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A QSAR-based mechanistic study on the combined toxicity of antibiotics and quorum sensing inhibitors against *Escherichia coli*

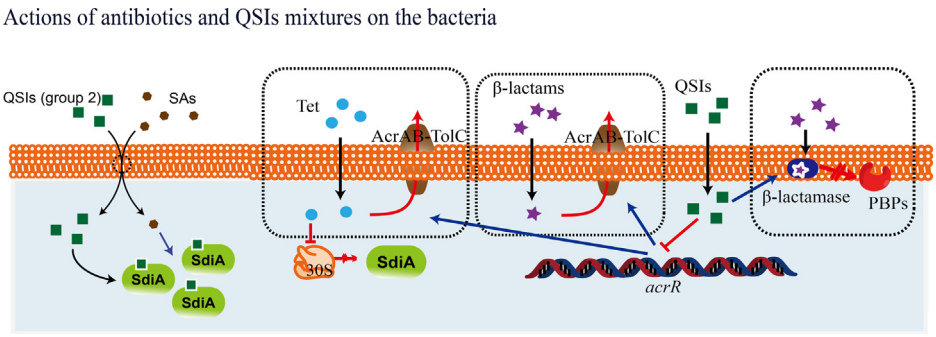
Dali Wang[a,b,1], Junyi Shi[c,1], Yanna Xiong[d], Jingyun Hu[a], Zhifen Lin[a,e,f,*], Yanling Qiu[f], Jinping Cheng[g]

[a] State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[b] Post-doctoral Research Station, College of Civil Engineering, Tongji University, Shanghai 200092, China
[c] College of Marine Ecology and Environment, Shanghai Ocean University, Shanghai 201306, China
[d] China Solid Waste and Chemical Management Technology Center, Ministry of Environmental Protection, Beijing 100029, China
[e] Collaborative Innovation Center for Regional Environmental Quality, China
[f] Shanghai Key Laboratory of Chemical Assessment and Sustainability, Shanghai, China
[g] School of Environmental Science and Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, China

HIGHLIGHTS

- Combined toxicities of antibiotics and QSIs on *E. coli* were determined.
- The joint effects of the mixtures varied with the type of the components.
- Distinct mechanisms were proposed for the joint effects of different mixtures.
- 4.QSIs in the mixtures can probably reduce the bioavailability of the antibiotics.
- The joint mechanisms were quantitatively analyzed based on QSAR models.

GRAPHICAL ABSTRACT



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ABSTRACT

Quorum sensing inhibitors (QSIs) have attracted increasing attention due to their potential roles as the antibiotic alternatives. The combination of QSIs and antibiotics in clinical use and their subsequent release into the environment may result in joint effects on the ecology and environment, which has not received enough concerns yet. In this study, eight potential QSIs and three types of commonly used antibiotics, i.e., sulfonamides (SAs), β-lactams and tetracyclines (TCs), were investigated for their combined toxicity on *Escherichia coli (E. coli)*. The QSAR models for the combined toxicity were constructed using the interaction energies between the chemicals and their target proteins as calculated by molecular docking. It was revealed that the SAs and QSIs presented either additive or antagonistic joint effects in the mixture toxicity test, while β-lactams and TCs showed only antagonistic effects with the QSIs. The analysis on the coefficients in the QSAR models suggested that the QSIs in the mixtures were more involved in the interaction with the proteins than the antibiotics. This study will help better understand the risks of joint exposure to the antibiotics and QSIs, and provide a new perspective for the study of the combined toxicity mechanism.

* Corresponding author at: College of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Shanghai 200092, China.
E-mail address: lzhifen@tongji.edu.cn (Z. Lin).
[1] These two authors contributed equally to this work.

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1. Introduction

The overuse of the antibiotics has brought about a series of environmental and health problems, among which the antibiotic resistance receives the most concerns [1,2]. So it is imperative to develop novel drugs against the constantly emerging resistant strains [3,4]. The discovery of bacterial quorum sensing (QS) provides an opportunity to develop novel anti-pathogenic drugs against bacteria. QS is a cell–cell communication through which the bacteria use small signal molecules (auto inducers, AIs) to coordinate certain gene expressions and thereby the bacterial behaviors, e.g. biofilm formation and virulence factors [5]. In Gram-negative bacteria, two QS systems have been identified, which are mediated by AI-1 and AI-2 respectively (Fig. 1A) [6]. Quorum sensing inhibitors (QSIs) are a class of chemicals that are structurally analogous to AIs (Fig. 1B). They can competitively bind to the sensing proteins and block the QS signal pathways. Since the QSIs do not pose selective stress that confers the drug resistance, they are deemed as an promising alternative to the antibiotics for bacterial infection treatment [7,8]. Given the broad prospects of QSIs in clinical application and their potential combined use with the antibiotics, there is a great possibility that QSIs and antibiotics coexist in the environment, posing joint effects on the environment and the ecological systems. Therefore, it is of great importance to investigate the potential combined toxicity of QSIs and antibiotics.

