Ecotoxicology and Environmental Safety 141 (2017) 25–29

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Novel fluorene-based supramolecular sensor for selective detection of amoxicillin in water and blood

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ARTICLE INFO

Keywords:
Fluorescence spectroscopy
Molecular recognition
Supramolecular hosts
Amoxicillin

ABSTRACT

Synthesis, characterization and molecular recognition properties of fluorene based supramolecular cleft **1** is reported. The cleft molecule **1** was prepared in a single-step with good yield (85% yield), by linking Fluorene with 1-ethyl piperazine. The cleft molecule **1** was carefully characterized using various spectroscopic techniques such as NMR and mass spectrometry. The supramolecular interaction of cleft **1** with amoxicillin, 6APA, aspirin, captopril, cefotaxime, ceftriaxone, cefuroxime, diclofenac, penicillin, and cephradine was evaluated by fluorescent spectroscopy. The molecular recognition studies showed that amoxicillin selectively binds with cleft **1** in the presence of other drugs. The analytical method developed for the supramolecular interaction of molecular cleft **1** and amoxicillin was validated at varying pH, concentration and temperature during recognition process. Job's plots indicated that the stochiometry of the interactions between the cleft **1** and the amoxicillin was 1:1.

1. Introduction

The key process in many biological processes is the molecular recognition. For instance, in organisms the reactions catalyzed by enzymes are based on recognition that takes place between the host (catalyst) and guest (substrate) (Setny et al., 2013). The synthesis of artificial molecular sensor has got significant importance to mimic the biological processes. These artificial sensors have been used as a chemosensor for the detection of varied range of species mainly charged ions, (Sahin and Yilmaz, 2012) neutral analytes, (Czarnik, 1994) globular proteins,(Rakshit et al., 2013) and organic molecules like resorcinol, nicotine and cotinine. (Antwi-Boampong et al., 2014; Bell and Hext, 2004; Goutam and Iyer, 2015). These chemosensors have been used in environmental, clinical and biological fields, because of their high selectivity, sensitivity, highly efficient binding behaviour and low cast of preparation. (Ahmad et al., 2015; Sharma et al., 2015).

Due to the high degree of sensitivity for the analyte detection, fluorosensors have got particular importance among different classes of chemosensors (Khan et al., 2015). For the quantitative determination and efficient detection of various target species, fluorescent chemosensors are powerful tools (Han et al., 2010). There are several advantages of fluorescence based detection over other analytical methods. For instance specificity, high sensitivity and real time monitoring with fast reaction time (Lee et al., 2010). An excellent fluorescent sensor generally contains three important components: namely a fluorophore, a binding-recognition unit, and a signal conducting mechanism (Yang et al., 2010).

One of the most commonly used antibacterial drugs is amoxicillin (i.e., D-α-amino-*p*-hydroxybenzylpenicillin trihydrate) (Goodman, 1996), (James et al., 1993). Worldwide this antibacterial drug is used for the treatment of humans as well as agricultural livestock to protect them against various diseases and also to enhance its food production and growth. (Bergamini et al., 2006). Due to the worldwide clinical as well as biological and pharmaceutical use of amoxicillin, methods for is quantifications in the environment are important. For the detection and quantification of amoxicillin several methods such as spectrophotometric, (Mohamed, 2001; Pasamontes and Callao, 2004; Salem, 2004; Salem and Saleh, 2002) high-performance liquid chromatography (HPLC), (Liu et al., 2011) fluorometry, (Ma et al., 1999) atomic absorption spectrophotometry, (Li et al., 2000) chromatography, (Aghazadeh and Kazemifard, 2001) mass spectrometry (Wen et al., 2008), flow injection chemi-luminescence, (Fuwei et al., 2010) and electrochemical techniques. (Fouladgar et al., 2011) are available. However, some of these methods have poor sensitivity while most of

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http://dx.doi.org/10.1016/j.ecoenv.2017.03.003
Received 11 October 2016; Received in revised form 28 February 2017; Accepted 6 March 2017

them require expensive instrumentation and involve complicated measurement procedures.

