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Research paper

Discovery of 2-aminothiazolyl berberine derivatives as effectively antibacterial agents toward clinically drug-resistant Gram-negative *Acinetobacter baumanii*

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ABSTRACT

Aminothiazolyl berberine derivatives as potentially antimicrobial agents were designed and synthesized in an effort to overcome drug resistance. The antimicrobial assay revealed that some target compounds exhibited significantly inhibitory efficiencies toward bacteria and fungi including drug-resistant pathogens, and the aminothiazole and Schiff base moieties were helpful structural fragments for aqueous solubility and antibacterial activity. Especially, aminothiazolyl 9-hexyl berberine **9c** and 2,4-dichlorobenzyl derivative **18a** exhibited good activities (MIC = 2 nmol/mL) against clinically drug-resistant Gram-negative *Acinetobacter baumanii* with low cytotoxicity to hepatocyte LO2 cells, rapidly bactericidal effects and quite slow development of bacterial resistance toward *A. baumanii*. Molecular modeling indicated that compounds **9c** and **18a** could bind with GLY-102, ARG-136 and/or ALA-100 residues of DNA gyrase through hydrogen bonds. It was found that compounds **9c** and **18a** were able to disturb the drug-resistant *A. baumanii* membrane effectively, and molecule **9c** could not only intercalate but also cleave bacterial DNA isolated from resistant *A. baumanii*, which might be the preliminary antibacterial action mechanism of inhibiting the growth of *A. baumanii* strain. In particular, the combination use of compound **9c** with norfloxacin could enhance the antibacterial activity, broaden antibacterial spectrum and overcome the drug resistance.

1. Introduction

The drug resistance for antibiotics and clinically synthetic antibacterial agents is becoming a major global issue and threatens to overburden healthcare systems because of the increasing occurrence of multi-drug resistant (MDR) pathogens worldwide [1–4]. *Acinetobacter baumannii* as one of the ESKAPE pathogens is an important cause for severe infections, particularly in immunocompromised patients [5,6], and has acquired resistance to a wide spectrum of antibiotics used in clinical practice, which often makes treatment extremely difficult and the eradication of this bacterium from the healthcare environment almost impossible [7,8]. *A. baumannii* has been listed by the World Health Organization first on their global priority list of MDR pathogens currently threatening human health [9]. Therefore, an increasingly urgent need is to discover new antibacterial compounds with novel or multi-target activity against drug resistant strains. It is well-known that the discovery of new scaffolds derived from natural sources can open the way to alternative classes of antibiotics with probably new mechanisms of action.

Berberine is a natural isoquinoline quaternary alkaloid from many kinds of naturally medicinal plants such as *Coptis chinensis* (a traditional Chinese herb Huanglian), *Hydrastis canadensis*, *Berberis aristata*, *Coptis japonica*, *Phellondendron amurense etc* [10,11]. It has paid extensive attention for the large clinical potential in treating a number of diseases including infection, Alzheimer's disease, hyperlipidemia, diabetes, metabolic syndrome, obesity, fatty liver

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disease and so on [12,13]. In particular, berberine as a validated antibacterial agent against gastroenteritis, abdominal pain and diarrhea has been used in China for more than 2000 years [14,15]. Despite of the long term clinical use, the genotoxic, cytotoxic or mutagenic effects, and drug-resistance are seldom reported [16]. However, berberine is present at a very low level in blood and its bioavailability *in vivo* is less than 1% [17,18], which seriously limits its useful profile. It has been found that the structural changes such as disruption of the symmetry or molecular planarity would be efficient strategies to improve the intrinsic aqueous solubility [19,20]. Tetrahydroprotoberberrubine as one of the berberine derivatives can improve the molecular flexibility to a certain extent, which may be conducive to enhancing the water solubility and bioavailability, and is a valid structural alternative with widely medicinal potentiality [21]. Thus, it is important and worthy to further develop tetrahydroprotoberberrubine-based agents for their medicinal value with broader spectrum of antimicrobial activity and more effective for infectious treatments.

