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Supramolecular strategy for reducing the cardiotoxicity of bedaquiline without compromising its antimycobacterial efficacy

Kit Ieng Kuok[a,1], Phoebe Choi In Ng[a,1], Xia Ji[b], Chunming Wang[a], Wing Wai Yew[c], Denise P.C. Chan[c], Jun Zheng[b], Simon M.Y. Lee[a], Ruibing Wang[a,*]

[a] State Key Laboratory of Quality Research in Chinese Medicine, and Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau, China
[b] Faculty of Health Sciences, University of Macau, Taipa, Macau, China
[c] Stanley Ho Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Hong Kong, China

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ABSTRACT

Bedaquiline (BDQ) is a newly approved anti-tuberculosis drug in treating multidrug-resistant tuberculosis. However, it has very poor aqueous solubility and several case reports have proposed that BDQ has potential risk of cardiotoxicity to patients. In this present study, we have explored into employing host-guest interactions between a synthetic receptor, cucurbit[7]uril (CB[7]), and BDQ aiming to improve the solubility and reduce the inherent cardiotoxicity of BDQ. HPLC-UV test on the solubility of BDQ in the absence and in the presence of increasing concentrations of CB[7] suggested a host-dependent guest-solubility enhancements. Cardiovascular studies using an *in vivo* zebrafish model demonstrated that the cardiotoxicity of BDQ was indeed alleviated upon its complexations by the synthetic receptor. Furthermore, our *in vitro* antibacterial studies suggested that CB[7] formulated BDQ preserved its antimycobacterial efficacy against *Mycobacterium smegmatis*. Therefore, CB[7] may become a suitable pharmaceutical excipient in formulating BDQ for improving its physiochemical properties (such as solubility), and for alleviating its side effects (such as cardiotoxicity), while the antimycobacterial efficacy of BDQ may be well maintained.

1. Introduction

Tuberculosis (TB) is a major threat to global health (WHO, 2016). Multiple drug-resistant tuberculosis (MDR-TB) is a form of TB infection caused by *Mycobacterium tuberculosis* that is resistant to treatment with at least two of the first-line drugs (WHO, 2016). Bedaquiline (BDQ, Fig. 1a) is a newly developed medicine for the treatment of MDR-TB, which is a long-awaited agent for anti-TB in more than forty years; both its action mechanism and structure are unique to the existing first-line regimens. BDQ is a first-in-class drug of diarylquinoline, by binding to the subunit c of ATP synthase, which is crucial for generating energy in mycobacteria, it suppresses mycobacterial energy metabolism (Andries and Guillemont, 2005; Haagsma et al., 2011). However, a black-box warning was issued in 2012 by the United States Food and Drug Administration for increased mortality and cardiotoxicity, as it may cause heart arrhythmias by prolonging the QT interval (SIRTURO, 2017). Moreover, BDQ has very poor intrinsic aqueous solubility. It is defined to be "very slightly soluble (VSS) in 1M HCl" by the European Medicines Agency (EMA). Some believed that the high lipophillicity of BDQ is responsible for the cardiotoxicity caused (Tong et al., 2017). And less lipophilic analogues have been synthesized and investigated with an attempt to resolve the toxic side effects while maintaining the promising efficacy of BDQ for the treatment of TB (Priebbenow et al., 2016). However, the chemical syntheses of new analogues are usually laborious and the efficacy can be challenging to retain. Therefore, these concerns inspired us to adapt a novel, facile supramolecular approach to formulate BDQ with a synthetic macrocyclic receptor.

Cucurbit[7]uril (CB[7], Fig. 1b), a synthetic macrocyclic receptor, is the most studied member in the cucurbit[n]uril (CB[n], n = 5–8, 10–14) family with regards to biomedical applications due to its superior water solubility and appropriate size to accommodate a variety of guest molecules of biomedical interest (Yin and Wang, 2017). Over the years, numerous investigations have demonstrated that upon complexation by CB[7], the solubility and stability of a variety of guest molecules were improved (Kuok et al., 2017). In addition, upon encapsulation by CB[7], the toxic side effects of guest drug molecules are often reduced and the therapeutic efficacies could be maintained or even improved (Kuok et al., 2017). For instance, a study reported the

* Corresponding author.
E-mail address: rwang@umac.mo (R. Wang).
[1] These authors contributed equally to this work.

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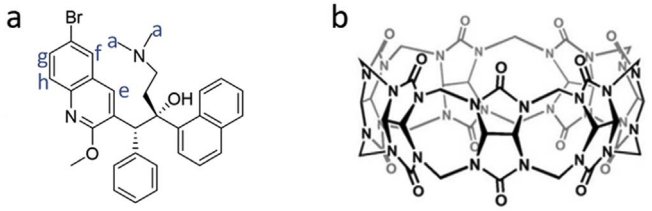

Fig. 1. Chemical structures of BDQ (a) and CB[7] (b) with key protons labeled.

