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ORIGINAL PAPER

A 4-Methylumbelliferone-based Fluorescent Probe for the Sensitive Detection of Captopril

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Abstract A highly sensitive fluorogenic probe for captopril, 4-methylumbelliferyl-2, 4-dinitrobenzenesulfonate (4-MUDNBS), was designed and synthesized. 4-MUDNBS is a nonfluorescent compound and was synthesized via the one-step reaction of 4-methylumbelliferone (4-MU) with 2,4-dinitrobenzenesulfonyl chloride. Upon mixing with captopril in basic solution, the 2,4-dinitrobenzenesulfonyl group of 4-MUDNBS was efficiently removed and highly fluorescent 4-MU was released, hence leading to the dramatic fluorescence increase of the reaction solution. The fluorescence intensity is linear with captopril concentration in the range 3.0–500 ng mL^{-1} with a detection limit of 2.2 ng mL^{-1} (3σ). The effect of substituents on the benzenesulfonyl moiety of the probe is discussed, and the presence of electronegative groups is favorable for the thiolate-induced cleavage reaction. The proposed method has been successfully applied to the captopril determination in pharmaceutical preparations.

Keywords Captopril · Benzenesulfonate · 4-Methylumbelliferone · Fluorescent probe

Introduction

Captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-l-proline (the structure is shown in Fig. 1), is an orally active inhibitor of the angiotensin-converting enzyme and has been widely used for the treatment of hypertensive diseases on its own or in combination with other drugs [1]. This compound can also be used to treat congestive heart failure. Several analytical methods have been proposed for the determination of captopril. These include high performance liquid chromatography [2–4], spectrophotometry [5–7], electroanalytical methods [8–12], chemiluminescence [13–17] and capillary electrophoresis [18, 19]. Fluorescence technique is becoming of an important detection method for pharmaceuticals as for its simplicity and high sensitivity. In recent years, some fluorimetric methods for captopril assays have been reported [20–24]. Among the reported methods for detecting captopril, the use of fluorescent probes has its apparent advantages over other methods in sensitivity and selectivity [24]. Although fluorimetric methods have been developed for assaying captopril, they suffered from some disadvantages, such as low sensitivity, poor selectivity or time consuming [20–24]. Therefore, there is still plenty of room for improvement in term of selectivity, sensitivity and performance with a new interaction mechanism.

It was reported that strongly electron-withdrawing nitro group is the most effective in labilizing the carbon–sulfur bond in benzenesulfonamide or (benzenesulfonate) [25]. The resulting sulfonate can be readily cleaved by thiolate anion, derived from thiol under basic conditions through nucleophilic aromatic substitution [26, 27]. We envisioned that this property might be utilized for selective analysis of thiol-containing drugs, and such an idea has been attempted in this work by designing a new fluorogenic probe, 4-methylumbelliferyl-2, 4-dinitrobenzenesulfonate (4-MUDNBS), for the selective determination of captopril in alkaline media. 4-methylumbelliferone (4-MU) has a hydroxyl group in its structure, which has been reported to be modified to get phosphatase and various glycosidases substrates [28]. In the present study, modification of the hydroxyl group in 4-MU with 2, 4-dinitrobenzenesulfonyl

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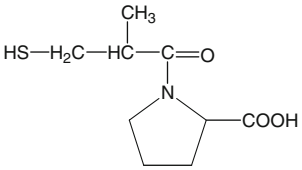
Fig. 1 The chemical structure of captopril



chloride gives a nonfluorescent 4-MUDNBS. Upon incubation with captopril in basic conditions at room temperature, the resulting sulfonate can be readily cleaved by thiolate anion through a nucleophilic substitution process, resulting in the formation of highly fluorescent 4-MU and hence leading to a dramatic increase in fluorescence intensity (Scheme 1). Based on the above mechanism, a new fluorescent probe for captopril was developed.

