

COLORIMETRIC DETECTION OF CARBENICILLIN USING CATIONIC POLYTHIOPHENE DERIVATIVES*

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Abstract A water-soluble, polythiophene-based colorimetric sensor was designed for selective and sensitive detection of carbenicillin in HEPES buffer solution. Quaternized quinine was linked to thiophene through bis-functionality benzyl group, which can interact with carbenicillin *via* electrostatic interaction and geometric match effect. The sensor exhibited a colorimetric signal change upon the addition of carbenicillin because of the formation of more nonplanar structures. However, the addition of other beta-lactam antibiotics or dicarboxylic acids into the sensor solution caused no obvious changes in absorbance intensity ratio. This result may be attributed to the cavity formed by the semi-rigid framework of PTQ2, which is suitable for the special binding with carbenicillin. This novel sensor can effectively distinguish carbenicillin from other beta-lactam antibiotics and has a wide linear range response in HEPES buffer solution. Linear calibration curves are obtained with 0 to 18 $\mu\text{mol/L}$ of HEPES buffer solution, with a limit of detection of 0.54 $\mu\text{mol/L}$.

Keywords: Polythiophene; Colorimetric sensor; Antibiotics.

INTRODUCTION

Antibiotics have been widely used to treat infectious diseases. Antibiotic residues induced by widespread usage are often encountered in animal-derived food products or drinking water; these residues are now becoming a serious problem because they may cause toxic or allergic reactions to some hypersensitive individuals^[1–5]. Long-term consumption of foodstuffs containing antibiotic residues might also result in the risk of spreading drug-resistant microorganisms. Therefore, highly selective and accurate quantification methods of determining antibiotic content in animal-derived food products or drinking water should be developed. To date, various microbiological, chromatographic, and immunochemical methods, fluorescence sensors and enzyme-based tests have been developed to detect antibiotic residues^[6–10]. However, these methods are limited by their complicated instrument, complex procedure, high detection concentration, narrow pH span and single signaling response. Thus, new assays for highly sensitive and selective quantification of antibiotics are highly desirable.

Water-soluble conjugated polythiophenes have received considerable attention as an emerging sensory material because of their meritorious signal amplification, convenient optical detection, readily tunable properties and easy fabrication^[11–14]. These conjugated polythiophenes can bind with analytes through cooperative interaction (*i.e.*, electrostatic and hydrophobic interactions), which causes conformational changes

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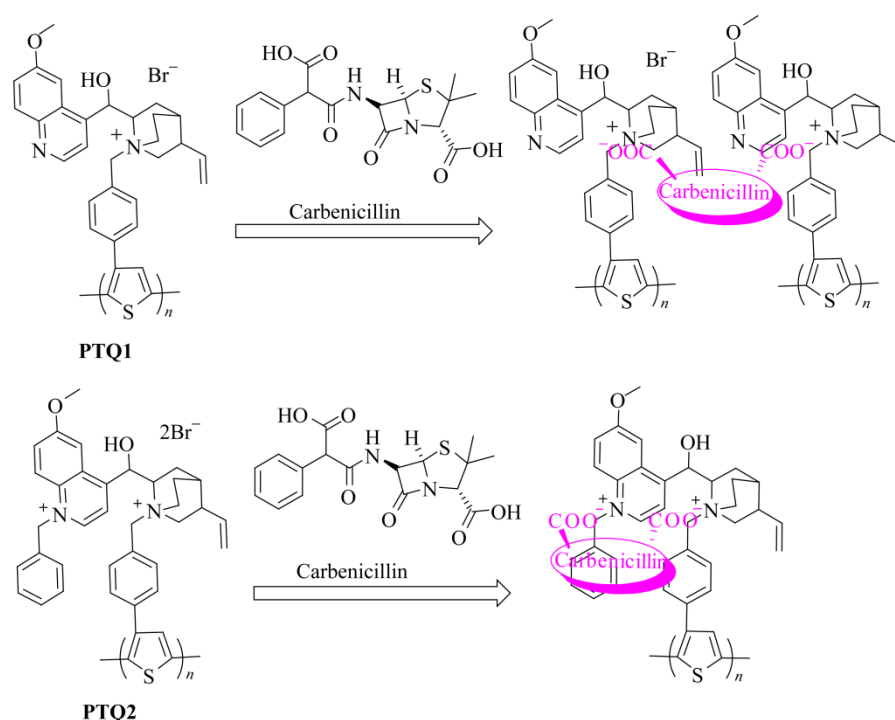
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in the molecules. Our previous studies explored polythiophene chemosensors for bioactive molecules, including antibiotics, lipopolysaccharides and lysophosphatidic acid^[15–18]. In the present study, we used a water-soluble conjugated polythiophene derivative (PTQ2) as colorimetric sensor for carbenicillin.

