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***ta*BOX**: A water-soluble tetraanionic rectangular molecular container for conjugated molecules and taste masking for berberine and palmatine

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ABSTRACT

A water-soluble macrocycle that bears four carboxylate anions has been designed and prepared, which forms a rectangular cavity that can efficiently encapsulate discrete electron-deficient aromatic compounds, including berberine and palmatine. This macrocycle is revealed to be highly biocompatible and able to inhibit the bitter taste of the two drugs.

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In host-guest chemistry, the design of new macrocycles plays a key role in the development of efficient hosts for efficient recognition of discrete guests [1–19]. The generation of the so-called "blue box" [20], the tetracationic cyclobis(paraquat-*p*-phenylene)$_2$, by Stoddart has remarkably promoted the construction of various cationic cyclophanes or cages for the binding of different guests [21–27]. More recently, several cationic cyclophanes have been successfully used to include bioactive porphyrin agents to inhibit their posttreatment phototoxicity after photodynamic therapy [28–30]. Typically, such multicationic cyclophanes have been constructed through the incorporation of pyridinium units into the macrocyclic backbones. In contrast, multianionic macrocyclic hosts have been mainly developed by introducing carboxylate or sulfate groups or side chains that bear these anionic units to the backbones to enable the required water-solubility. In this context, a variety of multianionic macrocyclic hosts [31–35], including cyclodextrin [36], calix[*n*]arene [37], pillar[*n*]arene [38–41], cucurbit[*n*]uril [42] derivatives as well as acyclic folded containers [43–45], have been prepared for investigating new host-guest chemistry. Given the wide clinic application of sugammadex [36], the cyclodextrin-derived multianionic reversal drug for neuromuscular blockers rocuronium and vecuronium, it is valuable to develop new anionic macrocycles to enable efficient binding of photo- and bioactive molecular guests. Here, we report the synthesis of a new tetraanionic rectangular macrocycle ***ta*BOX** that forms 1:1 host-guest complexes through unique parallel arrangement with a variety of electron-deficient aromatic compounds, with binding constants reaching up to 10^7 L/mol in water. We further demonstrate that the efficient encapsulation of berberine and palmatine by ***ta*BOX** can efficiently mask their bitter taste.

Macrocyclic tetraacid **TA** was prepared from the hydrolysis of tetraester **TE** (Fig. 1), which was produced from the coupling reaction of the corresponding diphenol and 4,4′-di(bromo–methyl) biphenyl. The crystal structures of **TE** and **TA** are shown in Figs. 2a and b. It can be found that the two biphenyl units of the ester adopted parallel arrangement with a spatial separation of ~9.4 Å. Two of the four methyl benzoates are nearly perpendicular to the two biphenyl units, with the two methyl groups seizing the cavity of the macrocycle. In contrast, tetraacid **TA** gave rise to an inclined rectangular cavity. The biphenyl units and two of the four benzene rings constituted the two longer planes with spatial separation of ~7.3 Å, while another two benzene rings act as the shorter planes which has a spatial separation of ~16.5 Å. Tetraanionic ***ta*BOX** was then prepared as Na^+ salt by treating the acid **TA** with aqueous NaOH solution. This multicharged macrocycle had a good solubility of 15 mmol/L (Fig. S1 in Supporting information). We envisioned that it should possess a similar rectangular cavity in water, which was expected to encapsulate large size-matching aromatic guests

