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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.

INTRODUCTION

In the last few years contamination of water by pharmaceuticals has become a serious concern by environmental scientists.¹⁻³ 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 manufacturers⁴⁻⁶

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),⁷⁻⁹ powdered activated carbon (slow kinetics of sorption,^{9,10} material becomes saturated over time, non-selective^{9,10}). Chlorination (by products formed^{9,10} of unknown toxicity, removal rate is pH dependent which could not be controlled in a treatment plant environment¹¹), ozonation¹²⁻¹⁴ (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.⁴), ion exchange (non-selective), reverse osmosis (90% of pharmaceuticals are removed but it is expensive⁴).

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. Clofibrilic 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 properties¹⁵. In small doses, aspirin is recommended to reduce the risks of heart attacks, angina and strokes.^{16,17} Side effects of this drug have been reported.¹⁸ These pharmaceuticals are widely detected in the environment.¹⁹ As far as diclofenac is concerned, methodologies used for its removal from water include electron beam radiation combined with a biological aerated filter,²⁰ pyrite catalysed Fenton oxidation,²¹ double templates- molecularly imprinted polymers,²² cork-based activated carbon,²³ poly-ethylenimine-modified chitosan beads,²⁴ membrane bioreactors,²⁵ and modified clays.²⁶ 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 negative impact on human health.²⁷ 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 Chemistry²⁸ 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 receptors²⁹⁻³². 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.^{33,34} 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 clofibrac 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.

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

EXPERIMENTAL

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

Scheme 1. Synthetic procedure used for the preparation of **L1** and **1**

The procedure applied by Danil de Namor and Shehab³⁵ for the synthesis of calix[4]pyrrole derivatives was adapted for the preparation of **L1** and **1**.

Synthesis of **L1** calix[4]arene derivative³⁸

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 $75^\circ C$ and refluxed for 24 hours 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 hours 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, $\geq 99\%$), 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 ^1H NMR spectroscopy: ^1H NMR (500 MHz, in CDCl_3) δ (ppm); 7.11 (s, Ar-H, 4H), 6.72 (s, Ar-H, 4H), 5.540 (s, OH, 2H), 4.80 (s, O-CH₂, 4H), 4.19 (d, CH_{ax}, 4H), 3.42 (d, CH_{ax}) 1.34 (s, CH₃, 18H), 0.87 (s, CH₃, 18 H).

Synthesis of 5, 11, 17, 23 tetra-*p*-*tert*-butyl, 25, 27- bis[aminoethoxy], 26, 28 dihydroxycalix[4]arene, 1³⁶

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, LiAlH₄ (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 four hours 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 ^1H NMR spectroscopy, (500 MHz, in CDCl_3) δ (ppm), 7.03 (s, Ar-H, 4H), 6.96 (s, Ar-H, 4H), 5.2 (s, OH, 2H), 4.34 (d, Ar-CH_{2ax}-Ar, 4H), 4.06 (t, OCH₂, 4H), 3.37 (d, Ar-CH_{2eq}-Ar, 4H), 3.3 (t, N-CH₂), 1.2 (s, CH₃, 18H), 1.09 (s, CH₃, 18 H).

NMR measurements

^1H NMR measurements were carried out at 298 K using a Bruker AC-500 MHz. ^1H 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 calculated from Eq.1

$$\Delta\delta = \delta_c - \delta_r \quad \text{Eq. 1}$$

Conductometric measurements

The conductivity cell constant was determined according to the method described by Jones and Bradshaw³⁷ as previously reported.³⁸

Conductometric titrations were carried out by the gradual addition of the receptor solution in acetonitrile to the conductivity cell containing the targeted guest in the same solvent at 298.15 K. For these measurements, the conductivity cell connected to a thermostated water bath and loaded with the guest solution in acetonitrile (25 ml). Then the electrodes were immersed into the cell containing the solution under continuous stirring during the course of the titration. Accurate aliquots of the receptor were gradually added to the guest solution in the same solvent, the resistance was recorded after each addition. The molar conductance, Λ_m (S cm² mol⁻¹) was calculated.

UV measurements

UV-visible spectrophotometric measurements were taken on a Cecil CE7200 spectrophotometer. UV-Vis spectra were recorded in the 200-400 nm range using 1 cm quartz cell to find the wavelength of maximum absorption. For the spectrophotometric titrations, aliquots of a solution of **1** in acetonitrile were added to the UV cell containing a solution of the targeted drug in the same solvent.