The presence of modern drugs in the water reservoirs has become an alarming issue due to the extensive use of it (Fatta-Kassinos et al., 2011; Redshaw et al., 2013). In the third world countries the problem of drug using is even more pronounced, as the regulations of the usage of these drugs are not properly managed. For example, in Karachi Pakistan due to contaminated water six children were died and about 200 were fill ill in 2005. A large number of pharmaceutical drugs in highly alarming amount were found in different components of drinking water (surface water, drainage, and effluent) of Karachi in the microgram-per-liter range during bioassay directed chemical analysis (Selke et al., 2010). A sensitive and selective method for the amoxicillin detection in the drinking water is desirable as it is widely used drug and its high concentration in the water reservoir including the environment make it unsafe.

In this paper we report the synthesis and photophysical evaluation of the fluorene based piperazine derivative, a supramolecular cleft **1** as a highly sensitive and selective sensor for the detection of amoxicillin. Particularly, this supramolecular cleft was used for the detection of amoxicillin in human blood plasma and in drinking water reservoir in Karachi. This molecular cleft **1** binds selectively and sensitively with amoxicillin in acetone/water (1:1, v/v). This novel fluorene based sensor exhibits rapid response, great selectivity, high sensitivity as well as applicability over a wide range of drug concentrations and pH.

2. Experimental

2.1. Materials and methods

9,9-Bis-(3-methyl-4-hydroxyphenyl) fluorene, 1-ethylpiperazine, acetic acid and all drugs were used as received without further purification. The solvents acetone, tetrahydrofuran, methanol, and dichloromethane were of HPLC grade and used as received without further purification.

2.2. Instrumentations

A mass spectrometer (JEOL EI-MS) was used for the molecular weight measurements. The ^{1}H NMR spectra were recorded on a Bruker AV-III 300- MHz spectrometer using $CDCl_3$ as the solvent. The infrared (IR) spectra were obtained on a Shimadzu IR-460 instrument using KBr plates. The fluorescent spectra were recorded on a Perkin Elmer LS 55 Spectrometer with a quartz cuvette (path length =1 cm) at room temperature (r.t). A digital pH meter (Model 510, Oakton, Eutech) equipped with a glass working electrode and an Ag/AgCl reference electrode was used for pH measurements. All of the spectroscopic titrations experiments were performed using a 100 μM solution of the molecular cleft **1** in acetone and a 100 μM drug solution in deionised water. The drug solutions prepared were those of amoxicillin, captopril, cefotaxime, diclofenac, aspirin, penicillin, cefuraxime, cephradine, ceftriaxone, 6-APA.

2.3. Synthesis of molecular cleft 1

0.1 mL Formaldehyde (2.7 mmol) and 0.15 mL acetic acid (2.6 mmol) were added to 40 mL tetrahydrofurane followed by the addition of 0.371 mL of 1-ethylpiperazine (2.9 mmol). The mixture was stirred for about 10 min at 50 °C. Then 500 mg of 9,9-Bis(3-methyl-4-hydroxy phenyl)fluorene (1.3 mmol) was added to the reaction mixture and the mixture was subsequently stirred for 12 h at same temperature. After the completion of the reaction monitored by TLC, the reaction solvent was removed by vacuum distillation, resulted a gum-like material. The crude product obtained was washed with 5.0% ethyl acetate in hexane (3×30 mL). The product was further purified by using DCM/methanol (9.5/0.5, v/v) as eluent in silica gel column chromatography. Which resulted purified 400 mg of the cleft **1** in about ~85% yield. The structure of the molecular cleft **1** was confirmed by EI-MS (Fig. 1s) and ^{1}H NMR (Fig. 2s).