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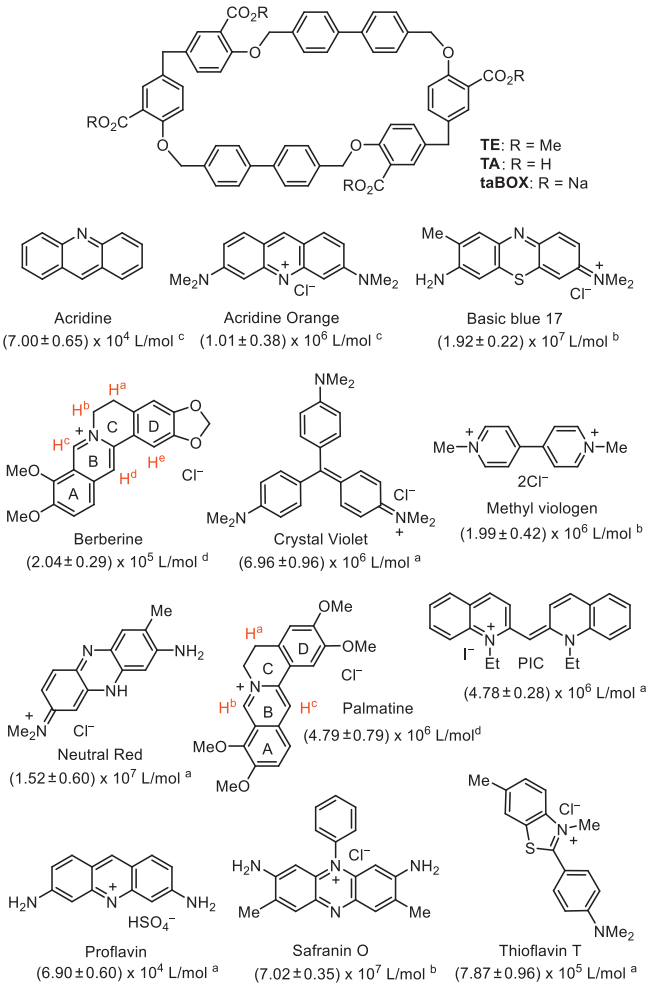

Fig. 1. The structures of macrocycles **TE**, **TA** and ***ta*BOX** and investigated aromatic guests. In the brackets are binding constant between the respective compound and ***ta*BOX** (methods used: [a] UV–vis titration; [b] UV–vis competition titration; [c] fluorescence titration; and [d] fluorescence competition titration).

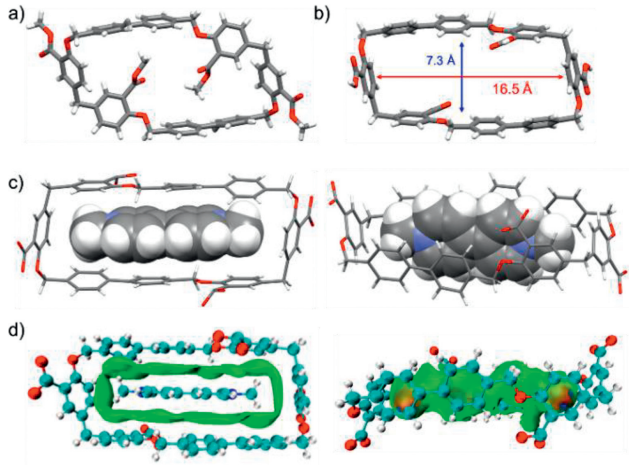

Fig. 2. Crystal structure of compounds (a) **TE**, (b) **TA**, (c) complex **MV⊂*ta*BOX** (left: side view, right: top view), which reveals the unique parallel arrangement of MV in the cavity of ***ta*BOX** (**TE** CCDC No.: 2262459, **TA** CCDC No.: 2262460, **MV⊂*ta*BOX** CCDC No.: 2262463). (d) IGM analyses ($\delta g^{inter} = 0.004$) for complex **MV⊂*ta*BOX** (left: side view, right: top view).

driven by cooperative hydrophobicity and ion-pairing electrostatic attraction.