Carbenicillin is a bacteriolytic antibiotic that belongs to the carboxypenicillin subgroup of penicillins. In our previous work^[17], we designed a polythiophene-derived ratiometric fluorescent sensor (PTQ1) based on intermolecular electrostatic interaction between polythiophene units linked by carbenicillin. As shown in Scheme 1, the difference between PTQ1 and PTQ2 is that the quinine in PTQ2 has two quaternary ammonium groups, which can form complexation with carbenicillin by intermolecular electrostatic interaction in each thiophene unit. Comparing with PTQ1, the intermolecular interaction between one thiophene unit and carbenicillin induces different conformational changes in the polythiophene chain. Upon the addition of carbenicillin, PTQ1 takes more rigid-rod-like amphiphilic macromolecules and tends to aggregate in water, exhibiting a relatively broader absorption and red-shifted fluorescence wavelength. By contrast, blue-shifted absorption spectra of PTQ2 can be obtained from the formation of nonplanar structures upon complexation with carbenicillin.



Scheme 1 Molecular structure of carbenicillin, PTQ1, PTQ2, and the proposed interaction mechanism of carbenicillin with PTQ1 and PTQ2

EXPERIMENTAL

Materials

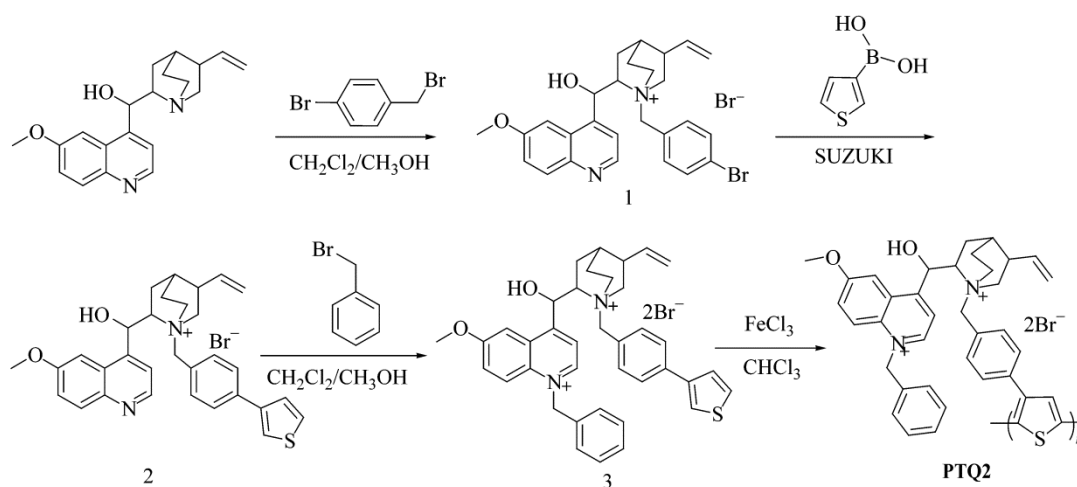
4-Bromobenzyl bromide, thiophene-3-boronic acid, tetrakis(triphenylphosphine)palladium(0), anhydrous quinine, oxalic acid, malonic acid, adipic acid, aspartic acid, glutamic acid, D-tartatic acid and L-tartatic acid, were purchased from Alfa Aesar and used without further purification. Streptomycin, chloramycetin, penicillin, neomycin, kanamycin sulfate, erythromycin, ampicillin and carbenicillin were purchased from INALCO. Other reagents were purchased from Beijing Chemical Regent Co. All reagents and chemicals were AR grade and used directly without further purification unless otherwise noted. CHCl_3 was distilled from CaH_2 under nitrogen. The water was purified by Millipore filtration system.