Calorimetric measurements

The reliability of the isothermal calorimeter (ITC) was checked by carrying out the standard reaction between Ba (II) and 18- crown- 6 in water at 298.15 K suggested in the literature³⁹

The Nano- ITC measurements were carried out in degassed acetonitrile at 298.15 K. For assessing complex formation, a solution of **1** (1 mM) in acetonitrile was placed in the calorimetric cell (1.8 cm³). Solutions of each guest (20 mM) in the same solvent were added (5 or 10 μ L) successively into the host solution in the same solvent with a 300 s interval between each injection. Corrections for the heat of dilution were applied. The Nano ITC is connected to a personal computer that included the software ITCRun which follows the information and the status of the reaction taking place.

Molecular modelling

Modelling was carried out using MOE 2015.10.⁴⁰ Models were built using the BUILDER module and energy minimised with the MMFF94X force field.⁴¹ A distance dependent van der Waals cut-off was used of 8 Å (below this distance atoms were treated normally) and van der Waals interactions were discounted after 10 Å. A dielectric constant of 1 was used for atoms below the lower cut-off distance and a value of 80 was used above. Minimisation was carried out using the smart minimiser that combines steepest descents, conjugate gradients and Newton-Raphson methods as appropriate. Minimisation was carried out to a convergence criterion of 0.0042 RMS (kJ/mol/Å²)

Extraction of pharmaceuticals from water

Preliminary experiments to assess the effect of the mass of the receptor, pH and temperature as well as the kinetics of the extraction process were carried out as previously described.⁴² Aliquots were taken and analysed by UV spectrophotometry at 280 nm. The uptake capacity of **1** was determined in batch experiments using a constant amount of the receptor (0.1 g) and aqueous solutions containing different concentrations of the pharmaceutical (9.10^{-5} – $4.4.10^{-4}$ mol.dm⁻³). The tubes containing the mixture were then placed in a water bath and the temperature was maintained at 298 K. Samples were left overnight to equilibrate and then analysed by UV spectrophotometry. The uptake capacity, q_{eq} (mmol g⁻¹), was calculated using eq.

$$q_{eq} = \frac{(c_i - c_{eq}) \times V}{m} \quad \text{Eq. 3}$$

In Eq.3, V denotes the sample volume (cm^3), c_i and c_{eq} are the initial and the equilibrium concentrations (mmol/dm^3) respectively, m is the amount of the receptor used.

RESULTS AND DISCUSSION

Interaction of **1** with pharmaceuticals

Several analytical techniques were used to assess the interaction of **1** with aspirin, clofibric acid and diclofenac in acetonitrile such as ^1H NMR, conductance, UV spectrophotometry and titration calorimetry. The lack of solubility of the receptor in water led us to select acetonitrile as the solvent. A further advantage of the use of this solvent is that the drugs are unlikely to be well solvated in this medium. In this way solvent-receptor competition for the drug decreases substantially and consequently the strength of guest-receptor interactions increases as previously discussed by Danil de Namor and co-workers.³³

As far as the ^1H NMR measurements are concerned, the results in CD_3CN listed in Table 1 show the chemical shifts of the free receptor, δ , and the chemical shift changes, $\Delta\delta$, for the receptor upon the addition of the pharmaceutical. No chemical shifts changes were found by the addition of other drugs containing carboxylic functionalities such as ciprofloxacin (antibiotic used against a wide range of bacterial infections)⁴³, aurintricarboxylic acid (inhibitor of RNA enzymes)⁴⁴, nalidixic acid⁴⁵ and ibuprofen (anti-inflammatory drug)⁴⁶. As far as the free receptor is concerned, quite clearly **1** adopts a *quasi* perfect 'cone' conformation in solution as reflected in the difference observed in the chemical shifts of the axial and equatorial protons ($\Delta\delta_{\text{ax-eq}} = 1.03 \text{ ppm}$).⁴⁷ This conformation is due to the presence of hydrogen bond formation between the phenolic hydrogen and the ethereal oxygen of the pendant arms of the receptor.

Table 1. Chemical shift changes (in ppm) for the receptor protons as a result of the addition of the drugs to the receptor solution in CD_3CN at 298 K.