The crystal structure of the complex of ***ta*BOX** and dicationic methyl viologen (MV) further confirmed this 1:1 stoichiometry (Fig. 2c). Remarkably, MV was incorporated completely in the cavity of ***ta*BOX**. That is, MV was paralleled with the two long planes of ***ta*BOX**, with its two methyl groups pointing to the short benzene planes of the rectangular cavity of ***ta*BOX**. This inclusion motif is quite different from that of the complexation between Stoddart's tetracationic blue box or its extended analogues and linear electron-rich guests, where the linear guests are typically interwined with and threaded through the electron-deficient macrocycle. The 4-(phenoxymethyl)-1,1′-biphenyl units of ***ta*BOX** may be considered as an unconventional electron-rich segment. Thus, the parallel arrangement of the 4-(phenoxymethyl)-1,1′-biphenyl units and MV in the complex indicated that the extended length of the new rectangular cavity enabled the inclusion of a linear guest to maximize the donor-acceptor interaction and minimize the exposure of hydrophobic MV to the polar aqueous medium. We further used independent gradient model (IGM) [46] to illustrate the noncovalent interactions that drove the 1:1 complexation between MV and ***ta*BOX** (Fig. 2d).

Totally twelve aromatic compounds (Fig. 1), including bitter drugs berberine and palmatine, were then evaluated for the encapsulating capacity of ***ta*BOX** in water. Since tetraacid **TA** formed a rectangular cavity with the longer planes having a spatial separation of 7.3 Å, which is ideal for forming sandwich complexes with long aromatic guests, we expected ***ta*BOX** formed 1:1 complexes with all these aromatic guests. To test this potential, Job's plots were obtained for its complexation with six organic dyes, *i.e.*, acridine orange, basic blue 17, crystal violet, PIC, safranin O, neutral red and thioflavin T, by using UV–vis absorption titrations (Figs. S2–S8 in Supporting information), which supported that all the complexations occurred in the 1:1 binding motif.

Direct or competitive UV–vis or fluorescence assays were then conducted to derive the binding constants (K_a) of the 1:1 complexes formed between ***ta*BOX** and the aromatic guests in water (Figs. S9–S24 in Supporting information) [43], which are provided in Fig. 1. Neutral acridine and proflavin gave the lowest values. The value of acridine orange was substantially higher than that of proflavine that bears two polar amino groups, which may be ascribed to the hydrophobic dimethylamino groups of acridine orange as well as the deprotonation of proflavine. The values of basic blue 17, neutral red and safranin O were at the level of 10^7 L/mol, indicating that ***ta*BOX** is more efficient than well-investigated macrocycles such as cucurbit [7] uril or β-cyclodextrin [47]. Another six guests afforded values of 10^5–10^6 L/mol, which are still among the higher ones compared with other macrocyclic receptors [47].

The K_as of berberine and palmatine were determined to be 2.04×10^5 L/mol or 4.79×10^6 L/mol. These two cationic alkaloids are clinically used for the treatment of enteritis or bacterial infection, but have an extremely bitter taste which disfavours patients during oral administration. Given the efficient binding of ***ta*BOX** for both drugs, we further evaluate the potential of the macrocycle for masking their bitterness. For this aim, we first studied the binding motif of ***ta*BOX** for berberine using ^{1}H NMR technique (Fig. S25 in Supporting information). Adding ***ta*BOX** (1.0 equiv.) to the solution of berberine in D_2O caused all the signals of berberine to shift upfield (see Fig. 1 for signal numbering), even though several signals were not assigned due to overlapping or low resolution. Remarkably, the Ha and Hb signals underwent upfield shifting of 1.84 or 1.23 ppm, respectively. Hc-He signals of the aromatic rings could be assigned, which had a upfielding shifting of 0.78, 0.65 and 0.22 ppm, respectively. Similar upfield shiftings were also observed for palmatine in the presence of ***ta*BOX** (Fig. S26 in

Supporting information). Three signals (Ha-Hc) could be assigned (Fig. 1), which again suffered substantial upfield shifting, *i.e.*, 1.66, 0.59 and 0.73 ppm, respectively. All these observations supported that these two guests formed 1:1 complexes with ***ta*BOX** through the parallel stacking motif as revealed above for the complex of ***ta*BOX** and MV. The structural difference of berberine and palmatine is only that their D rings bear a CH_2OCH_2 unit or two methoxy groups. However, the binding constant of palmatine is higher by 23.5 times than that of berberine, which might be considered as another evidence for the parallel encapsulation motif, through which the two more hydrophobic methoxy groups of palmatine could be more efficiently screened from the aqueous medium by the cavity of ***ta*BOX**. This result is also consistent with the observation by Meng and Li *et al.* that a terphen [3] arene macrocycle exhibited comparable binding affinity toward berberine and palmatine through a threading binding motif [35].

