Bioorganic & Medicinal Chemistry 22 (2014) 2409–2415

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis, molecular docking and biological evaluation of metronidazole derivatives containing piperazine skeleton as potential antibacterial agents

She-Feng Wang [†], Yong Yin [†], Fang Qiao, Xun Wu, Shao Sha, Li Zhang [*], Hai-Liang Zhu [*]

State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China

ARTICLE INFO

Article history:
Received 22 January 2014
Revised 3 March 2014
Accepted 4 March 2014
Available online 13 March 2014

Keywords:
Metronidazole derivatives
Antibacterial activities
S. aureus TyrRS
Molecular docking

ABSTRACT

Metronidazole has a broad-spectrum antibacterial activity. Hereby a series of novel metronidazole derivatives were designed and synthesized based on nitroimidazole scaffold in order to find some more potent antibacterial drugs. For these compounds which were reported for the first time, their antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* were tested. These compounds showed good antibacterial activities against Gram-positive strains. Compound **4m** represented the most potent antibacterial activity against *S. aureus* ATCC 25923 with MIC of 0.003 μg/mL and it showed the most potent activity against *S. aureus* TyrRS with IC_{50} of 0.0024 μM. Molecular docking of **4m** into *S. aureus* tyrosyl-tRNA synthetase active site were also performed to determine the probable binding mode.

1. Introduction

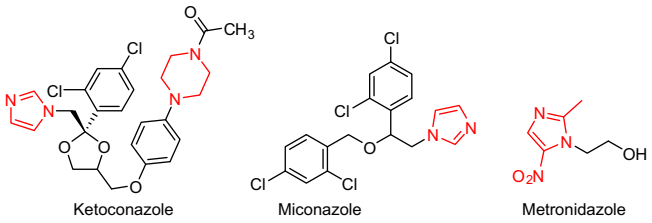
Infections induced by antibiotic-resistant pathogens have already led to a crisis in people's health care. With the advent of drug resistance, the microbes have developed several different kinds of resistance mechanisms. One of them is to reduce drug affinity by target-based mutation.[1] Many problems still need to be solved even though there are a lot of antimicrobial-drugs available such as ketoconazole, miconazole, metronidazole and so on (Scheme 1).[2–9] For example, it has already caused serious menace because of the appearance of multidrug resistant Gram-positive bacteria, in particular, methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant. In order to prevent this serious medical problem, it is urgent to elaborate new types of antibacterial agents.[10] Therefore, much of the research effort is put into the design of high efficiency antibacterial agents to combat resistant pathogens.[11]

Aminoacyl-tRNA synthetases (aaRSs) have been proved to be antimicrobial targets,[12] and they have been interesting targets in antibacterial drug design.[13,14] Aminoacyl-tRNAs can provide required substrates during protein synthesis. As in mRNA, the coded genetic information is translated into amino acid sequences which result proteins because the coded sequences complement with the anticodon sequences which are built into a respective aminoacyl-tRNA.[15] Because aminoacyl-tRNA synthetases can recognize these information including the coincident tRNA molecules and the amino acids' structures,[16] they are essential to translate the coded information into protein structures in nucleic acids.[17] As a member of aminoacyl-tRNA synthetase family, tyrosyl-tRNA synthetase also plays an important role in protein synthesis. And it is a promising target for antibacterial agents as the topology of the ATP binding site and the interaction of bacteria aaRSs are different from those of human.[18–22]

Nitroimidazole derivatives have showed broad varieties of biological activities especially antimicrobial activity.[23] 5-Nitroimidazole based drugs have been applied to treat the infections induced by bacteria and a range of pathogenic protozoan parasites for many years because nitroimidazole derivatives can undergo bioreduction to produce electrophilic substances that can damage protein and nucleic acids.[24] What's important is the toxicology and metabolism of nitroimidazoles have been characterized, especially metronidazole (Scheme 1).[25,26] As one of the important nitroimidazole derivatives, metronidazole has been widely used as antimicrobial medicine. In recent years, much attention has been paid to modify the pendant hydroxy group of metronidazole.[27] Since piperazines and its derivatives are important pharmacophores, they are effective ingredients in many marketed drugs like the merck HIV protease inhibitor Crixivan and Piperazinyl-linked ciprofloxacin dimers which was reported as potent antibacterial agents against resistant strains,[28,29] and imidazole is found in many marketed drugs like ketoconazole, miconazole and metronidazole and so on (Scheme 1),

* Corresponding authors. Tel.: +86 25 83592572; fax: +86 25 83592672.
E-mail address: zhuhl@nju.edu.cn (H.-L. Zhu).
† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bmc.2014.03.004
0968-0896/



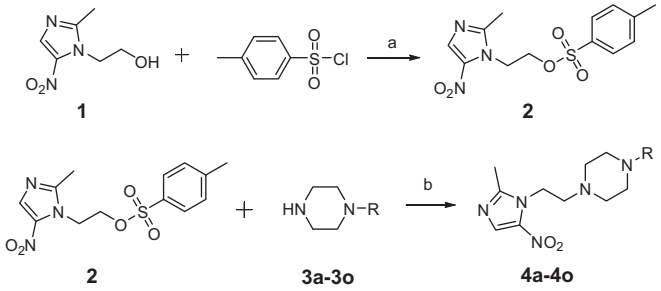
Scheme 1. Chemical structure of metronidazole.