Characterizations

All UV-Vis and fluorescence spectra in this work were recorded in Hitachi U3010 and Hitachi F-4500 fluorescence spectrometer. ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) spectra were determined on a Bruker Advance-400 spectrometer with chemical shifts (tetramethylsilane as internal standard), matrix assisted laser desorption ionization-time of flight ((MALDI-TOF) mass spectra were recorded on a Bruker Microflex mass spectrometer and electrospray ionization (ESI) mass spectra on a Shimadzu LC-MS 2010 instrument. All pH measurements were made with a Sartorius basic pH-meter PB-10. The gel-permeation chromatography was performed using gelatin as the standard, and the CH_3CN -water mixture solution containing NaNO_3 (25 mmol/L) was employed as an eluent.

Synthesis of PTQ2

The overall synthetic pathway was outlined in Scheme 2.



Scheme 2 Synthetic route of PTQ2

Synthesis of 1-(4-Bromobenzyl)-2-(hydroxy(6-methoxyquinolin-4-yl)methyl)-5-vinyl-1-azonia-bicyclo[2.2.2]octane bromide (Compound 1)

Anhydrous quinine (0.64 g, 2 mmol) was dissolved in 20 mL of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ ($V/V = 3/2$), then 4-bromobenzyl bromide (0.5 g, 2 mmol) was added. The mixture was stirred at room temperature for 24 h. After the reaction was complete, the reaction solution was concentrated to 5 mL. The residue was poured into 200 mL of absolute diethyl ether under stirring and then filtered. The crude product was further purified by column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH} = 20/1$) to give compound **1** (0.9 g, yield 78%) as a white solid.

^1H -NMR (400 MHz, CD_3OD , TMS), δ : 1.55–1.57 (m, 1H), 1.90 (m, 1H), 2.09 (m, 1H), 2.29–2.41 (m, 2H), 2.71–2.73 (m, 1H), 3.35–3.38 (m, 2H), 3.48–3.54 (m, 2H), 3.86–3.90 (m, 1H), 4.05 (s, 3H), 4.35–4.38 (m, 1H), 4.69–4.72 (d, 1H), 5.04–5.06 (d, 1H), 5.11–5.16 (d, 1H), 5.30–5.34 (d, 1H), 5.68–5.77 (m, 1H), 6.61 (s, 1H), 7.40 (d, 1H), 7.53–7.56 (d, 1H), 7.59–7.61 (d, 2H), 7.75–7.77 (d, 2H), 7.89–7.90 (d, 1H), 8.04–8.06 (d, 1H), 8.78–8.79 (d, 1H).

^{13}C -NMR (100 MHz, CDCl_3 , TMS), δ : 21.3, 24.7, 26.7, 37.7, 46.2, 51.2, 56.2, 60.0, 62.0, 62.3, 64.0, 68.8, 101.9, 117.6, 120.4, 121.2, 125.1, 125.8, 126.1, 131.3, 132.1, 135.4, 136.1, 143.2, 143.7, 146.9, 157.9. ESI-Mass spectra m/z : Calculated: 493.15 (100%), 495.15 (97.4%); Found: 493.3, 495.3.

Synthesis of 2-(Hydroxy(6-methoxyquinolin-4-yl)methyl)-1-(4-(thiophen-3-yl)benzyl)-5-vinyl-1-azonia-bicyclo[2.2.2]octane bromide (Compound 2)

To a mixture of compound **1** (0.58 g, 1 mmol), Na_2CO_3 (0.5 g, 4.7 mmol), $\text{Pd}(\text{PPh}_3)_4$ (200 mg, 0.17 mmol) and thiophene-3-boronic acid (0.15 g, 1.17 mmol) in 20 mL EtOH under nitrogen was added deionized water (10 mL) by syringes. After refluxing at 90 °C for 10 h, EtOH was removed under reduced pressure. The residue

solution was extracted with 3×20 mL CH_2Cl_2 . The collected organic layer was dried with MgSO_4 . The solution was concentrated to 5 mL. The residue was poured into 200 mL of absolute diethyl ether under stirring and then filtered. The curded product was further purified by column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH} = 20/1$) to give compound **2** (0.4 g, yield 70%) as a colorless solid.

