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Original article

Novel hybrids of metronidazole and quinolones: Synthesis, bioactive evaluation, cytotoxicity, preliminary antimicrobial mechanism and effect of metal ions on their transportation by human serum albumin

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ABSTRACT

A novel series of hybrids of metronidazole and quinolones as antimicrobial agents were designed and synthesized. Most prepared compounds exhibited good or even stronger antimicrobial activities in comparison with reference drugs. Furthermore, these highly active metronidazole–quinolone hybrids showed appropriate ranges of pKa, log *P* and aqueous solubility to pharmacokinetic behaviors and no obvious toxicity to A549 and human hepatocyte LO2 cells. Their competitive interactions with metal ions to HSA revealed that the participation of Mg^{2+} ion in compound **7d**–HSA association could result in a concentration increase of free compound **7d**. Molecular modeling and experimental investigation of compound **7d** with DNA suggested that possible antibacterial mechanism might be in relation with multiple binding sites between bioactive molecules and topo IV–DNA complex.

1. Introduction

Quinolones are an important kind of synthetic antibacterial agents as they are generally well tolerated with excellent safety profile, favorable pharmacokinetic characteristics, broad antibacterial spectrum and good treatment effectiveness [1–3]. Since the first generation of quinolones were discovered in 1960s, four generations of quinolones have been successfully developed in succession and a large number of quinolone drugs, such as norfloxacin, moxifloxacin, ofloxacin, temafloxacin, have been successfully and widely used in clinic to treat genitourinary infections and common respiratory tract pathogens. Furthermore, the antibacterial mechanism of quinolones has been elucidated clearly. These drugs primarily target type II topoisomerases or DNA gyrase in Gram-negative bacteria to stabilize the cleavage complex at specific sites on DNA, then block DNA replication and finally lead to a lethal lesion [4–5]. However, in recent years the emergence and spread of genitourinary infections, which have evolved mechanisms of resistance to quinolones, are becoming one of the most paramount public health threats. A growing medical concern on mechanisms of resistance to quinolones has drawn much attention, and much effort has been focusing on this topic [6]. Some researches [7] show that the resistance to quinolones is principally due to mutations in either ParC breakage-reunion or ParE TOPRIM domains of topo IV (topoisomerase IV) and in the analogous gyrase domains [8]. Further structure activity relationship (SAR) reveals that the groups at *N*-1 position of quinolones are in close proximity to the conserved ParC helix a4 residues Ser79 and Asp83 in the topo IV–DNA complex, whose mutation causes quinolone resistance in *pneumococci* and other bacteria [9]. Therefore, the structural modification of quinolones at *N*-1 position is a promising strategy to overcome the quinolone resistance. Many kinds of functional groups such as ethyl, cyclopropyl and various heterocyclic moieties have been successfully introduced into *N*-1 position, and produced many effective clinical quinolone drugs [10].

In recent years, azole heterocyclic compounds have been paid special attention due to their potential applications as medicinal agents [11–12], and an increasing effort has been directing towards their possible medicinal potentiality [13–16]. Particularly, the

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nitro-containing imidazoles are found to be a kind of important imidazole antimicrobial derivatives, and many of them such as benznidazole, secnidazole, metronidazole and ornidazole have been extensively used in clinic to treat anaerobic infections [17]. Especially the 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* [18]. These outstanding achievements encourage numerous researchers to focus on their research and development in nitroimidazoles with potential medicinal application. The mechanism researches indicate that the nitro fragment of metronidazole plays a significant role through the metabolic activation in exerting biological activities [19]. Furthermore, a lot of researches disclose that the reactive metabolic intermediates formed in microorganisms by reducing nitro group in nitroimidazoles can covalently bind with DNA and trigger the adverse effect [20]. The sterical protection of nitro group in metronidazole has been proved to be an effective strategy to improve the metabolism and physicochemical property of such compounds [21]. Moreover, the incidence of resistance in anaerobic bacteria is still very low. Therefore, the structural modification of metronidazole has been an increasingly attractive topic.

Our previous work has reported that the incorporation of nitroimidazoles into berberine not only enhance the antimicrobial activities, but also broaden antimicrobial spectrum. This type of hybrids of berberine and nitroimidazoles exhibited anti-Gram-negative efficacies with low MIC values ranging from 4 to 8 μg/mL. They were also proven to effectively bind with biomacromolecules like DNA and Human Serum Albumin (HSA) [22].