Experimental

Apparatus

The fluorescence spectra and relative fluorescence intensity were measured with a Sanco CRT-970 spectrofluorimeter (Shanghai, China) with a 10 mm quartz cuvette. The excitation and emission wavelength bandpasses were both set at 10 nm. The absorption spectra were measured with a Shimadzu UV-1700 spectrophotometer. The pH was measured with a Model pHs-3B meter (Shanghai, China). IR spectra were taken in KBr pellets on a Bruker Tensor 27 IR spectrometer. Mass spectra were obtained with AXIMA-CFR plus MALDI-TOF Mass Spectrometer. NMR spectra were measured with Varian unity INOVA-400 spectrometer (at 400 MHz for ^{1}H) with tetramethylsilane (TMS) as internal standard.

Reagents

A stock solution of 0.5 mg mL^{-1} captopril was prepared by dissolving appropriate amount of captopril in 50 mL 0.01 mol L^{-1} HCl. This stock solution was stored at 4 °C in the refrigerator. Standard test solutions were prepared daily by appropriate dilution of the stock solution. 4-MUDNBS solution (1.0 mmol L^{-1}) was prepared by dissolving 20.3 mg of the probe in 50 mL ethyl acetate. 4-MUTS solution (1.0 mmol L^{-1}) was prepared by dissolving 8.25 mg of 4-MUTS in 25 mL ethyl acetate. A 4-MU solution (4.0 mmol L^{-1}) was prepared by dissolving 17.6 mg of 4-MU in 25 mL ethanol. A 0.1 mol L^{-1} NH_3–NH_4Cl buffer solution (pH 9.5) was employed. The sources of reagents were as follows: Captopril was obtained from Drug Examination Bureau of Shaanxi Province, China; 4-MU was obtained from Beijing Hengye Zhongyuan Chemical Reagent Co.; 2,4-Dinitrobenzenesulfonyl chloride was obtained from Alfa Aesar; *p*-Toluenesulfonyl chloride was obtained from Tianjin Chemical Reagent Plant.

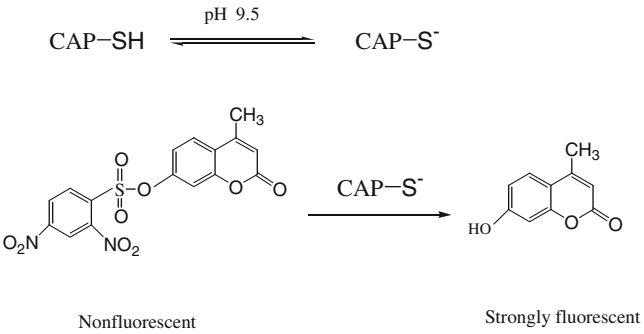
All the reagents were of analytical-reagent grade, and doubly distilled water was used throughout.

Synthesis of 4-MUDNBS

A mixture of 4-MU (0.1 g, 0.57 mmol) and 2,4-dinitrobenzenesulfonyl chloride (1.2 eq) in 5 mL dried pyridine was stirred overnight at room temperature (Scheme 2). The resulting reaction solution was poured into saturated brine and yellow precipitate was observed. The precipitate was filtered off and dried to give a light yellow solid. This solid was subjected to silica gel (200–300 mesh) chromatography (column dimensions: 30 cm×3 cm i.d., temperature: 25 °C) eluted with chloroform–ethyl acetate (7:1, *v*/*v*) to afford the desired product (0.16 g, 69% yield) as a white solid. The product was shown to consist of only one substance by thin layer chromatography (TLC) (stationary phase, GF254 silica gel), and its R_f value was 0.68 (chloroform /ethyl acetate, 4:1). IR (KBr, cm^{-1}), 1,732.4, 1,557.7, 1,537.7. MS (MALDI-TOF): *m/z* 406.1(M^+); M^+ calculated, 406.3. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.12 (d, 1 H, J=2.0 Hz), 8.60 (dd, 1 H, J_1=2.0 Hz, J_2=8.8 Hz), 8.29 (d, 1 H, J=8.4 Hz), 7.85 (d, 1 H, J=8.4 Hz), 7.35 (d, 1 H, J=2.4 Hz), 7.22 (dd, 1 H, J_1=2.4 Hz, J_2=8.8 Hz), 6.47 (d, 1 H, J=0.8 Hz), 2.41 (d, 3 H, J=1.2 Hz).

