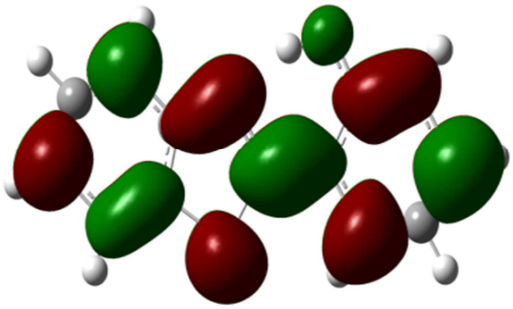
of Cys increases and can be easily detected through naked eyes. This confirmed that probe **2** could be used as a paper strip for the detection of cysteine through naked eyes.

DFT Study

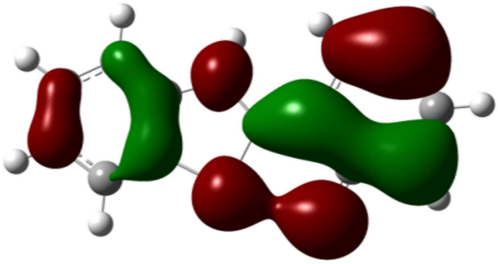
In a computational study, we have carried out the geometry optimization of Cys and Hcy substituted HBT molecules using the G09w software.[79] The minimization was carried out using the density functional theory (DFT) at the B3LYP/6-311G(d,p) level. From the viewpoint of quantum chemistry, excited-state properties are generally associated with the frontier molecular orbitals (FMOs) that are dispersed on the complete geometries of molecules. For a well understanding of the FMOs, the distribution patterns of HOMO (donor) and LUMO (acceptor) are presented in **Figure 7**.



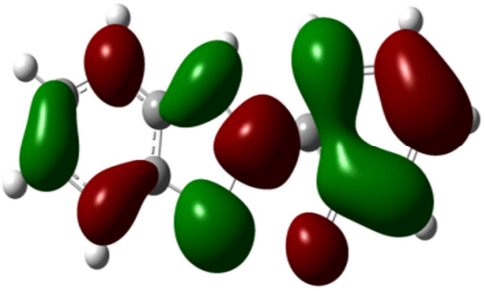
HBT-enol (HOMO)



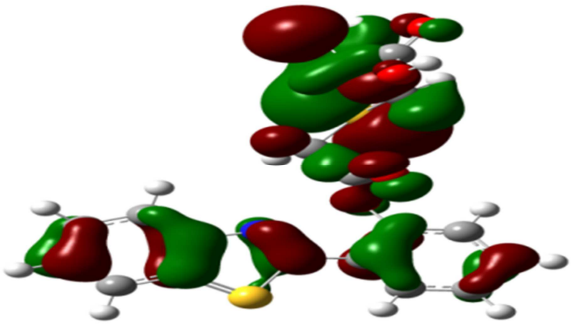
HBT-enol (LUMO)



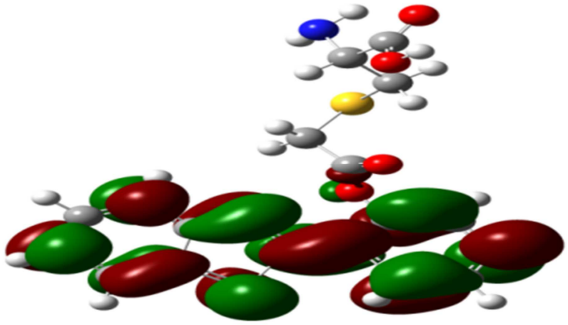
HBT-Keto (HOMO)