^1H -NMR (400 MHz, CDCl_3 , TMS), δ : 1.51–1.52 (m, 1H), 1.74 (m, 1H), 2.03 (m, 1H), 2.24–2.32 (m, 2H), 2.58 (m, 1H), 3.16–3.18 (m, 1H), 3.54–3.56 (m, 1H), 3.59 (m, 1H), 3.83–3.85 (m, 1H), 3.93 (s, 3H), 4.84–4.87 (d, 1H), 5.01–5.15 (m, 3H), 5.56–5.61 (m, 1H), 6.14–6.17 (d, 1H), 6.68–6.76 (m, 2H), 7.30–7.34 (m, 3H), 7.40–7.42 (m, 1H), 7.45–7.47 (m, 1H), 7.54–7.57 (m, 2H), 7.75–7.80 (m, 3H), 7.99–8.02 (d, 1H), 8.71–8.73 (d, 1H).

^{13}C -NMR (100 MHz, CDCl_3 , TMS), δ : 21.8, 25.0, 26.9, 30.9, 38.2, 51.3, 56.5, 61.0, 63.4, 64.4, 69.4, 102.4, 118.1, 120.6, 121.0, 121.7, 125.5, 126.1, 126.2, 126.9, 131.9, 134.4, 136.5, 137.9, 140.8, 143.5, 144.1, 147.5, 158.2.

MALDI-TOF mass spectra m/z : Calculated: 497.67; Found: 497.63.

Synthesis of Mono(2-((1-benzyl-6-methoxyquinolinium-4-yl)(hydroxy)methyl)-1-(4-(thiophen-3-yl)benzyl)-5-vinyl-1-azonia-bicyclo[2.2.2]octane) monobromide (Compound 3)

Compound **2** (0.3 g, 0.5 mmol) was dissolved in 20 mL of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ ($V/V = 3/2$), then benzyl bromide (0.1 g, 0.58 mmol) was added. The mixture was stirred at room temperature for 24 h. After the reaction was complete, the reaction solution was concentrated to 5 mL. The residue was poured into 200 mL of absolute diethyl ether under stirring and then filtered. The crude product was further purified by column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH} = 10/1$) to give compound **3** (0.15 g, yield 45%) as a colorless solid.

^1H -NMR (400 MHz, CDCl_3 , TMS), δ : 1.60 (m, 1H), 1.81 (m, 1H), 2.18 (m, 1H), 2.30 (m, 1H), 2.42–2.44 (m, 1H), 2.55–2.57 (m, 1H), 3.09–3.11 (m, 1H), 3.40–3.46 (t, 1H), 4.26 (s, 3H), 4.57 (m, 1H), 5.03 (t, 1H), 5.11–5.14 (d, 1H), 5.25–5.30 (m, 3H), 5.95–6.01 (m, 2H), 6.14–6.18 (d, 1H), 6.26–6.29 (d, 1H), 6.98–7.00 (d, 1H), 7.19–7.21 (d, 1H), 7.27–7.30 (m, 2H), 7.39–7.44 (m, 5H), 7.52–7.53 (m, 1H), 7.63–7.69 (m, 3H), 7.95–7.97 (d, 2H), 8.17–8.21 (m, 2H), 8.50–8.52 (d, 1H), 9.12–9.14 (d, 1H).

^{13}C -NMR (100 MHz, CD_3OD , TMS), δ : 22.0, 25.8, 28.4, 39.3, 52.9, 57.4, 61.6, 62.4, 65.4, 66.2, 69.8, 105.5, 117.9, 122.9, 126.9, 127.0, 128.0, 128.7, 129.0, 130.4, 130.5, 134.4, 135.0, 135.5, 138.6, 139.3, 142.1, 146.8, 157.8, 161.8.

