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A calix[4]arene derivative and its selective interaction with drugs (clofibric acid, diclofenac and aspirin)

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ABSTRACT

The synthesis and characterisation of a partially substituted calix[4]arene, namely, 5,11,17,23-tetra-*tert*-butyl,25,27-bis[aminoethoxy] 26,28-dihydroxycalix[4]arene are reported. Its interaction with commonly used pharmaceuticals (clofibric acid, diclofenac and aspirin) was investigated by spectroscopic (^{1}H NMR and UV), electrochemical (conductance measurements) and thermal (titration calorimetry) techniques. It is concluded on the basis of the experimental work and molecular simulation studies that the receptor interacts selectively with these drugs. Preliminary studies on the selective extraction of these pharmaceuticals from water by the calix receptor are reported and the potential for a carrier mediated sensor based on this ligand for '*on site*' monitoring of pharmaceuticals is discussed.

1. Introduction

In the last few years contamination of water by pharmaceuticals has become a serious concern by environmental scientists (Daughton and Ternes, 1999; Sorenson et al., 1998; Ternes et al., 1999). Pharmaceuticals can enter the aquatic environment through many sources resulting from their use for the treatment of humans and animals and can be excreted unchanged, as metabolites, as unused discharge or as residuals of drug manufacturers (Heberer, 2002; Winkler et al., 2001; Stumpf et al., 1999).

Several methods have been reported for the removal of pharmaceuticals from water such as metal salt coagulation and excess lime/soda ash softening (no significant removal), (Adams et al., 2002; Ternes et al., 2002; Boyd et al., 2003) powdered activated carbon (slow kinetics of sorption (Boyd et al., 2003; Westerhoff et al., 2005), material becomes saturated over time, non-selective (Boyd et al., 2003; Westerhoff et al., 2005)). Chlorination (by products formed (Boyd et al., 2003; Westerhoff et al., 2005) of unknown toxicity, removal rate is pH dependent which could not be controlled in a treatment plant environment (Pinkston and Sedlak, 2004)), ozonation (Huber et al., 2003; Huber et al., 2005; Ternes et al., 2003) (oxidation by ozone is effective to remove most pharmaceuticals from water but the toxicity of ozone and by-products resulting from this process has adverse implications on human health and the environment), UV photolysis (Up to 80% removal only with very high input of 3000 mJ cm^{-2}. Inefficient (Heberer, 2002)), ion exchange (non-selective), reverse osmosis (90% of pharmaceuticals are removed but it is expensive (Heberer, 2002)).

Diclofenac {2-[2-(2,6-dichlorophenyl)aminophenyl]ethanoic acid} is a non-steroidal anti-inflammatory drug currently used to treat painful conditions resulting from arthritis, sprains and strains, gout, migraine, and other illnesses. Clofibric acid [2-(4-chlorophenoxy)-2-methylpropanoic acid] is known as the active metabolite of a number of drugs currently used to reduce the levels of cholesterol in blood. Aspirin (acetyl salicylic acid) is one of the most frequently used drugs, it is an analgesic with pain relief and anti-inflammatory properties (Albert, 2010). In small doses, aspirin is recommended to reduce the risks of heart attacks, angina and strokes (Mundasad, 2016; Lewis et al., 1983). Side effects of this drug have been reported (Gorelick, 2009). These pharmaceuticals are widely detected in the environment (Nikolaou et al., 2007). As far as diclofenac is concerned, methodologies used for its removal from water include electron beam radiation combined with a biological aerated filter (Dal et al., 2011), pyrite catalysed Fenton oxidation (He et al., 2014), double templates- molecularly imprinted polymers (Bae et al., 2013), cork-based activated carbon (Dai et al., 2013), poly-ethylenimine-modified chitosan beads (Mestre et al., 2010), membrane bioreactors (Tambozi et al., 2010), and modified clays (Dordio et al., 2009). In deriving the Margin of Exposure (MOE) of pharmaceuticals, calculated guideline values were based on comparisons made with maximum concentrations found in secondary treated effluents. It was then assumed that values in drinking water are likely to be lower and therefore their presence may not lead to a

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negative impact on human health (Anon, 2012). This may be the case for highly developed Countries but not necessarily for the Developing World where often water treatments are not always applied.