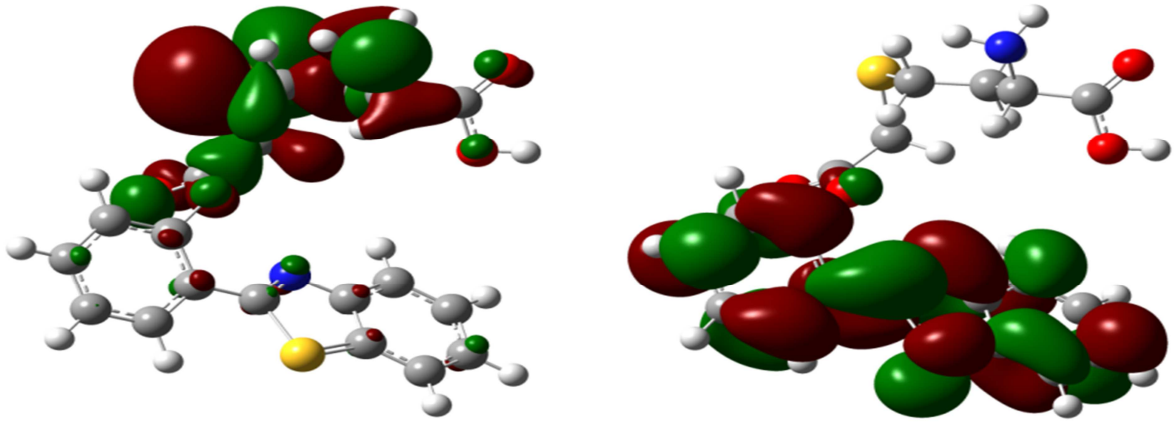
HBT-Keto (LUMO)



HBT-AcCl-Cys (HOMO)



HBT-AcCl-Cys (LUMO)



HBT-AcCl-Hcy (HOMO) HBT-AcCl-Hcy (LUMO)

Figure 7. HOMO and LUMO frontier orbitals of HBT(enol), HBT(keto), HBT-AcCl-Cys and HBT-AcCl-Hcy.

To enhance the electron transfer, the energy necessary for the transport of electrons from HOMO to LUMO must be smaller. The calculated HOMO and the LUMO energies of the selected molecules are listed in **Table 2**. In HBT-AcCl-Cys, the HOMO is mainly distributed along the side chain of the molecule with large densities on the S and N atoms, whereas the LUMO is more delocalized on the HBT area. In HBT-AcCl-Hcy, Hcy contributes to HOMO energies while LUMO is distributed among HBT molecules. The HOMO energies of HBT-AcCl-Cys and HBT-AcCl-Hcy lie at -6.51 and -6.44 eV, respectively, whereas their LUMO energies lie almost at the same level, and they are around -1.99 and -2.0 eV, respectively. The bandgap energy (ΔE) between the FMOs of HBT-AcCl-Cys and HBT-AcCl-Hcy are 4.52 and 4.44 eV, respectively and revealed the stability of HBT-AcCl-Cys over HBT-AcCl-Hcy. Also, with the help of long-range corrected DFT calculations i.e. CAM-B3LYP/ 6-311G(d,p) level of theory similar results

Table 2. B3LYP/6-311G(d,p) and CAM-B3LYP/ 6-311G(d,p) calculated energies of HOMO, LUMO, and energy gap (ΔE) for selected molecules.

Molecule	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV)
B3LYP/ 6-311G(d,p)			
HBT-Cysteine	-6.51	-1.99	4.52
HBT-Homocysteine	-6.44	-2.00	4.44
CAM-B3LYP/ 6-311G(d,p)			
HBT-Cysteine	-6.51	-2.01	4.50
HBT-Homocysteine	-6.44	-2.01	4.43

were obtained. During Nu-attack and cyclization of Cys and Hcy via intermediate formation of HBT-AcCl-Cys and HBT-AcCl-Hcy complexes, respectively both release HBT molecule which exist in the keto-enol form to exhibit fluorescence via ESIPT mechanism as shown above in **Scheme 3**.

