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Synthesis and characterization of triazole based supramolecule for interaction with cefuroxime in tap water and blood plasma

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ABSTRACT

In this study a new calix[4]arene triazole **5** was successfully synthesized using click reaction and characterized through UV–visible, FT-IR, ^{1}H NMR spectroscopes and Mass Spectrometry. The supramolecular interaction of compound **5** towards commonly used drugs has been carried out using UV–Visible spectroscopy. The supramolecule **5** showed characteristic enhancement in the absorbance intensity after mixing with Cefuroxime at pH (2–12). Compound **5** displayed considerably good interactions with cefuroxime in the presence of other drugs. Compound **5** exhibits linear relationship with cefuroxime concentration in the range of (10–80 μM) with regression value of 0.9954. The standard deviation for 50 μM Cefuroxime was found to be 0.01 and the limit of detection for cefuroxime was calculated to be 2 μM. Job's plot experiments showed 1:1 (**5:** cefuroxime) binding stoichiometry between compound **5** and cefuroxime. Supramolecule **5** displayed fairly good spectrophotometric recognition of Cefuroxime in human blood plasma and tap water thus showing that the ingredients of tap water and plasma sample was inert in the recognition of cefuroxime.

1. Introduction

The usage of antibacterial agents is increasing significantly in recent years causing serious concerns about potential ecological risks and the spread of antibacterial resistance among microorganisms. Most of the antibiotics can be approximately digested by humans and animals, they might be spread into the surroundings from human and agricultural sources, as excretory products, medicinal waste, release from wastewater treatment services, out-of-date prescriptions, leakages from infected systems, agricultural waste-storage structures and many others.

The existence of pharmaceuticals, especially antibiotics in the surroundings and in food gained increasing attention due to their severe side effects. The widespread use of antimicrobial agents is leaving medicine with few functioning therapeutic choices to treat several infections due to the fact that many organisms developed resistance to frequently used antibiotics (Laxminarayan et al., 2013; Tang et al., 2014). Antimicrobial resistance is a worldwide problem for both public and animal health. Cefuroxime [(6R,7R)-3-carbamoyloxymethyl-7-[Z-2-methyloxyimino-2-(2-furyl) acetamido]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2 carboxylic acid], is a well-known cephalosporin antibacterial agent used against several bacterial infections. It is effective to treat infections of ear, respiratory tract infection including *pneumonia*, sinuses, skin, throat, tonsils, and urinary tract (Bae et al., 2010; Bischoff et al., 2010). Cefuroxime is not fully absorbed and excreted without any change in urine and has no active components (Foord, 1976).

Since cefuroxime has been extensively used as antibacterial agents, it is critical to develop and validate novel approaches for the determination of cefuroxime in environmental and biological samples. Numerous analytical methods have been reported in the literature for the detection of cefuroxime in pharmaceutical and environmental samples like HPTLC (Ranjane et al., 2010), high performance liquid chromatography with UV–visible (Cheng and Chou, 2001) mass spectrometry (Carlier et al., 2012; Tuerk et al., 2006), microbiological techniques (Garton et al., 1997) thin-layer chromatography and densitometry (Krzek and Dąbrowska-Tylka, 2003) liquid chromatography tandem mass spectrometry(LC–MS/MS)(Partani et al., 2010) and capillary electrophoresis (Pajchel and Tyski, 2000).

Now a days chemosensors based on the concepts of supramolecular chemistry and photochemistry have been used extensively for the detection of various neutral molecules and ions due to their high sensitivity, selectivity and accessibility in application (Bell and Hext, 2004). Calix [4]arenes have been found to be a convenient molecular skeleton in the development of chemosensor, particularly for the detection of

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metal ions (Kim et al., 2002).