Therefore, the threshold of toxicological effects due to the presence of pharmaceuticals in water needs further research. Another issue to be addressed is the need for establishing standard protocols for sampling and monitoring pharmaceuticals in water. Advanced monitoring techniques such as gas chromatography-mass spectrometry (GC-MS), ion chromatography-mass spectrometry (IC-MS) and others have been used to detect trace amounts of these compounds. Again these techniques are not always available and all of them require the transport of samples from the site to the laboratory. Therefore, efforts are required for '*on site*' monitoring of pharmaceuticals in water.

In technologies and monitoring devices aiming for the removal/detection of pollutants from/in contaminated sources it is imperative to introduce the concept of selectivity (ability of the decontaminating agent to recognise one species relative to others), often confused with specificity (recognition of only one particular target while discriminating against others). Selectivity is one of the main features of Supramolecular Chemistry (Cabrera-Lafaurie et al., 2012) and therefore it is surprising to find that this field has not been explored for the removal/monitoring of pharmaceuticals from/in water. Calix[4]arenes (products of the condensation reaction of *p*- substituted phenol and formaldehyde in basic medium) are versatile receptors (Gutsche, 1989; Gutsche, 2001, 2007; Mandolini and Ungaro, 2000). These receptors can be easily modified through the lower and upper rims and a number of derivatives are known to interact with ionic (through the hydrophilic cavity) and neutral (through the hydrophobic cavity situated between the phenyl rings) species (Danil de Namor et al., 1998, 2013). The importance of fundamental studies in searching for applications is a relevant issue to address in the design of monitoring systems for the detection of pollutants as well as for the development of novel technologies for their removal from contaminated sources and most reports in the literature indicate that this is not often the case.

In this paper we report an investigation involving a calix[4]amine derivative, **1**, and its interaction with currently used pharmaceuticals, namely diclofenac, aspirin and clofibric acid (Fig. 1). The selection of the amine functionality in the structure of **1** is based on its great affinity for the proton.

Thus fundamental studies using a variety of techniques (spectroscopic, electrochemical and thermodynamic) involving this receptor and these pharmaceuticals are explored to assess the selectivity of **1** for these pharmaceuticals as the basis for environmental applications.

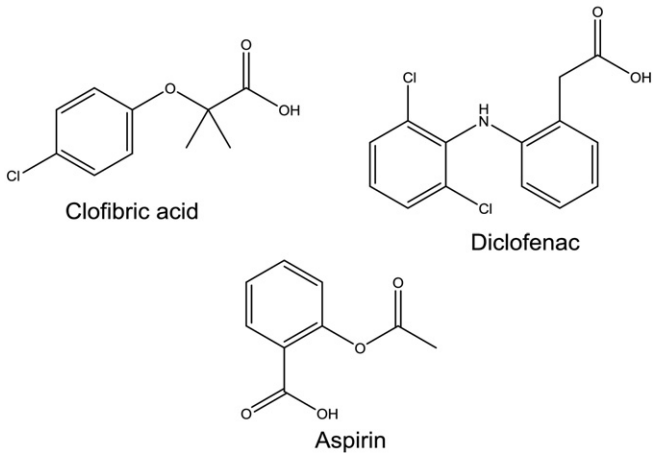

Fig. 1. Chemical structures of chlofibric acid, diclofenac and aspirin.

2. Experimental

The synthesis of **1** was carried out according to the following Scheme,

The procedure applied by Danil de Namor and Shehab (Shehab, 2005) for the synthesis of calix[4]pyrrole derivatives was adapted for the preparation of **L1** and **1** (Scheme 1).

