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Host-Guest Complexes of Carboxylated Pillar[*n*]arenes With Drugs

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ABSTRACT

Pillar[*n*]arenes are a new family of nanocapsules that have shown application in a number of areas, but because of their poor water solubility their biomedical applications are limited. Recently, a method of synthesizing water-soluble pillar[*n*]arenes was developed. In this study, carboxylated pillar[*n*]arenes (WP[*n*], $n = 6$ or 7) have been examined for their ability to form host-guest complexes with compounds relevant to drug delivery and biodiagnostic applications. Both pillar[*n*]arenes form host-guest complexes with memantine, chlorhexidine hydrochloride, and proflavine by ^{1}H nuclear magnetic resonance and modeling. Binding is stabilized by hydrophobic effects within the cavities, and hydrogen bonding and electrostatic interactions at the portals. Encapsulation within WP[6] results in the complete and efficient quenching of proflavine fluorescence, giving rise to "on" and "off" states that have potential in biodiagnostics. The toxicity of the pillar[*n*]arenes was examined using *in vitro* growth assays with the OVCAR-3 and HEK293 cell lines. The pillar[*n*]arenes are relatively nontoxic to cells except at high doses and after prolonged continuous exposure. Overall, the results show that there could be a potentially large range of medical applications for carboxylated pillar[*n*]arene nanocapsules.

Introduction

Nanocapsules are short polymers which close to form ring or barrel-shaped 3-dimensional (3D) structures which are useful in host-guest chemistry and have a range of important applications.[1] They are particularly useful in medicine and biodiagnostics where they have been shown to improve drug chemical and physical stability, as well as the solubility, dissolution, and effectiveness of drugs. They have also been shown to act as antidotes, work as *in vivo* chemical extractors, induce pKa shifts of drug acid and base functional groups, mask drug taste, reduce drug toxicity, and even reverse drug resistance.[1-3]

To date, 3 nanocapsule families have been extensively examined for their biomedical applications: *n*-cyclodextrins,[4] cucurbit[*n*]urils,[5] and calix[*n*]arenes,[6] where *n* indicates the number of subunits of each different-sized nanocapsule. *n*-Cyclodextrins are approved for use in medicines,[7] and are included in a number of pharmaceutical formulations, including the antifungal drug itraconazole, the antipsychotic drug aripiprazole, and maropitant which is used to control motion sickness and nausea in animals.

Pillar[*n*]arenes are a new nanocapsule family that was first reported in 2008 (Fig. 1a).[8] They are synthesized from the Lewis-catalyzed reaction of paraformaldehyde and 1,4-dimethoxybenzene and form a range of different-sized nanocapsules with a roughly symmetrical, pillar-like shape.[9] The cavities of the pillar[*n*]arenes are able to store and release small molecules and can be easily modified and functionalized. While they have been utilized as components in a variety of supramolecular- and nano-based drug delivery machines,[10-13] they have not been extensively studied in their own right as simple drug delivery vehicles, nor have they been examined for their use in biodiagnostics.

Native pillar[*n*]arenes are soluble only in organic solvents (methanol, acetone, acetonitrile, dimethylformamide, and dimethylsulfoxide) and display no significant water solubility.[8] This has recently been overcome through conversion of the hydroxyl groups to carboxylates on the pillar[*n*]arenes' rings (Fig. 1b).[14]