Calixarenes have been used as the basic molecular architecture in supramolecular chemistry, and modification of the calix[4]arene with the desired chromophores gives the Calixarenes with unique properties. After the discovery of click reaction in 2011 by Sharpless and co-worker significant progress were made in the synthesis of triazole based compounds for biological applications. The click generated 1, 2, 3-triazoles framework were also employed as sites for the binding of organic molecules. 1, 2, 3 triazole structure can interact with enzymes or receptor through hydrogen bond, coordination bond, pi–pi stacking, hydrophobic forces and many more thus displaying significant role in biological and pharmaceutical industries (Tron et al., 2008).

Herein we report straightforward synthesis and characterization of new calix[4]arene based triazole **5** which contain thiazole acetate moiety. Compound **5** interact with cefuroxime in DMF/H_2O (1:1) producing substantial enhancement in the absorption intensity. Compound **5** showed long time stability and quick response for Cefuroxime over a wide range of concentration without any interference.

2. Experimental

All of the chemicals, drugs and solvents were obtained from commercial sources and were used without any further purification. ^{1}H NMR and ^{13}C NMR spectroscopic studies were performed on a Bruker NMR spectrometer (Switzerland) at 300 and 75 MHz using tetramethylsilane as the internal standard. The chemical shifts values are expressed in units of ppm and coupling constant in Hz. Mass spectra were obtained using EI-MS, ESI Q-TOF and MALDI-TOF/TOF mass spectrometer. FT-IR spectra were recorded on a FT-IR 8900 Schimadzu using KBr disks. Stuart Apparatus (SMP10) was used for the measurement of melting point and were reported uncorrected. UV–visible spectra were performed using UV-1800 Shimadzu spectrophotometer (Tokyo, Japan) (1 cm quartz cell) at room temperature. Thin layer chromatography (TLC) analyses were carried out on silica gel plates and flash column chromatography was conducted using silica gel (300–400 mesh).

2.1. Synthesis and characterization

Calix[4]arene modified triazole **5** was synthesized using a click reaction [13] between ethyl 2-(2-(2-azidoacetamido)thiazol-4-yl)acetate and propargyl derivative of calix[4]arene in the presence of a copper (II) sulfate and sodium ascorbate as shown in Scheme 1. The structures of all synthesized compounds were established using ^{1}H NMR, ^{13}C NMR, spectra, and UV–visible as well as mass spectrometry techniques.

2.2. Synthesis of propargyl derivative of tert-butyl calix[4]arene(2)

In a 250 mL flask equipped with refluxed condenser and magnetic stirrer,4-tert-butylcalix[4]arene (600 mg, 0.92 mmol) and K_2CO_3(250 mg, 1.84 mmol) were dissolved in acetonitrile (30 mL) and was heated for 40 min at 60 ºC. After that, propargyl bromide (220 mg, 1.84 mmol) was added dropwise and reaction mixture was refluxed for additional 8 h. The progress of reaction was examined by thin layer chromatography (dichloromethane: Hexane (1:1 ratio)). After the accomplishment of reaction extra solvent was removed using rotary evaporator. The gel type mixture obtained was then diluted with cold water and extracted thrice with ethyl acetate (3 × 30 mL). The collective organic stuff was washed with brine solution, dehydrated using Na_2SO_4 and was concentrated under vacuum. The mixture was then purified using silica gel column chromatography (dichloromethane: Hexane 4:6 ratio) to get compound **2** as a white solid. Yield 420 mg, 70%.m.p. 166–168 ^{0}C, ESI-MS $[M+H]^+ m/z$ = 725.4567,^{1}H NMR (400 MHz,$CDCl_3$) δ: 0.87 (s,18H, CH_3), 1.28 (s, 18H, CH_3), 2.52 (t, 2H, CH), 3.31 (d, 4H, CH_2), 4.35 (d, 4H, CH_2), 4.72 (d, 4H, OCH_2), 6.45 (s,2H, OH), 6.70 (s, 4H, ArH),7.05 (s, 4H, ArH).