	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	N-H
Free L	1.20	1.16	7.24	7.17	3.39	4.32	4.01	3.22	2.17
Clofibric acid	-	-	-	-	0.06	-0.21	-0.09	0.15	0.32
Diclofenac	-	0.01	0.01	-	-	-0.21	0.14	0.17	0.21

Aspirin	-	-	-	-	0.08	-0.11	0.25	0.24	0.29
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It was previously demonstrated³⁸ for an homologous receptor, namely 5,11,17,23-*tetrakis*[(diethylamine)ethoxy]calix[4]arene, in the solid state (X ray diffraction studies) and in solution. that the $\Delta\delta_{ax-eq}$ = 1.02 ppm in CD₃CN for this homologous receptor is practically the same as that for **1**. Furthermore acetonitrile was found in the hydrophobic cavity of the receptor. Also the X ray structure of **2** showed acetonitrile in the hydrophobic cavity of the receptor. Previous studies from our group in this solvent demonstrated that this process is also observed in solution.⁴⁸ In fact, this is also the case for **1** as assessed from the chemical shift changes observed for the aromatic protons of this receptor in moving from CDCl₃ to CD₃CN where downfield chemical shifts of 0.21 and 0.19 ppm are observed.

Further conformational changes are also observed in the bridging methylene protons during the complexation or protonation process. The equatorial protons become less shielded relative to the free receptor particularly for clofibric acid and diclofenac (-0.21 ppm) while for aspirin this effect is less pronounced (-0.11 ppm) while the equatorial protons are slightly deshielded relative to the uncomplexed receptor. A direct consequence of that, is a decrease in the shift difference between each pair of doublets of the ArCH₂Ar system, from the free to the complex ligands (from 1.03 to 0.76 ppm for aspirin and to 0.66 ppm for clofibric acid and diclofenac), Significant downfield chemical shift changes are observed for H-7, H-8 and the NH proton as a result of an interaction between the amino group of the receptor and the proton of the carboxylic functional group of the drug. The most significant chemical shift changes are found for the NH proton. In an attempt to explain the differences observed in the $\Delta\delta$ values for the NH proton for these pharmaceuticals (Table 1) the pK_a values in water reported in the literature are considered⁴⁸ (pK_a, values are 3.0, 3.5 and 4.16 for clofibric acid, aspirin and diclofenac respectively) due to the unavailability of these data in acetonitrile. Although the absolute pK_a values of these drugs in the latter solvent are expected to differ significantly from those in water due to the medium effect, the order would be the same. It is therefore concluded that the downfield shift of the NH proton of **1** is strongly dependent of the degree of dissociation of the acid. This is further corroborated by i) ¹H NMR titrations carried out in CD₃CN. Representative examples for the titration of **1** with clofibric acid (a) and diclofenac (b) are given in Figure 2 where $\Delta\delta$ values obtained by the addition of these drugs to **1** are plotted against the drug/1

molar ratio where the most pronounced chemical shift changes are observed for the NH protons and to a lesser extent for H-7 and H-8.

ii)

Figure 2. ^1H NMR titration of 1 with clofibric acid (a) and diclofenac (b) in CD_3CN at 298 K

ii) The lack of interaction with other drugs tested given that these behave as relatively weak acids in water as reflected in their pK_a values which are 6.09, 6.1 and 4.54 respectively for cyprofloxacin⁴⁹, nalidixic acid⁵⁰ and ibuprofen⁵¹ and are expected to be undissociated in acetonitrile

Therefore we proceeded with conductometric titrations which as previously demonstrated^{49,33} can provide information on the composition of the complex as well as identifying whether the receptor-drug interaction is the result of hydrogen bond formation or a proton transfer reaction (from the pharmaceutical to the calixarene amine derivative) or both. To ensure that the receptor was not protonated its conductance was measured in MeCN prior to proceeding with

the titrations. No conductance was observed. However the conductometric curves for the titration of these drugs with the receptor in acetonitrile provide a great deal of information. Figure 3 shows the titration of clofibric acid (a), diclofenac (b) and aspirin (c) with **1** in MeCN and at 298,15 K where the molar conductances, Λ_m ($\text{S.cm}^2.\text{mol}^{-1}$) are plotted against the 1:drug concentration ratios.

Figure 3. Conductometric titrations of **1** with (a) clofibric acid (b) diclofenac and (c) aspirin in acetonitrile at 298.15 K

The conductometric titration curve for aspirin was found to show hardly any curvature to define the composition of the interaction with this drug and therefore a UV titration was carried out. As shown in Figure 4, a complex of 1:1 stoichiometry is formed between this drug and **1**

Figure 4. UV titration of aspirin with **1** in acetonitrile at 298 K.