MALDI-TOF mass spectra m/z : Calculated: 588.28; Found: 588.22.

Synthesis of PTQ2

PTQ2 was prepared *via* an oxidative polymerization under nitrogen in the presence of FeCl_3 . 4 Equiv of FeCl_3 was dissolved in 30 mL of dry CHCl_3 under nitrogen, and then 1 equiv of compound **3** dissolved in 20 mL of CHCl_3 was added drop wise. The reaction mixture was stirred at room temperature for 2 days. The resulting precipitate was collected, washed with a mount of methanol, and finally dried under vacuum to give the desired polymer as a dark red solid.

PTQ2 (yield: 60%) gel-permeation chromatography analysis (GPC): $M_n = 75.2$ KDa, PDI = 1.113.

^1H -NMR (400 MHz, $\text{CD}_3\text{CN}/\text{D}_2\text{O} = 1/1$, TMS), δ : 1.12 (s, br), 1.38 (s, br), 1.68–1.77 (br), 2.11 (s, br), 2.75 (br), 2.89 (br), 3.23 (br), 3.70 (br), 3.89 (br), 4.83 (s, br), 5.45 (s, br), 6.14 (s, br), 6.74 (s, br), 6.97 (s, br), 7.26 (s, br), 7.51 (br), 7.65 (br), 7.73 (br), 8.34 (s, br), 9.19 (s, br).

RESULTS AND DISCUSSION

We first investigated the interaction of PTQ2 with carbenicillin in 10 mmol/L HEPES buffer solution (50 $\mu\text{mol/L}$, pH 7.4, 10% CH_3CN) at room temperature by the absorption spectra. The UV-Vis spectra of PTQ2 in HEPES solution displays an absorption band from 400 nm to 550 nm, which corresponds to the polythiophene band (Fig. 1). Upon the addition of increasing concentrations of carbenicillin, the absorption intensity gradually decreased with slight blue-shift and finally displayed a broader peak at 400 nm to 500 nm. Such a spectral change is also clearly visible to the naked eyes — the solution color changed from yellow to

colorless (Fig. 1, inset). These results could be well explained by the carbenicillin-induced conformational change through the cooperative effects of the geometric match and electrostatic interaction.

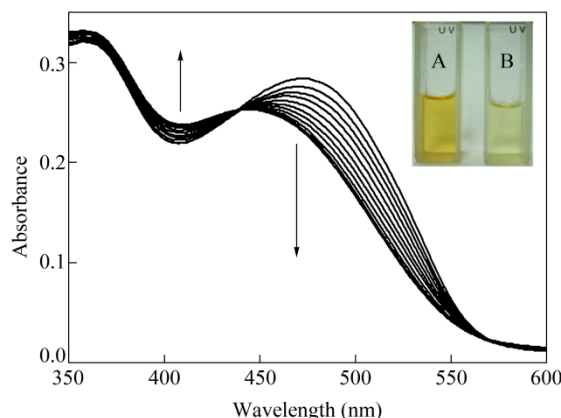


Fig. 1 UV-Vis absorption spectra of PTQ2 (50 $\mu\text{mol/L}$) in the presence of different concentrations of carbenicillin (0 to 20 $\mu\text{mol/L}$) in HEPES buffer solution (10 mmol/L, pH 7.4, 10% CH_3CN) The inset shows the digital images of PTQ2 (50 $\mu\text{mol/L}$) solution with carbenicillin solution of 0 (A) and 20 $\mu\text{mol/L}$ (B) at room temperature.