Further, we have calculated the electronic charge distribution over all the atoms by B3LYP/6-311G(d,p) optimized structure of the intermediates complexes formed by the Nu-attack of free NH_2 of the Cys and Hcy at the AcCl of the probe **2** to form HBT-AcCl-Cys or HBT-AcCl-Hcy as shown in **Figure 8** and **9**. It was observed that the 'N' of the amine in the intermediate complex HBT-AcCl-Cys is electronegative by a value of -0.431 while the 'C' of the carbonyl functional group in HBT-AcCl of the intermediate complex HBT-AcCl-Cys is electropositive by a value of 0.399. It revealed the favorable Nu-attack of NH_2 at the C=O carbon. Likewise, the 'N' of the amine present in intermediate complex HBT-AcCl-Hcy is electronegative by a value of -0.432 while the 'C' of the carbonyl functional group in HBT-AcCl of the intermediate complex HBT-AcCl-Cys is electropositive by a value of 0.395 to reveal the favorable Nu-attack of NH_2 at the C=O carbon. It was further supported by the Molecular Electrostatic Potential (MESP) on 0.001 a.u. molecular surface of the HBT-AcCl-Cys and HBT-AcCl-Hcy which have been computed at B3PW91/6-311G(d,p) level of theory and have been represented in **Figure 10** and **11**.

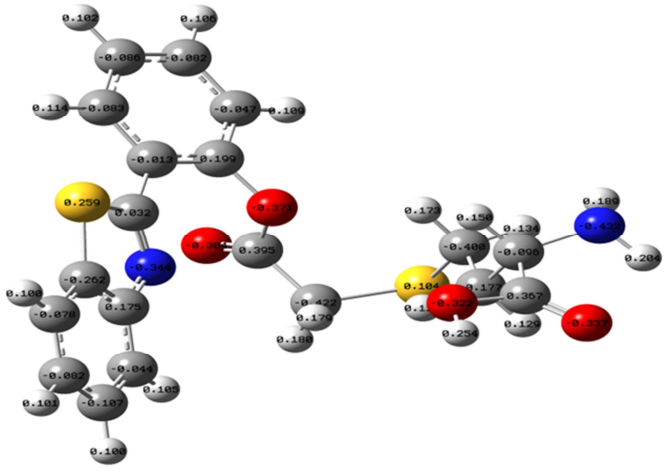


Figure 8: B3PW91/6-311G(d,p) optimized structure of HBTAcCl-Cys showing electronic charge distribution.

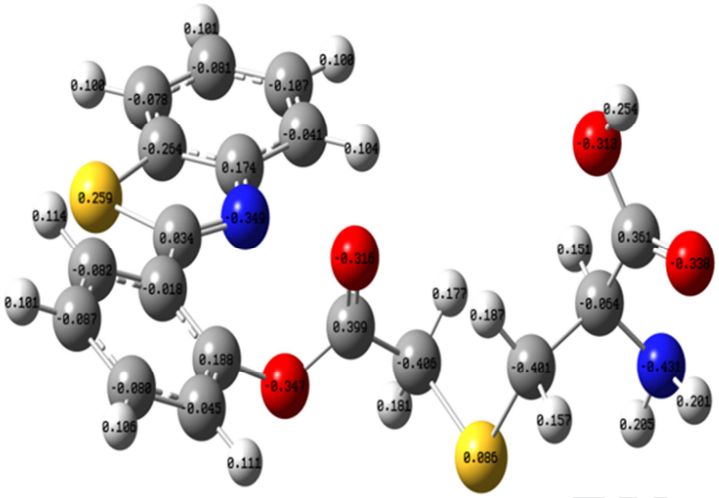


Figure 9: B3PW91/6-311G(d,p) optimized structure of HBTAcCl-Hcy showing electronic charge distribution.

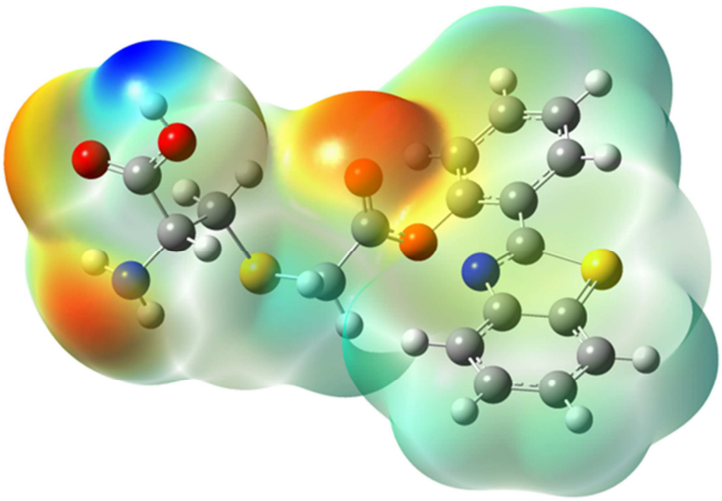


Figure 10. B3PW91/6-311G(d,p) calculated MESP on the 0.001 electron/bohr^3 molecular surface of HBT-AcCl-Cys.