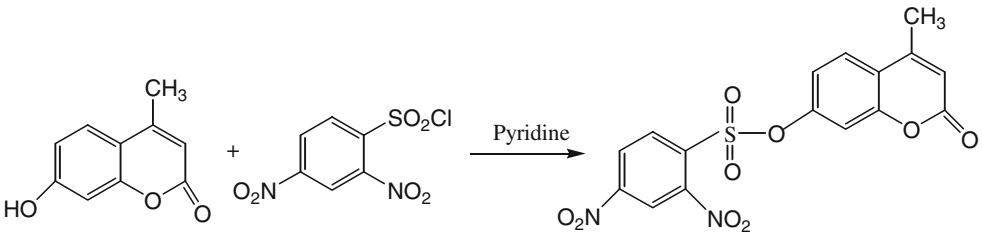
Synthesis of 4-methylumbelliferyl-*p*-toluenesulfonate (4-MUTS)

A mixture of 4-MU (0.2 g, 1.14 mmol) and *p*-Toluenesulfonyl chloride (1.5 eq) in 5 mL dried pyridine was stirred overnight at room temperature (Scheme 2). The resulting reaction solution was poured into saturated brine and white precipitate was observed. The precipitate was filtered off and dried to give a white solid. This solid was dissolved by 60 mL of CH_2Cl_2, washed with 0.1 mol L^{-1} Na_2CO_3 (2× 10 mL) and water (2×10 mL), and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation to dryness, giving the desired product as white solid (0.23 g,

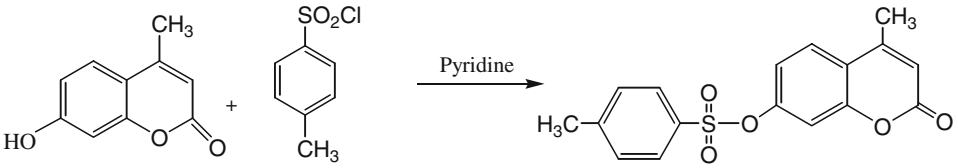


Scheme 1 Reaction of captopril with 4-MUDNBS at alkaline media (CAP-SH represents for captopril)

Scheme 2 Synthesis of 4-MUDNBS and 4-MUTS



4-MUDNBS



4-MUTS

61.3% yield). The product was shown to consist of only one substance by TLC (stationary phase, GF254 silica gel), and its R_f value was 0.72 (ethyl acetate /petroleum ether, 1:1). MS (MALDI-TOF): *m/z* 330.0(M^+); M^+ calculated, 330.3. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (dd, 2 H, J_1= 1.6 Hz, J_2=6.8 Hz), 7.57 (d, 1 H, J=8.4 Hz), 7.34 (dd, 2 H, J_1=0.8 Hz, J_2=8.0 Hz), 7.11 (dd, 1 H, J_1=2.4 Hz, J_2= 8.8 Hz), 6.83 (d, 1 H, J=1.2 Hz), 6.28 (d, 1 H, J=1.2 Hz), 2.48 (s, 3 H), 2.42 (d, 3 H, J=1.6 Hz).

Procedure

Procedure for calibration

The fluorogenic reaction was performed in a 10-mL volumetric tube. Typically, to a test tube containing 0.2 mL of 0.1 mmol L^{-1} 4-MUDNBS, different concentrations of captopril was added, then 2.0 mL of 0.1 mol L^{-1} NH_3–NH_4Cl buffer solution (pH 9.5) was added and the solution was diluted to 10 mL with water. The reaction solution was kept at room temperature (25 °C) for 15 min, and then the fluorescence intensity of the above solution was recorded at an emission wavelength of 446 nm with excitation wavelength set at 364 nm. In the meantime, a blank solution containing no captopril was prepared and measured at the same conditions for comparison.

Procedure for the pharmaceutical preparations

The proposed method was applied to the analysis of two commercial pharmaceutical tablets containing 25 mg captopril per tablet. The average tablet weight was calculated from weighting 20 tablets. They were finely powdered, homogenized and a portion of the powder, equivalent to approximately 20.0 mg of captopril was accurately weighed and shaken for 10 min with 20 ml of water, and the solution was filtered. Working solutions were prepared by appropriate dilution of the concentrated sample solution with water, so that the captopril concentration was in the working range and then analyzed according to the procedure described above.