Furthermore, the corresponding effect between PTQ2 and carbenicillin was evaluated by comparing the absorbance intensity ratio (A_{405}/A_{500}) values in the presence of different concentrations of carbenicillin for quantitative analysis. As shown in Fig. 2(a), A_{405}/A_{500} gradually increased upon the addition of carbenicillin. The saturation of A_{405}/A_{500} occurred at 18 $\mu\text{mol/L}$ carbenicillin. Further addition of carbenicillin did not induce obvious changes in the spectrum. By plotting this ratio versus the carbenicillin concentration, a good linear relationship ($A_{405}/A_{500} = 0.878 + 0.0315 \times [\text{carbenicillin}]$, $R^2 = 0.997$) was observed with increasing carbenicillin concentration from 0 to 18 $\mu\text{mol/L}$. (Fig. 2a). The limit of detection (LOD), defined as three times the standard deviation of background^[19, 20], was 0.54 $\mu\text{mol/L}$. In addition, upon gradual addition of carbenicillin, the negative circular dichroism signal of PTQ2 was remarkably decreased (Fig. 2b), indicating that the conformational change of the conjugated backbone was due to the binding of carbenicillin.

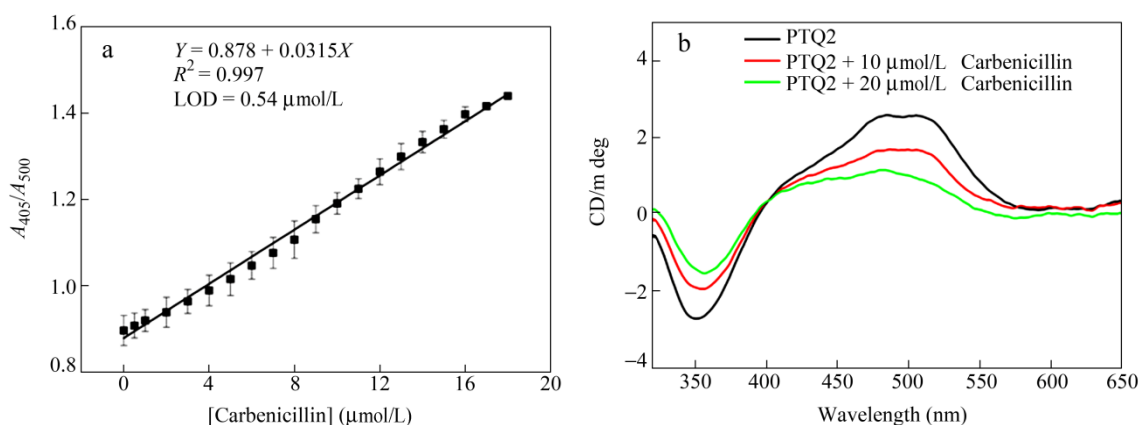


Fig. 2 (a) The changes of the UV-Vis absorbance intensity ratio of PTQ2 (A_{405}/A_{500}) versus the carbenicillin concentration ranging from 0 to 18 $\mu\text{mol/L}$; (b) Circular dichroism spectra of PTQ2 (50 $\mu\text{mol/L}$) at 25 $^{\circ}\text{C}$ in the absence and presence of carbenicillin (10 $\mu\text{mol/L}$ and 25 $\mu\text{mol/L}$, respectively)

To further study the practical application of this sensor, the effects of pH on the absorbance response to carbenicillin of PTQ2 were also investigated. As shown in Fig. 3(a), the A_{405}/A_{500} of PTQ2 showed no obvious

changes within a wide pH range (3.5 to 8). However, upon the addition of 20 $\mu\text{mol/L}$ carbenicillin, A_{405}/A_{500} increased from 1.0 to 1.4. Furthermore, the quaternary ammonium of PTQ2 may form an N^+-OH^- complex that can effectively weaken the electrostatic interactions between PTQ2 and carbenicillin at the base environment^[21]. Conversely, carbenicillin is more stable at lower pH. This result indicates that PTQ2 can effectively interact with carbenicillin under neutral medial pH; therefore, 7.4 is the optimum pH for experimental conditions.

