Bioorganic & Medicinal Chemistry Letters 24 (2014) 5279–5283

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis and biological evaluation of metronidazole–thiazole derivatives as antibacterial inhibitors

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ARTICLE INFO

Article history:
Received 31 March 2014
Revised 11 September 2014
Accepted 18 September 2014
Available online 28 September 2014

Keywords:
Metronidazole–thiazole derivatives
FabH inhibitors
Antibacterial activities
Cytotoxicity
Molecule docking

ABSTRACT

A series of metronidazole–thiazole derivatives has been designed, synthesized and evaluated as potential antibacterial inhibitors. All the synthesized compounds were determined by elemental analysis, ^{1}H NMR and MS. They were also tested for antibacterial activity against *Escherichia coli*, *Bacillus thuringiensis*, *Bacillus subtilis* and *Pseudomonas aeruginosa* as well as for the inhibition to FabH. The results showed that compound **5e** exhibited the most potent inhibitory activity against *E. coli* FabH with IC_{50} of 4.9 μM. Molecular modeling simulation studies were performed in order to predict the biological activity of proposed compounds. Toxicity assay of compounds **5a**, **5b**, **5d, 5e**, **5g** and **5i** showed that they were noncytotoxic against human macrophage. The results revealed that these compounds offered remarkable viability.

The traditional antibiotics have been known and commercially available like metronidazole and secnidazole (**a**, Fig. 1).[1] However, the major obstacle in the antimicrobial drug therapy has come to be the drug resistance. Therefore, the spread of antibiotic resistance among pathogenic bacteria has become a serious puzzle for the clinical management of infectious diseases and resulted in a clear need for novel antibacterial agents.[2] To solve this severe medical problem, the urgent task of seeking out new types of antibacterial agents should be accomplished.[3] In recent years, diverse targets in key areas of the bacterial cell cycle have been studied and correlative researches showed the prospect of finding a new approach against the challenge of drug resistance. Among all the targets, the fatty acid synthase (FAS) pathway in bacteria is a promising one. Fatty acid biosynthesis (FAB) is an essential metabolic process for prokaryotic organisms and is required for cell viability and growth. This pathway has been demonstrated to be essential for bacteria cell survival, and differs considerably from human FAS pathway.[4] A key enzyme responsible for initiation of bacterial fatty acid biosynthesis has so far escaped serious attention by the drug discovery industry. FabH is a β-keto-acyl-ACP synthase playing an essential and regulatory role in bacterial FAB.[5] As shown in Figure 2, FabH catalyzes condensation reaction between a CoA-attached

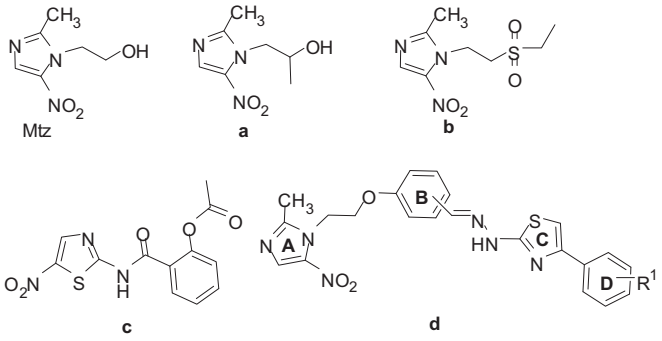

Figure 1. Chemical structures of the antigiardial 5-nitro drugs.

acetyl group and an ACP-attached malonyl group which yields acetoacetyl-ACP as its final product.[6]

FabH is a kind of condensing enzyme which initiates the fatty acid biosynthetic pathway.[7] It is different from FabB and FabF in structure which are also condensing enzyme elongating the chain of acyl ACP subsequently after the initiation. Wide interest has been arisen of FabH and it is also well accepted as specific target for drugs for almost no proteins in humans share remarkably homologous sequence.[8] Highly conserved at amino acid sequence and space structure level, FabH contains invariant residues in