Results and discussion

Spectral characteristics

The absorption spectra of 4-MU and 4-MUDNBS in pH 9.5 NH_3–NH_4Cl buffer were recorded and shown in Fig. 2. It can be seen that once the hydroxyl group of 4-MU was modified by 2,4-dinitrobenzenesulfonyl chloride, the absorbance at 364 nm ($\varepsilon_{364\ nm}=1.70\times10^4$ L mol^{-1} cm^{-1}) was disappeared and new absorption bands centered at 261 nm ($\varepsilon_{261\ nm}=1.57\times10^4$ L mol^{-1} cm^{-1}) and 308 nm ($\varepsilon_{308\ nm}=6.24\times10^3$ L mol^{-1} cm^{-1}) were formed. Upon incubation with captopril in basic media, the absorption spectra of the mixing solution were measured and the results are shown in Fig. 3. It can be observed that the characteristic absorption of initial 4-MU at about 364 nm was recorded, and the absorbance at 364 nm increased with increasing captopril concentration. Furthermore, the fluorescence spectra of 4-MUDNBS solution containing different concentrations of captopril were measured, and a characteristic fluorescence emission maximum centered at about 446 nm was recorded

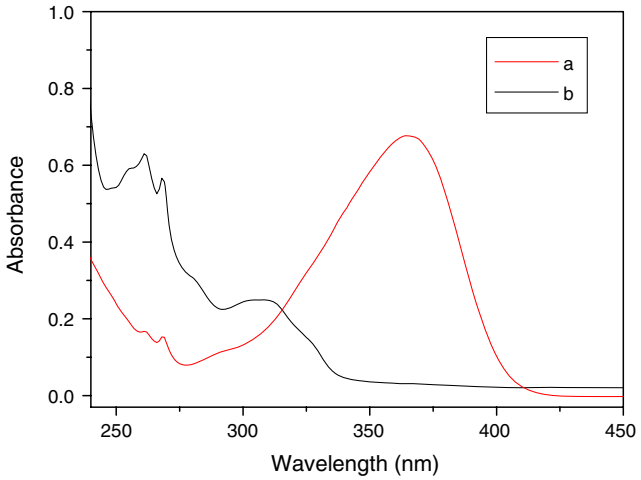


Fig. 2 The absorption spectra of 4-MU (*a*) and 4-MUDNBS (*b*) in ethanol–water (20/80, v/v) at pH 9.5 NH_3–NH_4Cl buffer. The concentrations of 4-MUDNBS and 4-MU were both 40 μmol L^{-1}

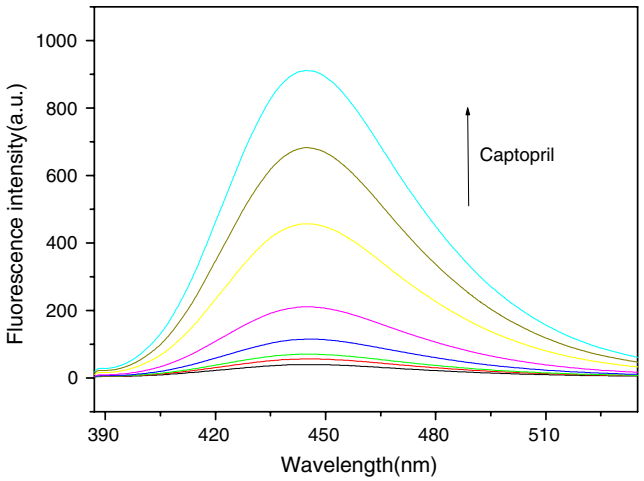


Fig. 4 Fluorescence emission spectra (excitation at 364 nm) of 4-MUDNBS (2.0 μmol L^{-1}) toward different concentrations of captopril (final concentration: 0, 10, 20, 50, 100, 300, 500, 800 ng mL^{-1}) after incubation at room temperature (25 °C) for 15 min in pH 9.5 NH_3–NH_4Cl buffer

(Fig. 4), consistent with the fluorescence emission maximum of 4-MU at the same conditions.