In view of the above considerations and as an extension of our previous work [23–28], herein we introduced the nitroimidazole moieties into *N*-1 position of quinolones to generate a novel series of hybrids of metronidazole and quinolone. Rational design and relevant synthons were shown in Fig. 1. The nitroimidazole groups at *N*-1 position of quinolones were expected to exert noncovalent interactions with DNA base, ParC breakage-reunion or ParE TOPRIM domains of topo IV–DNA complex to overcome the resistance and broaden the antimicrobial spectrum. Therefore, the antimicrobial activities for all newly synthesized compounds were evaluated *in vitro* against nine bacteria including *Methicillin-Resistant Staphylococcus aureus* N315 (MRSA) and five fungi [29]. Cytotoxicity, ionization constants (pKa), aqueous solubility and partition coefficients of some highly active target compounds were also evaluated to predict their pharmacokinetics behaviors. The effects of metal ions on their transportation by HSA were also investigated in order to preliminarily evaluate their transportation, distribution, and metabolism by fluorescence and UV–vis absorption spectroscopy on molecular level. Furthermore, flexible ligand–receptor docking investigation was undertaken to rationalize the antibacterial activity and understand the possible mechanism of the hybrids. The comparative study of preliminary interaction between compound **8d** and norfloxacin separately with calf thymus DNA were also evaluated to further predict the binding ability between these hybrids and topo IV–DNA complex.

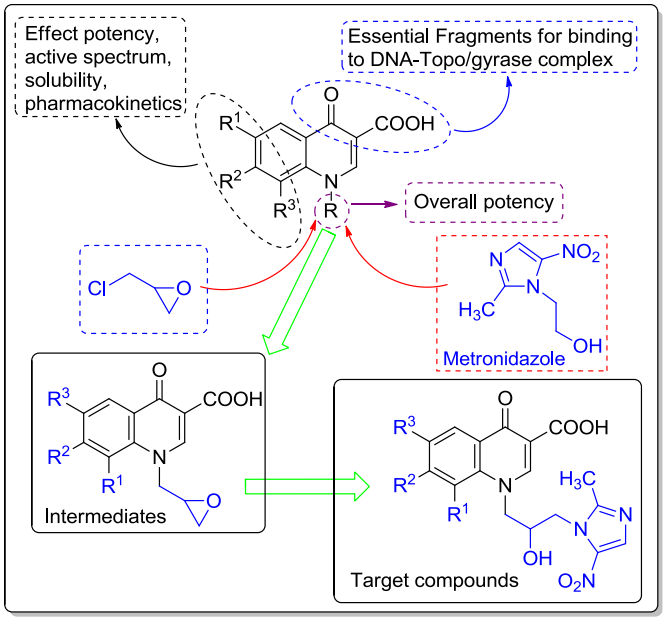

Fig. 1. Design of novel target hybrids of metronidazole and quinolones.

2. Results and discussion

2.1. Chemistry

The target hybrids were synthesized according to the synthetic route outlined in Scheme 1. Commercial ethoxymethylene malonic ester (EMME) was reacted with a series of substituted phenylamines in ethanol to afford intermediates **1a**–**h** in almost quantitative yields. The obtained compounds were then cyclized in phenoxybenzene under reflux to produce the desired quinolones **2a**–**h** in good yields ranging from 49.0% to 60.0%, which were further *N*-alkylated by commercial racemic 2-(chloromethyl)oxirane to yield ethyl 1-(oxiran-2-ylmethyl) quinoline-3-carboxylate derivatives **3a**–**h** in high yields (81.2–95.0%). The epoxy rings of compounds **3a**–**h** were opened by 4-nitroimidazole and 2-methyl-5-nitroimidazole respectively in ethanol using sodium bicarbonate as base to produce the corresponding racemates **4a**–**h** and **5a**–**h** in satisfactory yields ranging from 80.2% to 98.5%, and then the latter were further hydrolyzed by 3% sodium hydroxide at 100 °C to afford the target quinolone–metronidazole hybrids **6a**–**h** and **7a**–**h** in excellent yields.

In the preparation of compounds **3a**–**h**, it was found that the solvents and base exerted remarkable effect on their yields. In particular, 2-(chloromethyl)oxirane as both solvent and reactant led to relatively high yield in contrast to other common solvents such as ethanol, methanol and acetonitrile. It was found that suitable base in the synthesis of intermediates **3a**–**h** was potassium carbonate, since other bases resulted in the low yields of target compounds, and formation of various by-products was observed.

2.2. Analysis of spectra

All the new compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and HRMS spectra. Their spectral analyses were consistent with the assigned structures and listed in the experimental section. The mass spectra for new compounds gave a major fragment of $[M+H]^+$ or $[M+Na]^+$ according to their molecular formula.

2.2.1. ^{1}H NMR spectra

In ^{1}H NMR spectra, it was noticed that the protons of hydroxyl group and tertiary carbon in compounds **4**–**7** appeared as multiplet peaks at δ 5.20–5.95 ppm and δ 4.67–4.87 ppm respectively due to the adjacency of chiral tertiary carbon and the electron-withdrawing character of quinolone backbones and imidazole rings. For compounds **3a**–**h**, because of the effect of electron-withdrawing quinolones and epoxy rings, the protons of CH_2 linked to *N*-1 position gave large downfield chemical shifts at δ 4.40–5.04 ppm. The CH proton on the epoxy ring exhibited a