especially ketoconazole, it also contains piperazine skeleton. This promoted us to synthesize new derivatives of metronidazole with piperazine skeleton for the sake of finding new efficacy antibacterial agents. Hence, we synthesized a series of novel metronidazole derivatives owing piperazine skeleton (**4a**–**4o**). In our preliminary work, the molecular docking was performed to validate which antibacterial target protein the designed compounds can work, the compounds were fitted into the ATP binding site of FabH (PDB code: 1HNJ), bacterial DNA gyrase (PDB code: 3G75), bacterial thymidylate kinase (PDB code: 4HEJ) and *S. aureus* tyrosyl-tRNA synthetase (PDB code: 1JIJ).[30–34] The results have been plotted as a line-scatter graph and presented in Figure 1. It showed that the interaction energy between the designed compounds and protein 1JIJ is the lowest. It means that the designed compounds are likely to display more potent inhibitory activity against *S. aureus* tyrosyl-tRNA synthetase. So we studied their antibacterial activities against *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *S. aureus* tyrosyl-tRNA synthetase (PDB code: 1JIJ) inhibitory activities. The results showed that the compounds showed good activity against Gram-positive bacterial but no activity against the Gram-negative bacterial. Docking simulation was performed using the X-ray crystallographic structure of the TyrRS of *S. aureus* in complex with the most potent inhibitor to explore the binding mode of the compound at the active site.

2. Results and discussion

2.1. Chemistry

Compounds **4a**–**4o** were synthesized by the routes outlined in Scheme 2. They were first designed and synthesized from metronidazole and piperazine derivatives. The key compound MET-OTs (2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 4-methylbenzenesulfonate, compound **1**) was synthesized by the method in our previous paper.[35] The research showed when the ratio of MET-OTs and piperazine derivatives was 1:1.2, the reaction temperature was 80 °C, and the reaction time was 24 h, compounds **4a**–**4o** were obtained with the higher yield. The chemical structures of these metronidazole derivatives were summarized in Table 1. All of the synthetic compounds were characterized by ^{1}H NMR, elemental analysis and mass spectrum, and they gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.



Scheme 2. Reagents and conditions: (a) CH_2Cl_2, TEA, 0 °C, 5 h; (b) K_2CO_3, DMF, 80 °C, 22–24 h.

2.2. Biological activity

2.2.1. Antibacterial activity

The synthesized compounds were tested for their antibacterial activity against two Gram-negative bacterial strains: *E. coli* and *P. aeruginosa* and two Gram-positive bacterial strains: *B. subtilis* and *S. aureus* by MTT method with MH medium (Mueller-Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The MICs (minimum inhibitory concentrations) of the compounds against these bacteria were reported in Table 2, the activity of reference drugs Penicillin G and Chloramphenicol was also included. The results revealed that those compounds showed good activity against Gram-positive bacterial but inactive against Gram-negative bacterial.

Most of the new compounds exhibited good inhibitory activities against both *B. Subtilis* and *S. Aureus*. As shown in Table 2. Compounds **4l** and **4m** showed most potent activities with MIC values of 0.033,

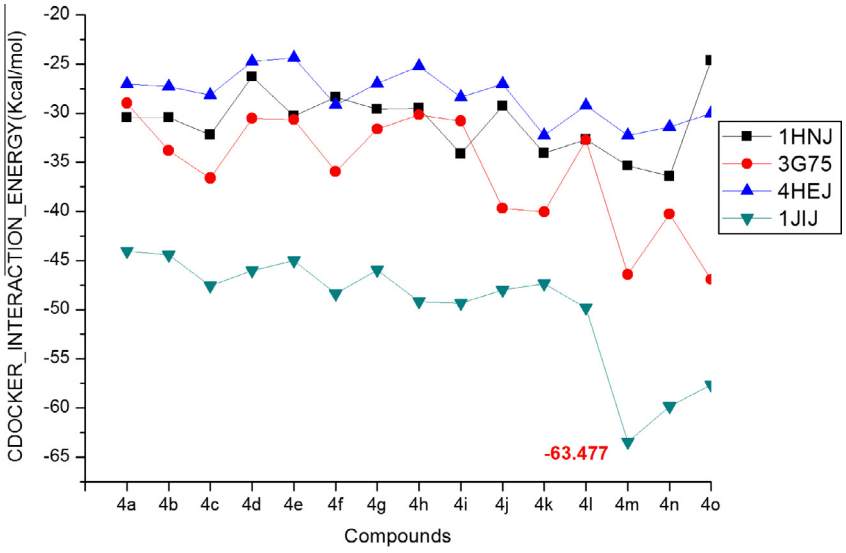


Figure 1. The CDOCKER_INTERACTION_ENERGY (kcal/mol) obtained from the docking study of all synthesized compounds by the CDOCKER protocol (Discovery Studio 3.5, Accelrys, Co. Ltd).