The effect of substituents on the thiolate-induced cleavage reaction

The effect of substituents on the benzenesulfonyl moiety of the probe on the cleavage reaction was discussed. In the present study, both 4-MUDNBS and 4-MUTS were prepared and their fluorescence responses toward captopril at the same conditions were compared. As can be seen from Fig. 5, the fluorescence signal of 4-MUDNBS increased dramatically upon mixing with captopril, whereas the fluorescence signal of 4-MUTS remained almost unchanged. The significant difference between them lies in the substituents on the benzenesulfonyl moiety of the probe. For 4-MUDNBS, the nitro group in its structure can effectively labilize the C–S bond [25], and hence the thiolate-induced cleavage reaction can proceed readily. While for 4-MUTS, because of the lack of electronegative groups in its benzenesulfonyl group, the cleavage of the sulfonate by thiolate anion is generally unfavorable. Based on the above results, it can be concluded that the electro-withdrawing groups on the benzenesulfonyl moiety of the probe markedly facilitate the thiolate-induced nucleophilic reaction.

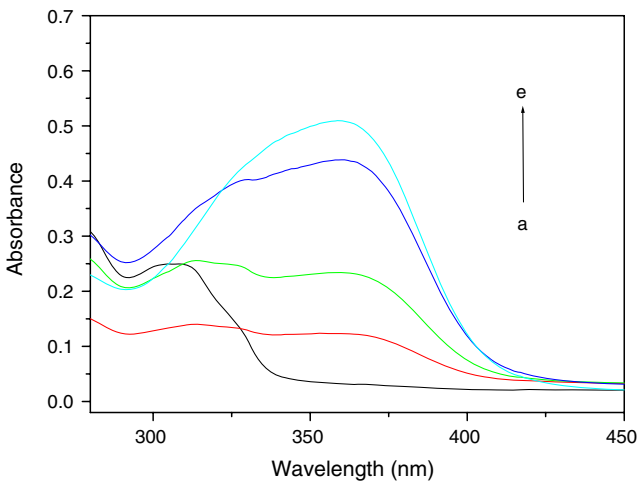


Fig. 3 UV–vis absorption of 4-MUDNBS (40 μmol L^{-1}) toward different concentrations of captopril after incubation at room temperature for 30 min in ethanol–water (20/80, *v*/*v*) at pH 9.5 NH_3–NH_4Cl buffer. Captopril concentration: (*a*) 0; (*b*) 1.0; (*c*) 2.0; (*d*) 5.0; (*e*) 10.0 μg mL^{-1}

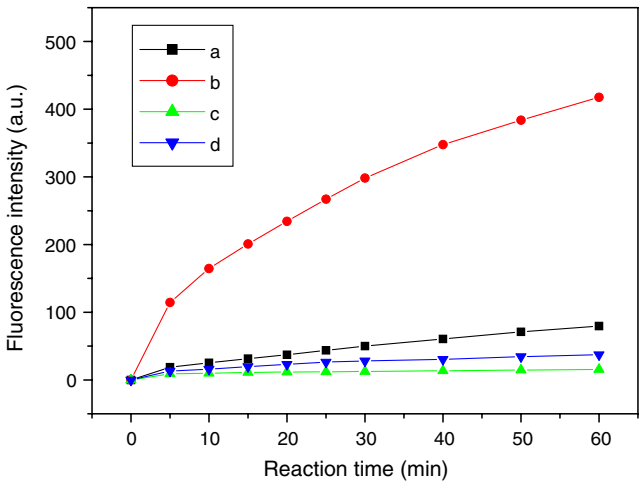


Fig. 5 Time dependent fluorescence intensity changes observed during reaction of 4-MUDNBS (or 4-MUTS) with captopril (100 ng mL^{-1}) in pH 9.5 NH_3–NH_4Cl buffer. (*a*), 4-MUDNBS; (*b*), 4-MUDNBS + captopril; (*c*), 4-MUTS; (*d*), 4-MUTS + captopril. The concentration of 4-MUDNBS and 4-MUTS are both 2.0 μmol L^{-1}