At pH 1.2, the typical acidity of gastrointestinal tract, ***ta*BOX** was acidified to insoluble **TA**. Accordingly, the ^{1}H NMR spectrum of berberine and palmatine (0.3 mmol/L) in D_2O in the absence and presence (0.3 mmol/L) of ***ta*BOX** gave rise to the same set of signals of the two drugs (Figs. S27 and S28 in Supporting information), and the signals of ***ta*BOX** disappeared completely. These observations indicated that in the above acidic medium, ***ta*BOX** was acidified to insoluble **TA** and thus lost the ability of including the two drugs, which meant that the drugs included by ***ta*BOX** in neutral water would be released in gastrointestinal tract.

Encouraged by the above experimental results, we then evaluate the biocompatibility of ***ta*BOX**. The CCK-8 assay was first utilized to evaluate the cytotoxicity of ***ta*BOX** for L02 and H9C2 cells. The results demonstrated a high survival rate (>90%) of the cells at the high concentration of 0.25 mmol/L (Fig. 3a). To reveal the safety profile *in vivo* of ***ta*BOX**, we further chose healthy BALB/c mice (5 male and 5 female) of close body weights to do the acute gavage test by using the high dose of 1000 mg/kg. The body weights and behaviours of the mice were monitored for two weeks (Fig. 3b). Compared with control group which was treated with distilled water, the body weights, physical and social behaviours of the ***ta*BOX**-treated group had no obvious difference. Moreover, no detectable toxicity was observed histopathologically in major organ samples of the mice after 14 days (Fig. S29 in Supporting information). All the results indicated that ***ta*BOX** had a relatively high biocompatibility.

Mice have bitter taste receptors similar to those of human beings. We thus used mice model to evaluate if ***ta*BOX** could mask the bitterness of berberine and palmatine by using drinking preference tests. Thus, three groups of BALB/c mice ($n = 5$) were firstly housed individually and accessed to two drinking bottles containing distilled water for 1 week to acclimatize to the environment. Then, they were subjected to two drinking bottles. One of the bottles contained distilled water, while another one contained ***ta*BOX**, the mixture of ***ta*BOX** and berberine or palmatine, and berberine or palmatine (Fig. 4), respectively. The concentration of both ***ta*BOX** and the drugs was kept at 50 μmol/L by following the reported method [35]. The difference of the preference of the mice in choosing the bottles for drinking, which was determined by the amounts of the different liquids consumed, was used for evaluating the masking of ***ta*BOX** for the bitterness of berberine. It can be seen that, for distilled water and ***ta*BOX**, mice had no preference (Figs. 4e and f), which indicated that the ***ta*BOX** had no aversive taste. In contrast, berberine and palmatine had only a 24% and 27% preference, respectively (Figs. 4a and b), which can be reasonably ascribed to their bitter taste. However, in the presence of the identical concentration of ***ta*BOX** (Figs. 4c and d), the mice did not ex-