As it can be seen from the structures of HBT-AcCl-Cys and HBT-AcCl-Hcy, the amine nitrogen in both the structures are showing red color over its surrounding which is indicative of electronegative nature of nitrogen of the amine. Also, the oxygen of the carbonyl is electronegative whereby carbon atom of carbonyl became more electropositive for the attack of nucleophile or electronegative group/atom like nitrogen of amine.

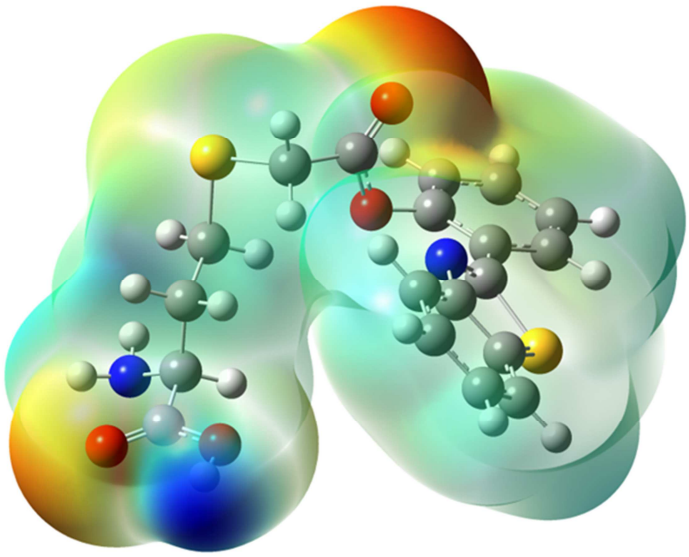


Figure 11: B3PW91/6-311G(d,p) calculated MESP on the 0.001 electron/bohr^3 molecular surface of HBT-AcCl-Hcy.

Conclusion

A new simpler, sensitive, selective turn-on fluorescent probe chloro-acetic acid 2-benzothiazol-2-yl-phenyl ester (HBT-AcCl) has been developed from the negative research results of some other scheme. The probe was synthesized by simple chloroacylation of 2-hydrohyphenyl benzothiazole (HBT) and efficiently utilized for selective detection of Cys and Hcy over other amino acids. The %atom economy of the presented probe **2** was found to be better than other similar probes. It conserves complexity, reduces the use of extra chloroacetate and has improved %atom economy. The selective nature of probe **2** for Cys and Hcy and nucleophilic attack of amine in HBT-AcCl-Cys and HBT-AcCl-Hcy were supported by the DFT results. The potential application of probe **2** was explored by using a paper strip test for the naked detection of Cys under UV lamp.

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Supplementary Material available

Supplementary data associated with this article can be found in the online version.

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Highlights:

1) A new simpler, sensitive, selective turn-on ESIPT-based fluorescent probe chloro-acetic acid 2-benzothiazol-2-yl-phenyl ester (HBT-AcCl) developed from the negative research results of some other scheme.
2) The HBT-AcCl probe was synthesized by simple monochloroacylation of 2-hydrohyphenyl benzothiazole (HBT) to avoid wastage of chemicals.
3) The HBT-AcCl probe was efficiently utilized for selective detection of Cys and Hcy over other Amino Acids.
4) The %atom economy of the presented probe was found better than other similar but complex reported probes.
5) Paper strip tests were carried out to explore the potenitial application for naked eye detection of Cys under UV lamp.
6) Selectivity of HBT-AcCl probe for Cys and Hcy is supported by theoritical DFT calculations

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The authors declare no competing financial interest.