non-specific hepatotoxicity of isoniazid, could be potentially reduced by forming host-guest inclusion complex with CB[7] and by altering the amount of CB[7], the rate of isoniazid acetylation could be controlled, thus potentially reducing the hepatotoxic effect associated with N-acetylated isoniazid to patients with the fast-acetylator phenotype (Cong et al., 2011). On the other hand, in the presence of CB[7], the solubility of clofazimine, another anti-TB drug, could be improved reaching a concentration of up to approximately 0.53-fold of the maximum solubility of CB[7]. While the antimycobacterial efficacy of clofazimine was maintained with the MIC_{50} in the order of 10^{-6} M towards *Mycobacterium smegmatis* (*MS*), the *in vivo* experiment in zebrafish demonstrated that the cardiotoxicity caused by clofazimine was reduced when formulated with CB[7] (Yew et al., 2017) (Li et al., 2016). Likewise, a recent study established that CB[7] also improved the solubility of sorafenib in an NMR phase solubility analysis, by forming CB[7]-sorafenib complex with K_a = (2.87 ± 0.13) × 10^5 M^{-1}. Analysis of zebrafish cardiac function and morphology showed that the complex reduced the cardiotoxicity caused in zebrafish as compared to the free drug, while the therapeutic efficacy was not affected upon complexation by CB[7] shown in *in vitro* anti-angiogenic activity studies (Yang et al., 2017). We have also previously demonstrated that the complexation of a general anesthetic agent by CB[7] reversed its anesthesia effects on a zebrafish model (Chen et al., 2015).

Encouraged by these results, the aim of this research was to investigate the host-guest complexations between CB[7] and BDQ, as well as the potential benefits of such supramolecular formulation on the solubility, nonspecific toxicity, and therapeutic efficacy of the guest drug. This study may provide new insights on novel formulation strategies of MDR-TB drugs.

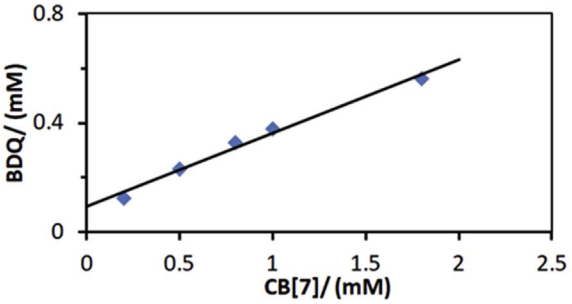

Fig. 3. Phase solubility diagram of BDQ in the presence of increasing concentrations of CB[7] (0.2, 0.5, 0.8, 1.0, 1.6 and 2.0 mM), determined from HPLC-UV chromatogram integrations (the linear fit equation: y = 0.270 x + 0.093, R^2 = 0.987).

2. Materials and methods

2.1. Reagents and materials

CB[7] was synthesized according to the reported method (Day et al., 2001). BDQ was purchased from International Laboratory (IL, CA 94080, USA) and used as received. Ammonium acetate, NaCl, KCl, $CaCl_2$, Mg_SO_4, HPLC grade methanol, acetonitrile and all other chemicals were commercially available and purchased from Sigma Aldrich (St. Louis, USA). Ultrapure water was obtained from a Millipore water system (Millipore, Bedford, Massachusetts).

2.2. Nuclear magnetic resonance (NMR) study

^{1}H NMR and COSY NMR spectra were obtained using Bruker 600 MHz NMR spectrometer. Briefly, a solution of BDQ was dissolved in D_2O with minimum amount of DCl, in which different equivalents of CB[7] (0.5 eq, 1.2 eq and 2.2 eq) was added. These samples were sonicated before NMR measurements.

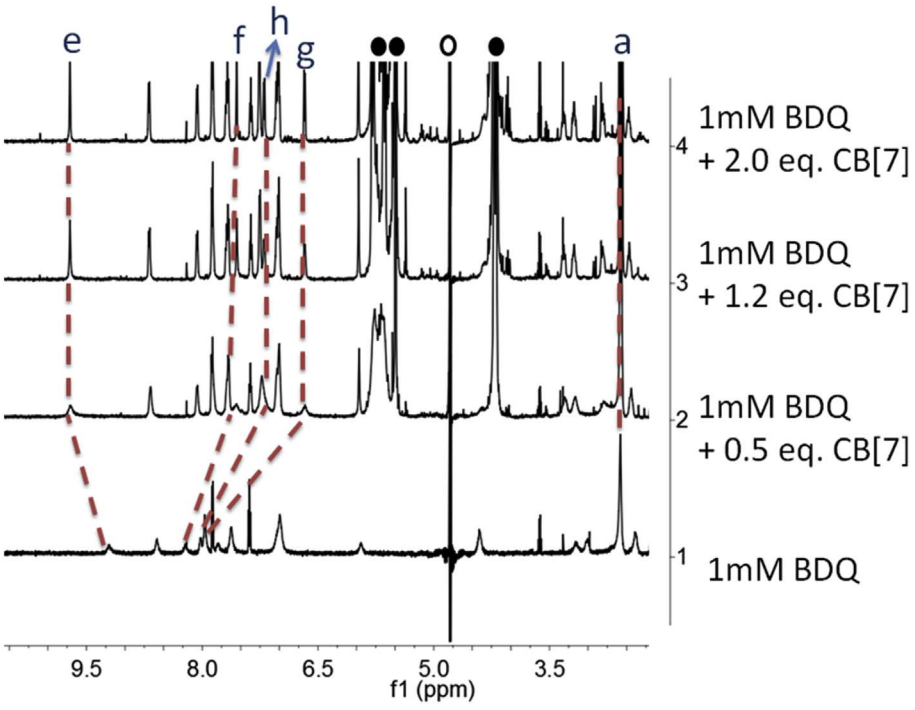

Fig. 2. The stacked ^{1}H NMR spectra of BDQ (1 mM) in the absence and in the presence of CB[7] (0.5 and 1.2 and 2.0 equivalents) at p*D* = 2. The peaks corresponding to CB[7] are labeled as (●) and the HOD peaks as (○). Dotted lines indicated that the protons on BDQ experienced upfield or downfield shifts with increasing amounts of CB[7].