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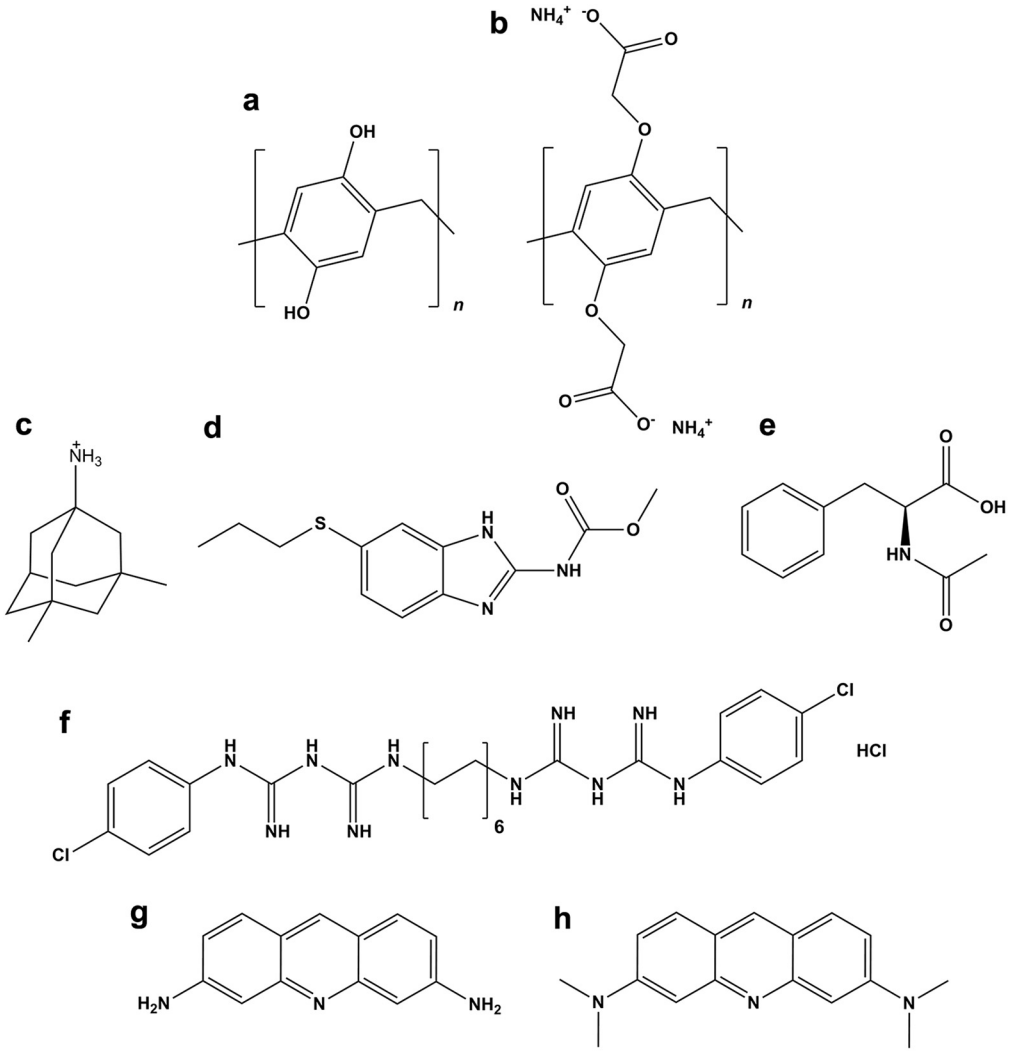

Figure 1. The chemical structures of (a) native and (b) carboxylated pillar[*n*]arenes (WP[*n*], $n = 5$, 6, or 7), and the biorelevant compounds used in this study: (c) memantine, (d) albendazole, (e) *N*-acetyl-phenylalanine, (f) chlorhexidine hydrochloride, (g) proflavine, and (h) acridine orange.

These carboxylated pillar[*n*]arenes (WP[*n*]) are anionic and can have a charge up to 10^- or 14^- depending on the number of subunits and the pH of the solution. As such, they display excellent water solubility, and because of this, may have conceivable medical applications.

Carboxylated pillar[*n*]arenes have several potential benefits over other nanocapsule families. First, they are more easily functionalized and are more water soluble compared with cucurbit[*n*]urils. Second, they can form stronger host-guest complexes, and it is easier to monitor their host-guest complex interactions, compared with *n*-cyclodextrins. Third, the barrel shape of carboxylated pillar[*n*]arenes provides a more suitable cavity for the storage, protection, and release of guests, compared with the bowl shape of the calix[*n*]arenes.

In this study, we have examined the potential of water-soluble carboxylated pillar[*n*]arenes with respect to their application in drug delivery and biodiagnostics. The formation of host-guest complexes with a range of biorelevant molecules was examined by ^{1}H nuclear magnetic resonance (NMR), molecular modeling, and fluorescence spectrophotometry, and their toxicity determined using *in vitro* growth inhibition assays.

Materials and Methods

Materials

The carboxylated pillar[*n*]arenes ($n = 6$ or 7) were made as previously described.[14] Memantine, proflavine, acridine orange, and *N*-acetyl-phenylalanine were purchased from Sigma-Aldrich (Sydney, Australia). Chlorhexidine hydrochloride was purchased from Imperial Chemical Laboratories Ltd (London, UK). Albendazole was provided by Dr. Anthony Day (UNSW, Australia). Deuterium chloride (99.9%) was purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA).

NMR

^{1}H NMR spectra were recorded on a Varian Avance 400 in D_2O. One-dimensional spectra were obtained using 128-512 scans. Two-dimensional rotating frame Overhauser effect spectroscopy (ROESY) experiments were undertaken using 200 $t1$ increments with 64 scans per increment, a mix time of 200 ms and a relaxation delay of 1 s.