*2.1. Synthesis of **L1** calix[4]arene derivative (Danil de Namor et al., 2002)*

Thus in a three-neck round bottom flask (500 ml) containing a magnetic stirrer at room temperature under an inert atmosphere, *p-tert*-butyl calix[4]arene (97% HPLC, Sigma- Aldrich,10.293 g, 15.862 mmol), 18-crown-6 (Fluka, 99%, 1.004 g, 3.799 mmol) and potassium carbonate (Fisher Scientific, 99.99%, 18.087 g, 130.869 mmol) were dissolved in dry acetonitrile (Fisher Scientific, HPLC grade, 99.99%, 200 ml). Bromoacetonitrile (Aldrich, 97%., 7.394 g, 61.643 mmol) dissolved in acetonitrile (20 ml) was added drop-wise. The reaction mixture was heated in an oil bath at 75 °C and refluxed for 24 h under vigorous stirring. The reaction was monitored by thin layer chromatography using a mixture of hexane: ethyl acetate (8:2) as the developing solvent. After 24 h the reaction mixture was allowed to cool down to room temperature. The solvent was removed under reduced pressure and a yellow solid was obtained. The solid was dissolved in dichloromethane (Sigma-Aldrich, ≥ 99%), filtered gravitationally and the solvent was removed under reduced pressure. Methanol (Sigma-Aldrich, HPLC grade, 99.7%) was added to the resulting oil which was broken by sonication. Afterwards the solid was filtered and washed with cold methanol. The solid was left in a vacuum drier at 90 °C. The product obtained in 70% yield was characterised by ^{1}H NMR spectroscopy: ^{1}H NMR (500 MHz, in $CDCl_3$) δ (ppm); 7.11 (s, Ar-**H**, 4H), 6.72 (s, Ar-**H**, 4H), 5.540 (s, O**H**, 2H), 4.80 (s, O-C**H**$_2$, 4H), 4.19 (d, C**H**$_{ax}$, 4H), 3.42 (d,C**H**$_{ax}$)1.34 (s, C**H**$_3$, 18H), 0.87 (s, C**H**$_3$, 18H).

*2.2. Synthesis of 5, 11, 17, 23 tetra-p-tert-butyl, 25, 27-bis[aminoethoxy], 26, 28 dihydroxycalix[4]arene, **1** (Villanueva Salas, 2005)*

A solution of **L1** (9 g, 21.45 mmol) was dissolved in dry THF (Scientific, HPLC grade, 99.99%, 100 ml) and stirred in an ice bath until the temperature reached 0 °C, $LiAlH_4$ (95%, Sigma-Aldrich, 1.896 g, 49.95 mmol) was dissolved in freshly dried THF then added drop-wise to the reaction mixture with continuous stirring for 4 h and the temperature was kept at 0 °C. The reaction was monitored using TLC and a hexane:ethyl acetate (8:2) mixture as the developing solvent, (10 ml, 20%). Then an aqueous solution of NaOH was added, followed by distilled water (10 ml). A white precipitate was obtained. The undesired white precipitate was filtered gravitationally and the solvent was evaporated by using a rotary evaporator. A white solid was obtained and dried under vacuum at 90 °C. The product obtained in 60% yield was characterised by ^{1}H NMR spectroscopy, (500 MHz, in $CDCl_3$), δ (ppm), 7.03 (s, Ar-**H**, 4H), 6.96 (s, Ar-**H**, 4H), 5.2 (s, O**H**, 2H), 4.34 (d, Ar-C**H**$_{2ax}$-Ar, 4H), 4.06 (t, OC**H**$_2$, 4H), 3.37 (d, Ar-C**H**$_{2eq}$-Ar, 4H), 3.3 (t,N-C**H**$_2$), 1.2 (s, C**H**$_3$, 18H),1.09(s,CH_3,18H).

2.3. NMR measurements

^{1}H NMR measurements were carried out at 298 K using a Bruker AC-500 MHz. ^{1}H NMR titrations were carried out by adding increasing amounts of known concentrations of the pharmaceuticals (diclofenac, aspirin and clofibric acid, all from Aldrich) dissolved in CD_3CN (Cambridge Isotope Laboratories, 99.8%) to the NMR tube containing a known concentration of the receptor in the same deuterated solvent. Chemical shift changes of the receptor after each addition of the drug were recorded. Subtracting the chemical shifts for the free receptor δ_r from those of the complex, δ_C, the chemical shift changes $\Delta\delta$ were