Nevertheless, researches on the combined toxicity of antibiotics and QSIs are very limited currently. Some researches [9,10] indicated that tobramycin, clindamycin and vancomycin showed synergetic joint effects with QSIs on the bacteria, because the susceptibility of the bacterial biofilm to the antibiotics was increased by the QSIs. Whereas, Wang et al. [11] revealed that the QSIs may interact with sulfonamides (SAs) and penicillin, and presented antagonistic effects with the two types of antibiotics. These researches imply that the joint effects of the antibiotics-QSIs mixtures differ with the type of the components, and the mechanisms for their combined toxicity can be different as well. Therefore, more researches should be conducted to explore the joint effects between different types of antibiotics and QSIs.

Quantitative structure-activity relationship (QSAR) has been widely applied to predict the biological effects of chemicals [12]. Recent years witnessed an increasing trend of the non-empirical QSAR models that are based on quantum chemistry and molecular docking process [13]. For instance, predictive QSAR models using quantum chemistry and molecular docking-based descriptors have been developed for single antibiotics [14] and QSIs [15], respectively. The non-empirical methods also facilitate the QSAR models for chemical mixtures. For example, QSAR models based on E_{bind} (interaction energy between chemicals and proteins obtained through molecular docking) were developed for the mixtures of diverse antibiotics [16–19]. In a more recent research, Wang et al. [20] developed a QSAR model for the antibiotic-QSI mixtures, using E_{bind} and a hormesis-related parameter Y_{max} (Maximal hormetic stimulation).

In this paper, a QSAR-based mechanistic study on the combined toxicity of antibiotics and QSIs against *E. coli* was reported. Three commonly used antibiotics, i.e., SAs, β–lactams and TCs, and some potential QSIs (including furanones, fyrrolidones and pyrroles) were investigated for their joint effects on *E. coli*. The QSAR models of the combined toxicity were constructed using E_{bind} as the structural descriptors. The mechanism of combined toxicity was then discussed based on the models. This study will help better understand the risk of the joint exposure to antibiotics and QSIs, and provide a reference for the prediction and mechanism study of the combined toxicity.

2. Materials and methods

2.1. Chemicals and organisms

The chemicals, including twelve SAs, four β–lactams, two TCs, and eight potential QSIs are shown in Table 1. All of the drugs were purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA). The model bacterium *E. coli* MG1655 was obtained from Biovector Co., LTD. (Beijing, China). The bacteria were reconstituted and maintained on LB agar slants at 4 °C.

2.2. Toxicity test

Prior to each toxicity analysis, the bacteria were inoculated in 5 ml LB broth and incubated at 37 °C until the OD_{600} (optical density at 600 nm) reached 0.5. Then the bacteria in the log growth phase were diluted by 1×10^5 times and added to the 96-well plates that contained a series of concentrations of the test compounds prepared in LB broth in each well. After 12 h of incubation, the OD_{600} value of each well was measured by a 96-well plate reader (Luminoskan Ascent, Thermo Scientific). Each concentration was tested in triplicate, and wells with no compound were measured as the controls. The compounds' inhibition on the growth of the bacteria was calculated according to Eq. (1):

$$\text{Inhibition}\% = \frac{OD_{600,0} - OD_{600,i}}{OD_{600,0}} \quad (1)$$

The median effective concentration (EC_{50}), i.e., the concentration of a chemical that inhibits 50% of the bacterial growth, was then calculated based on the decrease in OD_{600} using a probit model [21].

Based on the EC_{50}s of the individual compounds, binary equitoxic mixtures of the antibiotics and QSIs were prepared. Then, their bacterial toxicity was determined according to the above method. The joint effects of the binary mixtures were characterized by the sum of the toxic unit indexes as follows:

$$TU = \sum \frac{C_{i,50\%}}{EC_{50,i}} \quad (2)$$

where $C_{i,50\%}$ denotes the concentrations of components i in the binary mixture that provoked an inhibition equal to 50%. $EC_{50,i}$ denotes the EC_{50} value of components i. According to the literature [22], a simple addition is characterized by $1.2 > TU > 0.8$, whereas $TU \geq 1.2$ represents antagonism and $TU \leq 0.8$ indicates synergism. The addition implies that the mixture toxicity is equal to the sum of the individual toxicity, while the synergism and antagonism indicate a greater or reduced effect of the mixture than the sum of each component separately.

2.3. Molecular docking

Molecular docking was conducted by the CDOCKER method performed on Discovery studio 3.1 (Accelrys Software Inc., San Diego, CA.). The lowest CDOCKER interaction energy (E_{bind}) obtained for each compound was selected to represent its binding affinity with the receptor and the negative peak for connecting corresponds to the most stable conformation [23]. The 3D structure of the protein used in the molecular docking is either obtained from the Protein Data Base (PDB) or constructed using Homology Modeling. *E. coli* receptor proteins, i.e., SdiA (PDB entry 4Y15), DHPS (PDB entry 1AJ0), PBPs (PDB entry 3ITA), LsrB (PDB entry 1TJY) and 30S ribosomal subunit (PDB entry 4ABZ) respectively.