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http://dx.doi.org/10.1016/j.bmcl.2014.09.054
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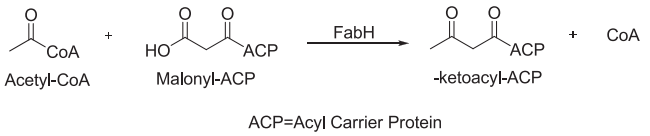

Figure 2. FabH-catalyzed initiation reaction of fatty acid biosynthesis.

active site in diverse bacteria, which makes specific inhibitors of FabH enzyme promising drug candidates possessing property of nontoxicity, high selectivity and wide antibacterial spectrum.[9] Designed using enzymatic assays and optimized through structure-guided drug design methods, quite a few of novel compounds have been synthesized to inhibit bacteria by cocrystallization with diverse pathogenic FabH enzymes, which is key to the initiation of fatty acid biosynthesis.[10] Among those, 5-nitroimidazoles play important role in antibacterial drugs as heterocyclic nucleus.[11] Nitroimidazoles and their derivatives have been extensively used as antimicrobial chemotherapeutics and as antiangiogenic hypoxic cell radiosensitizers.[12] Therefore, nitroimidazole derivatives have attracted considerable attention as they show a tendency to penetrate and accumulate in bacteria.[13] In fact, 5-nitroimidazoles are the most common antimicrobial drugs, particularly secnidazole and metronidazole are accepted as drug of choice for anti-infectious chemotherapy against bacteria. In the previous researches, we have already gained several series of 5-nitroimidazole containing compounds and examined their bioactivity and toxicity. Also the target was proved to be FabH enzyme and the structure of 5-nitroimidazole essential.[14–16] Importantly, the toxicology and metabolism of nitroimidazoles have already been characterized.[17]

Metronidazoles are rapidly and completely absorbed after oral administration and has a longer terminal elimination half-life (17–29 h) than commonly used drugs in this class.[18] In this case, the treatment with secnidazole is shorter and more effective than the treatment using other imidazole drugs and the adverse effects are not very drastic.[19] On the basis of the therapeutic utility of metronidazole, several other 5-Nis[a] has been developed recently. For example, tinidazole (**b**, Fig. 1), a N1-position modified 5-NI, has been approved by the FDA for the treatment of giardiasis. Not all antigiardial nitro drugs are based on the 5-NI scaffold. Nitazoxanide (**c**, Fig. 1) belongs to an emerging class of 5-nitrothiazole compounds with potent antigiardial activity.[20] Overall, metronidazole remains the standard treatment for giardiasis to date, making the discovery and development of new therapeutics an important goal in expanding the arsenal of antibiotics for controlling the infection.[21]

Thiazoles and their derivatives have attracted continuing interest over the years because of their varied biological activities such as anti-inflammatory, antimicrobial, antiproliferative, antiviral, anticonvulsant, antifungal and antibacterial.[22]

As a result, excellent works have been published on the activities and pharmacokinetics of metronidazole derivatives and their determination in pharmaceutical is of great importance.[23] At present, our group has designed and synthesized several kinds of FabH inhibitors.[4b] All of these compounds have 5-nitroimidazole in skeleton. According to this, we continued our previous study and designed new heterocycles containing imidazole and thiazole moieties in single scaffold as FabH inhibitors. In this paper, we have designed and synthesized a series of metronidazole derivatives which was evaluated for antibacterial activity against *Escherichia coli*, *Bacillus thuringiensis*, *Bacillus subtilis* and *Pseudomonas aeruginosa* and cytotoxicity in human cells. The structure–activity relationships (SAR) were discussed with respect to four key structural features of these compounds: (i) metronidazole (A); (ii) position of formyl group on the phenyl ring (B); (iii) thiazole ring (C) and (iv) substituent on the benzene ring (D) (**d**, Fig. 1). Initial studies were performed by modification of the parent compounds to determine whether any of the subunits which comprise the 2-substituent group on secnidazole enhanced antibacterial activity or not. Furthermore, docking simulations were performed using the X-ray crystallographic structure of the FabH in complex with an inhibitor to explore the binding modes of these compounds at the active site.