Azole compounds like imidazoles [22], triazoles [23,24], tetrazoles [25,26], thiazoles [27,28] and benzene-fused derivatives benzimidazoles [29,30], benzotriazoles [31] and benzothiazoles [32,33] are one of the most important kinds of *N*-heterocycles with various bioactivities and have been extensively employed in the development of antimicrobial agents, especially the aminothiazole derivatives. Aminothiazole moiety is present in a variety of clinical drugs, such as antibacterial sulfathiazole and cephalosporins cefodizime, cefmenoxime, anticancer dasatinib, and antiinflammatory meloxicam [34–36]. The successful development of many clinical aminothiazole-based drugs has been motivating more and more efforts to develop new bioactive molecules on the basis of this fragment [37]. The structural modifications of clinical drugs by 2-aminothiazole is one of the most convenient and rewarding methods to exploit new medicinal agents [38]. Very recently, the introduction of 2-aminothiazole moiety into the 3-position of antibacterial quinolone skeleton was found to not only improve antimicrobial potency but also afford multi-targeting agents with both membrane active and DNA intercalating potency [39–41]. Aminothiazole fragment can easily interact with DNA, enzymes and other biomacromolecules through noncovalent interactions such as hydrogen bonds, π–π stacking, coordination, ion dipole, hydrophobic effect as well as van der Waals force, because of the unique structural fragment aminothiazole with both electron-donating groups (-NH- or -NH_2, -S-) and the electron-accepting C═N group [42,43]. Therefore, the aminothiazole modified derivatives were endowed to possess multi-targeting binding with multiple biological active sites in biological system, thus possibly overcoming the severe resistance and exhibiting strong bioactivity [44].

Our previous research revealed that the introduction of azoles such as imidazoles [45], benzimidazoles [46,47] and triazoles [48] into the natural berberine scaffold not only improved biological potencies but also broadened the antimicrobial spectrum, including against methicillin-resistant *Staphylococcus aureus* (MRSA). However, to our surprise, aminothiazole, an important structural fragment which is extensively present in a large number of clinical drugs and candidates, so far has not been observed to modify the naturally antibacterial berberine. As continuous efforts in discovering and developing novel small molecule compounds with new or multiple targets effectively inhibiting resistant bacterial strains, herein we have overwhelming interest in combining the naturally antibacterial berberine backbone and the important antibacterial structural aminothiazole to develop a novel series of potentially antibacterial hybrids. The new hybrids of aminothiazole and berberine derivative are expected to exert multi-targeting properties including the validated berberine targeting DNA and membrane activity *via* multiple binding to biological system by using electron-donating groups (-NH- or -NH_2, -S-), electron-accepting bond (C═N) and aromatic moiety in aminothiazole and berberine fragments. More importantly, the aminothiazole functional group may also be helpful for enhancing the water solubility of target molecules *via* forming hydrogen bonds by using its amino and imino groups. Reasonably, the designed target compounds may have larger potential in treating antimicrobial infections including clinically drug-resistant strains because of potential multi-targeting. Therefore, in this work, a series of aminothiazolyl berberine derivatives were constructed. In order to investigate the structure-activity relationships, various substituents, including halophenyl groups and aliphatic chains like alkyl, hydroxyalkyl, alkenyl, alkynyl and cyano ones for regulating the molecular rigidity and flexibility with the validated remarkable influence in biological activities, were introduced to modify the aminothiazolyl berberine backbone. The Schiff base moiety was employed as a bridge linker between aminothiazolyl fragment and berberine backbone because it is a validated fragment to beneficially improve the water solubility [49,50] (Fig. 1).

The antimicrobial activities *in vitro* for the target 2-aminothiazolyl berberine derivatives and their new precursor compounds were evaluated against clinically Gram-positive and Gram-negative bacteria and fungi including drug-resistant strains. The aqueous solubility of compounds was investigated to helpfully explain the differences in antimicrobial activities. To identify the safety profile, the cytotoxic test for the highly active compound was done against normal human cells. The further experimental studies including bactericidal kinetic assay, drug resistance development, bacterial membrane permeabilization and theoretical exploration of molecular modeling was also performed to verify the research values and foreground of the derivatives. Moreover, the genomic DNA was isolated from the sensitive resistant strains in order to explore the possible antibacterial mechanism by the use of UV-visible absorption spectra and along with Agarose gel electrophoresis. Finally, the combination use of the most active molecule with clinically antibacterial drugs was evaluated to further excavate its potential in enhancing the antimicrobial efficiency and overcoming drug resistance.

2. Results and discussion

2.1. Chemistry

The target 2-aminothiazolyl berberine derivatives were conveniently synthesized from natural berberine according to the routes outlined in Schemes 1–3. Commercially available berberine chloride **1** was easily converted into tetrahydroprotoberberrubine-12-carbaldehyde **4** through demethylation, reduction and formylation in an excellent yield of 82.0% (Scheme 1). The condensation of compound **4** with hydrazinecarbothioamide in anhydrous alcohol conveniently afforded carbothioamide derivative **5**, and then further cyclization with 2-chloroacetaldehyde efficiently produced hydroxyl aminothiazolyl berberine derivative **6** in 45.0% yield.

The desired 2-aminothiazolyl berberine derivative **6** was further structurally modified with an attempt to investigate the effects of various substituents on bioactivities. Much effort failed in modifying the 9-hydroxyl group of compound **6**. An alternative strategy had to start from 9-hydroxyl berberine-derived aldehyde **4**. The synthetic route was shown in Scheme 2.

The substitution of compound **4** with a series of aliphatic bromides or chlorides in *N*,*N*-dimethylformamide (DMF) at 80 °C using