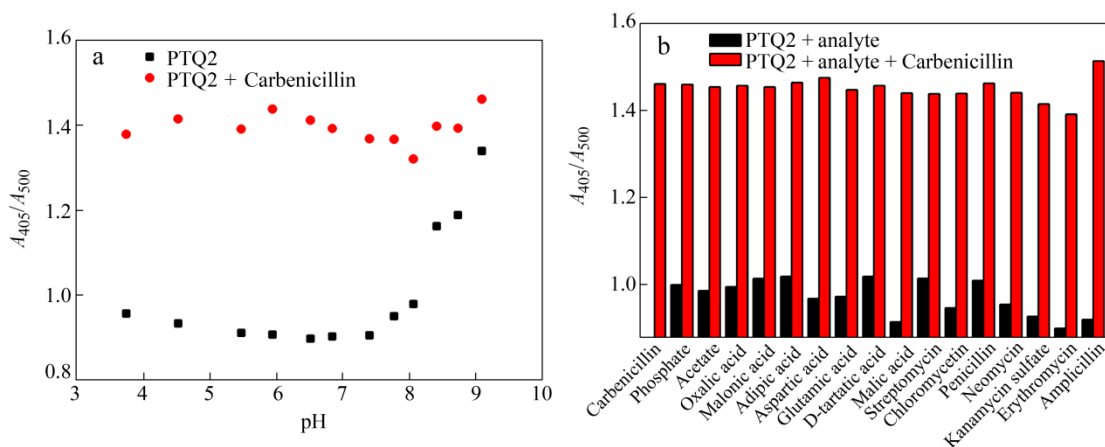


Fig. 3 (a) Effect of pH on the absorbance intensity ratio (A_{405}/A_{500}) of free PTQ2 (50 $\mu\text{mol/L}$) (black squares) and PTQ2 (50 $\mu\text{mol/L}$) / carbenicillin (20 $\mu\text{mol/L}$) mixtures (red dots) at room temperature; (b) Selectivity of PTQ2 (50 $\mu\text{mol/L}$) to carbenicillin (20 $\mu\text{mol/L}$) over competing some antibiotics and other important biological species in 10 mmol/L $\text{CH}_3\text{CN/HEPES}$ buffer solution ($V/V = 1/9$, pH = 7.4) (Interfering species: phosphate, acetate, oxalic acid, malonic acid, adipic acid, aspartic acid, glutamic acid, D-tartaric acid, streptomycin, chloromycetin, penicillin, neomycin, kanamycin, erythromycin and ampicillin.)

In numerous situations, several antibiotics may be used together in research or any clinical application. The selectivity of PTQ2 was evaluated by monitoring A_{405}/A_{500} response in the presence of other antibiotics and biologically important species in HEPES buffer solution. As depicted in Fig. 3(b), at the same concentration as carbenicillin (20 $\mu\text{mol/L}$), other antibiotics including streptomycin, chloromycetin, penicillin, neomycin, kanamycin, erythromycin, and ampicillin did not significantly increase in the absence of carbenicillin. By contrast, only the addition of an equimolar amount of carbenicillin increased A_{405}/A_{500} to 1.4. Otherwise, A_{405}/A_{500} did not induce obvious changes in the presence of dicarboxylic acids, such as sodium acetate, oxalic acid, malonic acid, adipic acid, aspartic acid, glutamic acid, and D-tartaric acid. Phosphate was also tested and showed only slight interference. Moreover, the presence of other coexisting species did not interfere with the selective detection of carbenicillin. The reason might be attributed to the molecular structure of PTQ2 that is topologically complementary with the structure of carbenicillin. As illustrated in Scheme 1, the rigid polythiophene backbone linked with quaternary ammonium quinine derivative forms an appropriate cavity, which is propitious to the special binding with carbenicillin *via* electrostatic interaction and geometric match effect. This finding indicates that the water-soluble polythiophene PTQ2 is highly selective in response to carbenicillin.

CONCLUSIONS

In summary, we have successfully developed a colorimetric sensor for the selective detection and quantification of carbenicillin at the submicromole level through the intermolecular interaction between PTQ2 and carbenicillin. The appealing performance of the sensor was attributed to electrostatic interaction and geometric match effect. PTQ2 takes more nonplanar structure upon complexation with carbenicillin, which exhibits a relatively blue-shifted absorption wavelength. We believe that this newly proposed design strategy can also be extended to design other highly sensitive and selective biomolecular sensors.

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