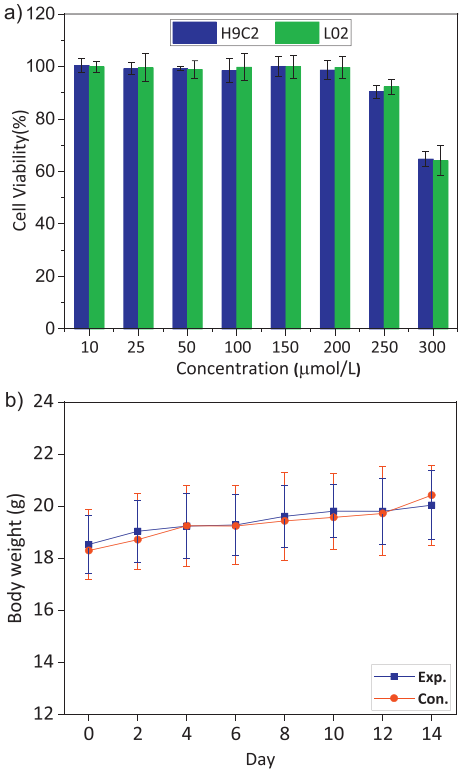

Fig. 3. (a) Relative viabilities of H9C2 and L02 cells after incubation for 24 h with macrocycle ***ta*BOX** at the indicated concentrations measured by CCK-8 assay (mean ± SD, $n = 6$). (b) Changes in body weight of mice (blue) after acute gavage test to ***ta*BOX** (1.0 g/kg) as compared with the control group (distilled water, red). Data were represented as mean ± SD, $n = 10$, which had no statistically significant difference in body change over 14 days.

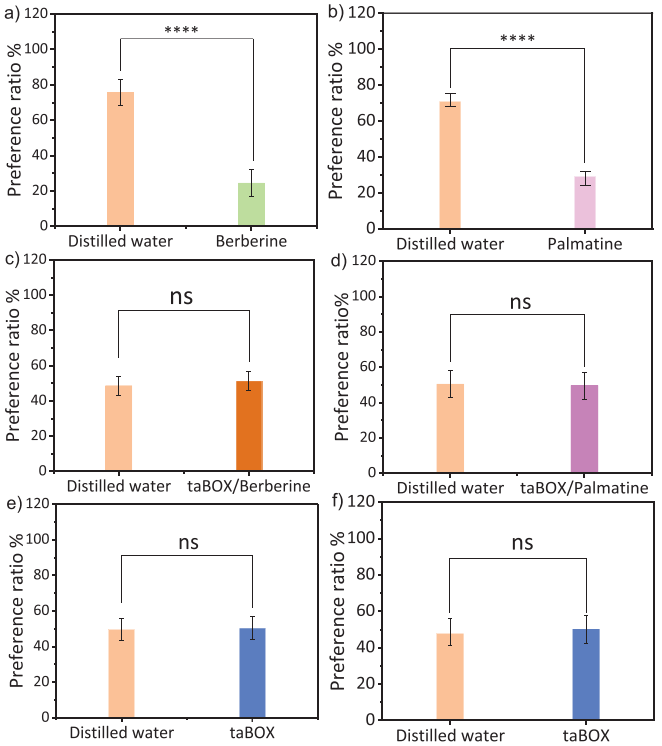

Fig. 4. Preference ratios of mice between two drinking bottles that contained distilled water and (a) berberine, (b) palmatine, (c) ***ta*BOX** and berberine, (d) ***ta*BOX** and palmatine, and (e, f) ***ta*BOX**. All examples had the same concentration of 50 μmol/L. The distilled water group was used as control (mean ± SD, $n = 5$). Significant differences were assessed in (a–c) using unpaired t – tests; ns = not significant; $^{****}P < 0.0001$.

hibit observable preference for distilled water and the mixing liquids. Clearly, the inclusion by macrocycle efficiently screened the bitterness of the drugs. It is noteworthy that, although the ability of the macrocycle for including berberine was considerably lower than that for palmatine, ***ta*BOX** still completely suppressed its bitterness, which may be regarded as evidence for the unique parallel encapsulation mechanism which maximized the screening of the macrocycle for the drug to approach the taste receptors of the mice.

In summary, we have synthesized a tetraanionic macrocycle that has a rectangular hydrophobic cavity, which can strongly include large hydrophobic aromatic molecules through a unique parallel encapsulation mechanism, which is expected to maximize the screening of the included aromatic guests from the aqueous medium. For bitter drugs berberine and palmatine, this encapsulation mechanism enables the masking of their bitterness. The macrocycle can be prepared from readily available precursors and allows for modifications for both the backbone and the side chains. Introducing alkylsufonate side chains is expected to achieve increased water-solubility. Future endeavours will point to the design of macrocycles that have increased height for including stacking, dimeric aromatic guests or increased width for larger bioactive molecules or linear supramolecular or dynamical covalent guests.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.109119.

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