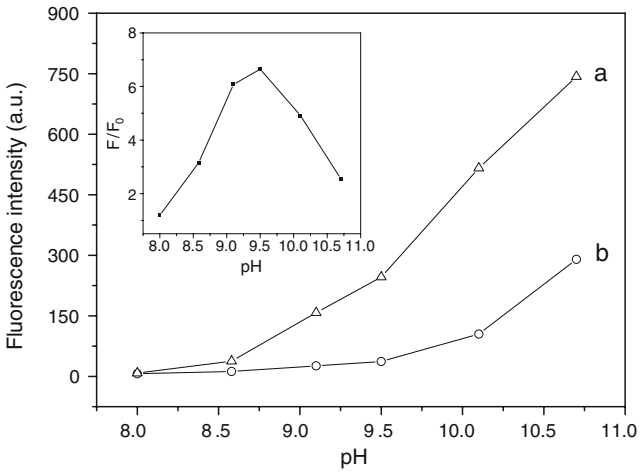


Fig. 6 Effect of pH on the fluorescence intensity of the system. (*a*), 4-MUDNBS + captopril; (*b*), 4-MUDNBS. 4-MUDNBS, 2.0 μmol L^{-1}; Captopril, 100 ng mL^{-1}. The fluorogenic reaction was carried out in different pH of NH_3–NH_4Cl buffer solution for 15 min, and then the fluorescence intensity was recorded. *Inset*: fluorescence enhancement factor F/F_0 as a function pH value. Where F and F_0 are the fluorescence signals of the reaction mixture in the presence and absence of captopril, respectively

Effect of pH

The pH had a large effect on the sensitivity of the proposed method and was selected considering the following three aspects: (1) it was reported that captopril has pK_a values of 3.7 (carboxyl group) and 9.8 (thiol group) [18]. Therefore, to maintain the predominant form of captopril was present in its corresponding thiolate anion, an essentially reactive form for the proposed fluorogenic reaction, the reaction solution should be kept at basic conditions. (2) As the pK_a of the 4-MU phenolic proton is 7.8 [29], it requires relatively high pH medium (pH~9) to achieve maximum fluorescence intensity. (3) Because the nucleophilic attack by hydroxide can take place and thus leading to the cleavage of the sulfonate [25], a high background signal may be recorded under high pH media.

The effect of pH on the fluorogenic reaction was studied in the range 8.0–10.7, and the results were shown in Fig. 6. It can be seen that both the fluorescence signal of reaction system and blank solution increased with increasing pH. It can also be observed from Fig. 6 (insert curve) that F/F_0 increased with increasing pH, remained the maximum value at pH 9.5, and decreased when pH was above 9.5. Hence, to obtain a high F/F_0 value, pH 9.5 NH_3–NH_4Cl buffer was selected for the fluorogenic reaction in the subsequent experiments.

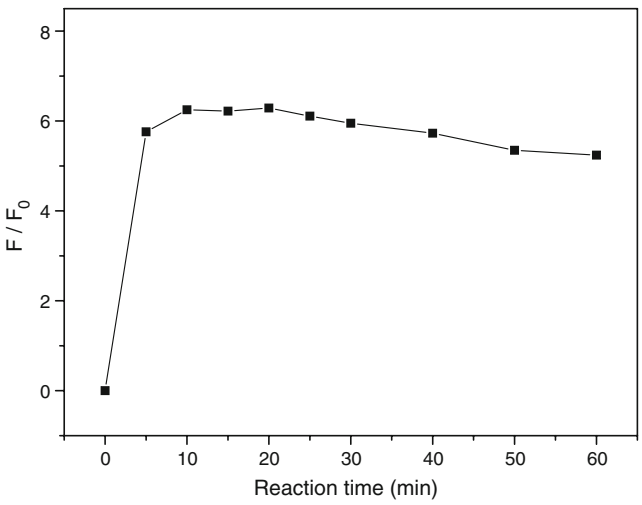


Fig. 7 Effect of reaction time on the fluorescence increase of the system. 4-MUDNBS, 2.0 μmol L^{-1}; Captopril, 100 ng mL^{-1}. The fluorogenic reaction was carried out in pH 9.5 NH_3–NH_4Cl buffer solution for different time, and then the fluorescence intensity was recorded