2.3. Synthesis of ethyl 2-(2-(2-azidoacetamido) hiazole-4-yl) acetate (4)

In a 100 mL round bottom flask ethyl 2-(2-(2-chloroacetamido) thiazole-4-yl) acetate (400 mg, 1.48 mmol) and NaN_3 (96 mg, 1.14 mmol) were mixed in dry acetone (25 mL). The resulting mixture was then refluxed for 6 h. After the complete conversion of reactants (monitored by TLC) the excess solvent was removed under vacuum. The solid product was diluted with water and extracted with dichloromethane (3 × 20 mL). The combined organic stuff was washed with saturated brine solution (20 mL), dried and concentrated using rotary evaporator which gives compound **4** as a white solid. Yield 300 mg, 90%. M.p. 82–84 °C, EI-MS m/z= 269.0, ^{1}H NMR (300 MHz, DMSO) δ: 1.17 (t, 3H, CH_3), 3.68 (s, 2H, CH_2CO), 4.05 (q, 2H), 4.10 (s, 2H), 7.02 (s, 1H) 12.36 (s, 1H).

2.4. Synthesis of calix[4]arene based bis-triazole (5)

To a solution of compound **2** (350 mg 0.48 mmol) and compound **4** (260 mg 0.95 mmol) in dry DMF (15 mL) fresh prepared solution of $CuSO_4$ (3.50 mg, 5 mol%) and sodium ascorbate (17 mg, 20 mol%) was added. The reaction mixture was then stirred at 80 ºC till the complete conversion of reactants. The reaction progress was monitored using TLC (Dichloromethane: Hexane 8:2). After the completion, reaction mixture was quenched using excess cooled water. The organic product was precipitate out which was then filtered washed with water, ammonia solution and dried under vacuum. The mixture was then subjected for column chromatography for purification (ethyl acetate: Hexane 4:6 ratio). Compound **5** was obtained as white solid. Yield 340 mg, 65%.m.p. 148–150 °C, MALDI-TOF/TOE $[M+Na]^+ m/z$ = 1285.55,^{1}H NMR (400 MHz, DMSO) δ: 1.12 (s, 18H, t-butyl H), 1.15 (t, 18H, t-butyl H, CH_3H,6H), 3.50 (s, 2H, CH_2), 3.68 (s, 4H, CH_2), 4.04 (overlapped, 10H, OCH_2, CH_2), 5.16 (s, 4H, $PhOCH_2$), 5.53 (s, 4H, $PhOCH_2$), 7.01 (s, 2H, OH), 7.04 (s, 4H, ArH), 7.08 (s, 4H, ArH), 7.92 (s, 2H, CH), 8.30 (s, 2H, CH), 12.69 (s, 2H, NH).

3. Results and discussions

The molecular recognition profile of **5** toward a number of common drugs were established by UV–visible spectroscopic analysis. Compound **5** showed a major absorption bands at 280 nm in DMF/H_2O (1:1 v/v) which is assigned to π → π* transition (Fig. 1).

DMF was used as a solvent for the preparation of 1 mM solution of triazole **5**, for experimental purpose diluted solution of **5** i.e. 50 μM was used, same concentration (50 μM) of the drugs solution were used which were prepared in deionized water.

3.1. Spectroscopic recognition of drugs

To evaluate the photophysical potential of compound **5** towards commonly used drugs, we examined the absorption changes upon addition of the one equivalent of several drugs as shown in Fig. 1. It can be seen from Fig. 1 that absorption intensity of compound **5** (50 μM) at 280 nm enhanced dramatically upon addition of cefuroxime (50 μM). By contrast, addition of all other drugs produced a slight spectral change in the absorption intensity of compound **5**. This change in the absorption intensity of compound **5** may be due to the possible hydrogen bonding or π-π stacking interaction between compound **5** and Cefuroxime. Results of this study reveals that compound **5** have specific binding site for cefuroxime.

3.2. Concentration dependent experiments

The binding affinity of compound **5** towards cefuroxime was further explored using concentration dependent experiments. The change in absorption intensity of **5** (50 μM) with different concentration of cefuroxime were recorded as shown in Fig. 2a. The concentration of **5** was