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Synthesis and bioactive evaluation of novel hybrids of metronidazole and berberine as new type of antimicrobial agents and their transportation behavior by human serum albumin

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ABSTRACT

A series of novel hybrids of metronidazole and berberine as new type of antimicrobial agents were synthesized and characterized by ^{1}H NMR, ^{13}C NMR, IR, MS and HRMS spectra. Bioactive assay manifested that most of the prepared compounds exhibited effective antibacterial and antifungal activities and some showed comparable or superior potency against *Methicillin-resistant Staphylococcus aureus* to reference drugs Norfloxacin, Chloromycin and Berberine. The transportation behavior of human serum albumin (HSA) to the highly active compound **5g** was evaluated and revealed that the association of imidazole derivative **5g** with HSA was spontaneous and the electrostatic interactions played important roles in the transportation of HSA to **5g**. The calculated parameters indicated that compound **5g** could be effectively stored and carried by HSA.

1. Introduction

Imidazole derivatives have been attracting increasing interest due to their large potentiality in medicinal chemistry.[1–6] Nitro modified imidazoles are a unique type of imidazole derivatives in which nitro fragment could not only enhance lipophilicity of the target compounds which is favorable for tissue penetration, but also induce bioactivities through the metabolic activation of nitro group.[7] Nitroimidazole-based compounds exhibited various bioactivities like antibacterial,[8,9] anti-tubercular,[10] antiparasitic[11] and anticancer[12,13] ones. Especially, as antimicrobial agents, many nitroimidazoles such as metronidazole, benznidazole, ornidazole, secnidazole, nimorazole and tinidazole have been in widespread clinical use to treat diseases caused by anaerobic bacteria.[4,14] Notably, despite of their long term clinical use, the incidence of resistance in anaerobic bacteria is still very low. This encourages continuous researches to focus on the development of such nitroimidazoles with potential medicinal application. Particularly, structurally simple metronidazole as an effective synthetic drug introduced in 1960 possesses strong inhibitory efficacies against Gram-negative anaerobic bacteria like *Helicobacter pylori* and protozoa such as *Giardia*, *Lamblia*, and *Entomoeba histolytic*.[15] However, with the extensive investigations towards nitroimidazoles, researches disclosed that the reactive intermediates formed in microorganisms by the reduction of nitro group in nitroimidazoles could covalently bind with DNA and trigger the adverse effect. The sterical protection of nitro group in metronidazole was proved to be an effective way to improve the metabolism and physicochemical property of such compounds.[16] Furthermore, some frequently used clinical antimicrobial nitroimidazole drugs like nitroimidazooxazine PA-824 were reported to be subjected to poor aqueous solubility and unsuitable binding propensity to proteins in human plasma (Fig. 1).[17–19] Therefore, the development of new types of nitroimidazoles with broad antimicrobial spectrum, good metabolism and physicochemical property, and suitable binding affinity to serum albumins has been recently attracting special attention. Much effort has been oriented towards novel nitroimidazole-based antimicrobial agents with high efficiency.

Berberine has been commonly used in the clinic as therapeutic agent to treat infectious diseases such as acute gastroenteritis, cholera and bacillary dysentery for many years. Its importantly clinical uses stimulate the continuing researches in antimicrobial field[20,21] and other related investigations such as anticancer,[22] antiviral,[23] antiinflammatory,[24] antiparasitic[25,26] activities. The special structure of berberine with a quaternary nitrogen and large desirable π-conjugated backbone could exert noncovalent forces like π–π stacking and electronic interactions. Thereby, it not only could improve the physicochemical properties of its derivatives,

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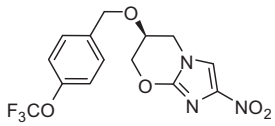

Nitroimidazooxazine (PA-824)

Figure 1. Structure of nitroimidazooxazine (PA-824).

their affinity to transport proteins, and thus enhance their antimicrobial activities, but also easily interact with diverse enzymes and receptors in biological system resulting in broad bioactive spectrum. In our previous work,[27] a type of amine-derived bis-azoles has been successfully developed as newly structural Fluconazole analogues. The tertiary alcohol moiety in Fluconazole was replaced by a tertiary amino group as bioisoster, and the methylene bridge between the tertiary alcohol and the triazolyl moieties was substituted by an ethylene chain (Fig. 2). Bioactive study manifested that this type of compounds exhibited good or even better antimicrobial activities in comparison to reference drugs Chloromycin, Norfloxacin and Fluconazole. Here, we would like to replace the azolyl ethylamino moiety with the clinical metronidazole as bioisoster fragment and further substitute the other azolyl ring by the berberine skeleton to generate a series of novel hybrids of berberine and nitroimidazoles as potential antimicrobial agents. This special type of hybrids might not only be helpful to spatially protect the nitro group with the potential to improve the metabolism and physicochemical property, but also improve the water solubility and binding affinity by the introduction of tertiary amine moiety thereby effectively increase their biological activities and broaden active spectrum. In addition, various halobenzyl moieties were introduced into target compounds to investigate the effect of halobenzyl moiety on biological activities, since many halobenzyl incorporated molecules gave good antimicrobial activities.[28,29] The designed structures of this series of novel hybrids of berberine and nitroimidazole moiety including 4-nitroimidazole, 5-nitroimidazole and 2-methyl-5-nitroimidazole derivatives are shown in Scheme 1.