All the metronidazole–thiazole derivatives (**5a–5j**) described herein were synthesized by the following synthetic pathway depicted in Scheme 1. The starting MET-OTs (2-(2-methyl-5-nitro-1*H*-imidazole-1-*yl*) ethyl 4-methylbenzene-sulfonate, compound **2**) has been synthesized from the metronidazole using the proper excess of tosyl chloride in the presence of triethylamine in dichloromethane.[24] The syntheses of metronidazole–benzaldehydes **3a**–**3b** were performed by nucleophilic displacement reactions of the corresponding MET-OTs in the presence of *o*- or *p*-hydroxybenzaldehyde and K_2CO_3 in DMF. Condensation of hydrazine-carbothioamide and the corresponding metronidazole–benzaldehyde in isopropanol afforded the desired **4a**–**4b** in high yields and short reaction times. Subsequently, compounds **5a**–**5j** were prepared from reaction of **4a**–**4b** with the corresponding different substituted 2-bromoacetophenone in isopropanol at room temperature. The solvent was removed by evaporation. The reactions were monitored by thin layer chromatography (TLC) and the crude products were purified by recrystallization with ethanol, ethyl acetate and acetone (1:1:0.05). All of the target compounds gave satisfactory analytical and spectroscopic data, which were in accordance with their depicted structures by ^{1}H NMR, ESI MS.

Compounds **5a**–**5j** were assayed against two Gram-negative bacterial strains (*E. coli* and *P. aeruginosa*) and two Gram-positive bacterial strains (*B. subtilis* and *B. thuringiensis*) by MTT method. In this assay, the IC_{50} of sufficiently potent antibacterial activity compounds were shown in Table 1. The results were compared with that provided by the known antibiotic: Kanamycin under identical conditions, and proved that most of the synthesized compounds exhibited significant antibacterial activities.

The results obtained from in vitro antibacterial evaluation of compounds **5a**–**5j** against susceptible and resistant strains were moderate. Most compounds exhibited moderate to good activity against all Gram-positive and Gram-negative strains tested. Most potent activity in vitro was observed for compound **5e** against *E. coli* with corresponding IC_{50} of 26.9 μg/mL, which was superior as compared to the activity of positive control Kanamycin. In addition, these data suggested that compound **5i** and **5g** possessed potent activities with IC_{50} values of 3.6, 5.9, 1.9 μg/mL against *P. aeruginosa*, *B. subtilis*, *B. thuringiensis*, respectively.

Based on the data obtained, we have changed a variety of substituents at different positions on **D** ring and found that various substituents such as halogen, methoxyl and trifluoromethyl leaded to different antibacterial activities of these target compounds. Compound **5e** with a bromo atom at 4-position of **D** ring and a metronidazole at 4-position of **B** ring showed higher antibacterial activity with IC_{50} values of 26.9 μg/mL against *E. coli* than compound **5b** with a methoxy group at 4-position (50.8 μg/mL) of **D** ring. However, the most active compound **5g** from this group was the methoxy group at 4-position of **D** ring and a metronidazole at 3-position of **B** ring.

In comparison to these target compounds, we found that these compounds with electron-donating group on the benzene ring (such as –Br, $–OCH_3$) exhibited more potent antibacterial activities than those have electron-withdrawing substituents (such as $–CF_3$). From the above-mentioned analysis, it could be concluded that the compounds with methoxyl or halogen substituted benzene ring were the most favorable for the antibacterial activity.

To generate data concerning the broad spectrum potential of these compounds in Table 1, the IC_{50} values of selected compounds against FabH enzymes were summarized in Table 1. Reference data