Interpretation of these data shows that

- i) Prior to the addition of the receptor, the drugs are highly associated in this solvent as reflected by the low conductance observed even at low concentrations.
- ii) Upon addition of **1**, a significant increase in conductance is observed which must be attributed to a proton transfer reaction from the drug to the amine functional group of the receptor
- iii) The composition of the complex appears to be 1:1 for aspirin which differs from those for clofibric acid and diclofenac where two slight breaks are found in the conductometric titration curves at concentrations ratios of 0.5 and 1 which appear to indicate the formation of 1:2 (A to B) and 1:1 (B to C) complexes .

Having characterised the sites of interaction of the receptor and the composition of the complexes in MeCN at 298 K, we proceeded with the determination of the thermodynamic parameters in this solvent using isothermal titration calorimetry (ITC). These are now reported in Table 2 where the stability constants (expressed as $\log K_s$), the standard enthalpy, $\Delta_c H^\circ$ and entropy, $\Delta_c S^\circ$ for the interaction with aspirin, clofibric acid and diclofenac in acetonitrile at 298.15 K are listed in this Table. The standard deviation of the data are also included.

Table 2. Thermodynamic parameters for the complexation of **1** with pharmaceuticals in acetonitrile at 298.15 K

Drug	$\log K_s$	$\Delta_c G^\circ$ (kJ mol ⁻¹)	$\Delta_c H^\circ$ (kJ mol ⁻¹)	$\Delta_c S^\circ$ (J mol ⁻¹ K ⁻¹)
Clofibric acid	7.30 ± 0.05 (Eq. 4)	-41.4 ± 0.3	-48.1 ± 0.7	-23
	6.1 ± 0.1 (Eq. 5)	-35.7 ± 0.1	14 ± 3	168
Diclofenac	5.8 ± 0.7 (Eq. 4)	-33 ± 4	-34.5 ± 0.1	-4
	5.1 ± 0.6 (Eq. 5)	-29 ± 3	17 ± 3	150

Aspirin	3.9 ± 0.1 (Eq. 4)	-22.4 ± 0.6	-23.0 ± 0.3	-2
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The data for aspirin fits into a 1:1 (host: guest) complex while those for clofibric acid and diclofenac into 1:2 complexes. As previously stated thermodynamics does not provide structural information. However, it is indisputable that any model proposed must fit the experimental data. Therefore, the thermodynamic data are referred to the following processes,

For 1:1 complexes of aspirin, clofibric acid and diclofenac, eq. 4 is representative of the process taking place in solution on the results obtained from conductance data that these pharmaceuticals predominantly exist as ion pairs in acetonitrile

For 1:2 complexes of clofibric acid and diclofenac,

For aspirin the process is enthalpy controlled. The same applies for the 1:1 complexes of clofibric acid and diclofenac. However the formation of 1:2 complexes is enthalpically unfavoured and entropically controlled. These results are striking and suggest that a great disorder in the structure of the receptor occurs to allow the entrance of the second proton. X ray crystallographic studies of an analogous receptor,**2**, with perchloric acid³⁶ has shown the double protonation of the receptor as well as the loss of the solvent upon protonation while the hydrogen bond between the ethereal oxygen and the phenolic oxygen still remains. The conformational change that **1** undergoes in moving from the free to the protonated ligand as shown by ¹H NMR studies in CD₃CN indicates a flattened 'cone' conformation for the latter relative to the *quasi* 'cone' conformation of the former and as a result the hydrophobic cavity is able to lose the solvent. This process will lead to an increase in entropy. Given that energy would be required to remove the solvent from the cavity, the process would be enthalpically unfavoured. This interpretation is corroborated by the data reported in Table 2. Comparison of the protonation constants of **1** with clofibric acid and diclofenac in acetonitrile at 298.15 K relative to those for **2** and HClO₄ in the same solvent and temperature (log K_{s1}=15.68, log K_{s2}=14.36) demonstrates the effect of the strength of the acid on the protonation process as well as the basic nature of the amine moieties in the pendant arms given that the basicity of the tertiary

amine in **2** is higher than that of **1** (primary amine). However the most interesting finding of this research is that this receptor besides having a higher capacity for clofibric acid and diclofenac relative to aspirin is able to recognise selectively these drug to the extent that in terms of a 1:1 receptor: drug interaction **1** is more selective for clofibric acid relative to diclofenac and aspirin by factors of 32 and 2399 respectively in acetonitrile while the overall selectivity of the receptor for clofibric acid with respect to diclofenac is greater by a factor of 316.