Table 1 Tolerance limit of some foreign substances on the determination of captopril

Substance	Molar ratio to captopril[a]
Benzoate, Ca^{2+}, Mg^{2+}, Mn^{2+}, $C_2O_4^{2-}$, F^-, I^-	1,000
Sucrose, Starch, Na^+, Urea	500
Glycine, L-threonine, L-leucine, L-aspartate, L-arginine, L-norvaline, Citrate, Co^{2+}, Br^-	100
Glucose, NO_3^-, CO_3^{2-}	50
Ascorbic acid, Zn^{2+}	10

[a] Captopril concentration:0.5 μmol L^{-1}

Effect of reaction time

The effect of the reaction time on the fluorescence signal of the system was studied. It can be seen from Fig. 5 that upon mixing with captopril, the fluorescence signal of the system increased with the reaction time prolonged. The effect of the reaction time on the F/F_0 value was studied and the results were shown in Fig. 7. It can be seen that F/F_0 value of the reaction solution increased rapidly at the beginning, and remained almost constant in 10–25 min, and decreased

Table 2 Results of the determination of captopril in pharmaceuticals

Captopril tablets	Label amount (mg)	Found (mg)[a]	Added (mg)	Recovery (%)[a]
Sample 1	25.0	24.9±0.9	25.0	96.5±2.9
			50.0	105.9±5.8
Sample 2	25.0	24.1±0.7	25.0	98.5±2.3
			50.0	106.0±0.6

[a] Average of three measurements (±S.D.)

slowly when the reaction time was above 30 min. Therefore, a 15-min reaction was selected in the following experiment.

Interference study

In order to assess the proposed method to the analysis of captopril pharmaceutical dosage forms, the interference of commonly used excipients and additives, co-existing ions or the other compounds was tested by analyzing a standard solution of captopril (0.5 μmol L^{-1}) to which increasing amounts of interfering species were added, using an error <5% as the criterion. The results are shown in Table 1. It can be seen that most of compounds have little interference with the determination of captopril. As the proposed probe is selective to the nucleophilic thiolate anion, some other thiol-containing compounds, such as biological interesting cysteine and glutathione, can also lead to the cleavage of 4-MUDNBS at the present conditions and may cause interference for captopril determination.

Analytical characteristics of 4-MUDNBS for captopril

Under the selected experimental conditions, the $(F-F_0)/F_0$ value is directly proportional to the captopril concentration in the range 3.0–500 ng mL^{-1}. The linear regression equation was determined to be $(F-F_0)/F_0=4.31C+0.39$ ($n=8$, $r=0.9989$), where the concentration (C) is measured in 10^{-7} g mL^{-1}. According to IUPAC, the detection limit was determined from three times the standard deviation of the blank signal (3σ) as 2.2 ng mL^{-1}. The relative standard deviation ($n=7$) was 3.9% for 20 ng mL^{-1} and 4.1% for 100 ng mL^{-1} of captopril, respectively.

Sample analysis

Following the procedure detailed above, the proposed method was applied to the determination of captopril in commercially available captopril tablets (Changzhou Pharmaceutical Factory, Changzhou). The results were in good agreement with the labeled amounts given by the manufacturer, indicating the utility of the present probe for routine analytical control in commercial formulations (Table 2). A recovery test was also carried out to check the accuracy of the proposed method, leading to recovery values ranging from 96.5 to 106.0%.

Conclusions

In summary, a 4-MU-based fluorogenic probe for captopril was prepared and its fluorogenic behavior was investigated. The method is based on the strong nucleophilicity of thiolate anion to cleave the 2, 4-dinitrobenzenesulfonyl group from 4-MUDNBS which is nonfluorescent in aqueous solution, resulting in the formation of the strongly fluorescent 4-MU, hence leading to a dramatic increase in fluorescence intensity of the reaction solution. Because of the strong nucleophilic ability of thiolate, the proposed probe shows excellent selectivity toward thiol-containing compounds. The method is proved to be simple, selective and highly sensitive. As the selectivity of the proposed method is due to the strong nucleophilicity of thiolate anion, the present probe can also show similar response to other thiol-containing compounds, such as cysteine, homocysteine and glutathione.

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