Serum albumins as the most important and abundant macromolecule proteins in the circulatory system have received much attention for that they could deliver drugs or other bioactive small molecules to the binding sites.[30,31] A thorough binding analysis between drugs or bioactive small molecules and human serum albumin (HSA) may beneficially provide useful information for the absorption, transportation, distribution, metabolism and excretion properties of drugs. It might also be significant to the design, modification and screening of drug molecules. In view of above observations, it is considerably reasonable for us to further investigate the transportation behavior by HSA to the highly active prepared compounds in order to preliminarily evaluate their transportation and pharmacokinetic properties by fluorescence and UV–vis absorption spectroscopy on molecular level.

2. Results and discussion

2.1. Chemistry

The target hybrids of berberine nitroimidazoles were prepared from commercial halobenzyl chlorides, diethanolamine and berberrubine (Scheme 1). Diethanolamine was *N*-alkylated with different halobenzyl chlorides **1a–e** to produce intermediates **2a–e** in excellent yields (92.2–97.8%). The resulting diols **2a–e** were treated in chloroform with phosphorus tribromide to afford dibromides **3a–e** in good yields ranging from 88.3% to 97.4%.[27] Further reactions of compounds **3a–e** with 4-nitroimidazole afforded the corresponding mono-azole bromides **4b–k** in 18.5–80.2% yields. Possible reactions of 4-nitroimidazole in the presence of potassium carbonate are shown in Figure 3. It has been documented that tautomeric interconversion of the 5-nitro and 4-nitro imidazoles takes place under either acidic or basic conditions. During the *N*-alkylations of 4-nitroimidazole with alkyl halides, the acidic conditions favored the 5-nitro orientation while basic conditions favored the 4-nitro orientation.[32] In this work, potassium carbonate was selected to produce our target compounds, since the yields were generally poor under acidic conditions. In the presence of potassium carbonate, 4-nitroimidazole displays four resonance forms (**A–D**) (Fig. 3). Since patterns **A** and **C** are much more stable than patterns **B** and **D**, the *N*-alkylation of 4-nitroimidazole could yield *N*-1 and *N*-3 alkylated products, and the former is the predominant one due

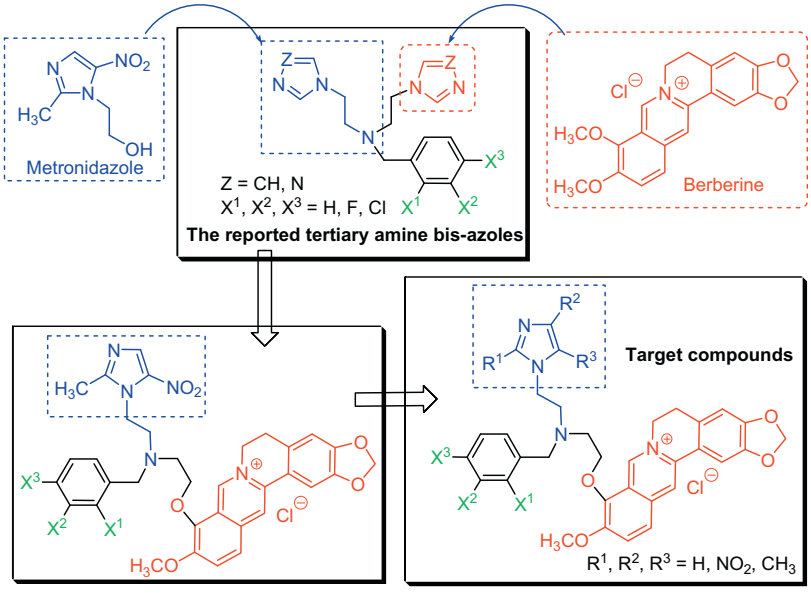

Figure 2. Design of novel hybrids of berberine and nitroimidazoles.