In order to get further insight regarding the different hosting capacity of **1** for aspirin relative to clofibric acid and diclofenac, molecular simulation calculations were carried out. The outcome is shown in Figure 5 where the presence of a carbonyl oxygen in the *ortho* position with respect to the carboxylic group in aspirin leads to two contact points involving the amine functionalities in each of the pendant arms of the receptor. Thus a proton transfer reaction occurs where one of the amino functionalities is protonated (as shown from conductance measurements) while the remaining one enters hydrogen bond formation with the carbonyl oxygen of the ester group of aspirin. It seems that the presence of acetonitrile in the hydrophobic cavity of the ligand pre-organise the receptor to interact with the drug. For clofibric acid and diclofenac each amino group of the receptor is protonated by two units of drug.

CLOFIBRIC ACID

Figure 5. Molecular Simulation on the interaction of **1** with clofibric acid, diclofenac and aspirin

Preliminary Extraction Studies of Drugs by the Calix[4]arene Receptor and Scope for the Design of Sensing Devices

Given that **1** is a non-soluble receptor in water and to avoid the use of organic solvents which are environmentally unfriendly, preliminary batch experiments on solid-aqueous solution extractions using these pharmaceuticals were carried out. The results so far obtained showed that this receptor is able to remove 82 ± 2 and 71 ± 2 % of clofibric acid and diclofenac respectively from water (optimum pH range=7-8) while the % E was found to be very low for aspirin. It should be emphasised that although it was shown that aspirin interacts with **1** in

acetonitrile, it is well established that the solvation properties of this solvent are very poor relative to water. In the latter solvent the drug is bound to be strongly hydrated and therefore a high competition between the solvent and the receptor for the drug occurs. As the drug is comfortable in water it is reluctant to interact with the receptor, reducing its capacity to remove the drug from water. The trend observed is in accord with that observed for the stability constant data obtained from thermodynamics (Table 2) and the pK_a values discussed above. Again the capacity values obtained (mmoles of drug/ gram of material), follows the same trend with values of 0.20 and 0.035 mmol/g for clofibric acid and diclofenac respectively. The extraction process was found to be fast (10 minutes). No changes in the capacity of the receptor were found with temperature, this is expected given that it would depend on the number of sites in the material. Recycling of the material can be carried out *via* a pH switching mechanism. Indeed hardly any % E is found at pH 2 due to the protonation of the receptor.

We have recently reported⁵² the following correlation for a carrier mediated mercury (II) ion selective electrode

$$\log K_s M(II) - \log K_s Hg(II) = \log k_{Hg(II), M(II)} \quad \text{Eq.6}$$

In Eq.6, K_s denotes the stability constant of the receptor and the appropriate metal cation and k is the selectivity coefficient of the metal cation relative to Hg(II). This correlation demonstrated for the first time that selectivity could be established through solution screening (thermodynamics) without having to construct an electrode each time. Further investigations will be carried out to develop a carrier mediated electrode based on **1** and to assess whether eq.6 is applicable to the systems reported in this paper.

Conclusions

From the above discussion it follows that

- i) The calix[4]arene receptor is able to recognise selectively clofibric acid, diclofenac and aspirin and discriminates against other drugs containing carboxylic functional groups (cyprofloxacin, nalidixic acid and ibuprofen). The interaction is controlled by the dissociation constants of the acids

- ii) The hosting ability of the receptor is greater for clofibric acid and diclofenac given that two protons are taken up per unit of receptor. For aspirin only one proton is transferred to the receptor. Molecular simulation studies suggest that for the latter drug, one of the amino functionalities is protonated while the remaining one enters hydrogen bond formation with the carbonyl oxygen of the ester group of aspirin.
- iii) Thermodynamics provides a quantitative assessment of the strength of interaction through the stability constant. The availability of enthalpy and entropy data allows to assess whether the process is enthalpy or entropically controlled.
- iv) Preliminary extraction data are encouraging as far as the use of this receptor for the selective removal of these drugs from water

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Graphical abstract

SUPRAMOLECULAR RECEPTORS