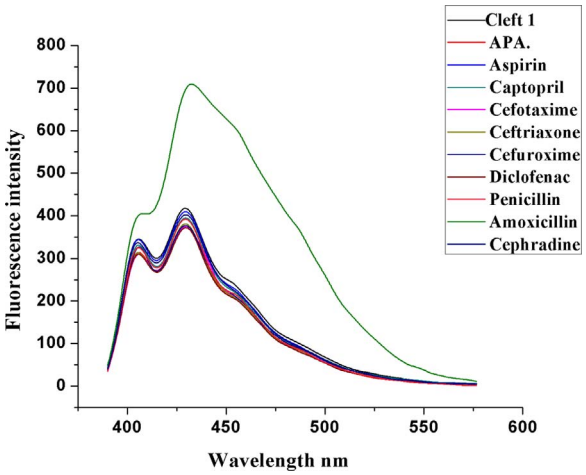

Fig. 1. Fluorescence emission spectra of molecular cleft **1** (100 μM) after it has been mixed with various drugs (100 μM) at pH 8.

EI-MS: (M^+) *m/z* 631.3 (calc. *m/z* 630.86).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72 (d, *J* =7.2 Hz, 2H, Ar-H), δ 7.32 (t, *J* =7.5 Hz, 4H, Ar-H), δ 6.79 (s, 2H, Ar-H), δ 6.61 (s, 2H, Ar-H), δ 3.54 (s, 4H, Ar-CH_2-N-piperazine), δ 2.56 (m, 20H), δ 2.06 (s, 6H, Ar-CH_3), δ 1.08 (s, 6H, Ar-CH_2-N-piperazine-N-CH_2-CH_3) (Scheme 1).

3. Results and discussion

Molecular cleft **1** is composed of fluorene-based piperazine derivative. The fluorene and piperazine are connected by a methylene (CH_2) unit via Mannich reaction. The molecular cleft **1** contains four nitrogen atoms above the CH_2 in the piperazine moieties and two phenolic groups. For the complexation with guest acceptor species, these are the potential donor sites.

Fig. 1 shows the binding capacity and fluorescent recognition behaviour of cleft **1** which was investigated with a range of drug molecules, including aspirin, 6APA, captopril, ceftriaxone, cefotaxime, cefuroxime, penicilline, diclofenac, cephradin and amoxicillin. Acetone/water (1/1 v/v) solutions was used for the spectroscopic titrations. Both amoxicillin and molecular cleft **1** exhibited emission maxima at 450 and 431 nm respectively. When equimolar solution of both amoxicillin and molecular cleft **1** was mixed a strong enhanced fluorescent emission band was observed at 431 nm. As Fig. 1 show, that except amoxicillin, the presence of other drugs, the fluorescence spectra of cleft **1** exhibited no significant change. Whereas with the addition of amoxicillin the fluorescence spectra of cleft **1** enhanced dramatically. This higher selectivity for amoxicillin of cleft **1** may be endorsed to the perfect fitting of amoxicillin into the room between the piperazine moieties.

The detection limit of molecular cleft **1** for amoxicillin was also explored. As shown in Fig. 2a that with the successive decrease in the concentration of amoxicillin the fluorescent emission spectra recorded keeping the concentration of cleft **1** constant. By decreasing the concentration of amoxicillin down to 800 nM, a gradual decrease in the emission was also observed. By plotting the normalized emission of cleft **1** versus the concentration of amoxicillin a linear relationship was observed Fig. 2b. At the same time, during the titration, in the emission spectra of molecular cleft **1**, no noticeable change was observed.

To find the selectivity for amoxicillin of cleft **1**, in the presence of other various drugs, the recognition studies of cleft **1**with amoxicillin (100 μM) were performed as shown in Fig. 3. The enhancement in the fluorescent emission at 431 nm may be due to the formation of hydrogen bonds between the amoxicillin and molecular cleft **1**. From