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Synthesis and antibacterial evaluation of amino acid–antibiotic conjugates

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ABSTRACT

Amino acid conjugates of quinolone, metronidazole and sulfadiazine antibiotics were synthesized in good yields using benzotriazole methodology. All the conjugates were screened for their antibacterial activity using methods adapted from the Clinical and Laboratory Standards Institute. Antibiotic conjugates were tested for activity in four medically relevant organisms; *Staphylococcus aureus* (RN4220), *Escherichia coli* (DH5α), *Pseudomonas aeruginosa* (PAO1), and *Bacillus subtilis* (168). Several antibiotic conjugates show promising results against several of the strains screened.

The increasing incidence of infection caused by the rapid onset of bacterial resistance to available antibiotics is a serious health problem.[1] While many factors may cause mutations in microbial genomes, it has been demonstrated that the incorrect use of antibiotics can greatly increase the development of resistant genotypes.[2] As multidrug-resistant bacterial strains proliferate, the necessity for effective therapy has stimulated research into the design and synthesis of novel antimicrobial molecules. Many versatile bioactive molecules are peptides and many peptide hormones and analogous shorter peptides exert their action by binding to membrane receptors.[3] Peptide derivatives can exhibit antimicrobial,[4] antiviral,[5] anticancer activity[6] etc. and can open up new perspectives in drug design as highly specific and non-toxic pharmaceuticals. In recent years, these synthesis-based derivatives have received considerable attention.[7,8] Currently there is much interest in conjugates of amino acid or peptide residues with bioactive heterocyclic motifs in the field of biomedical research taking advantage of the low toxicity, biocompatibility and structural diversity of amino acids.[9]

Cell-permeating antimicrobial agents can potentially play an important role in eliminating infections by intracellular pathogens. Unfortunately, many antibiotic classes do not penetrate the plasma membrane effectively (for example C/E ratio of fluoroquinolones is 4–10; β-Lactams is <1; metronidazole is 1).

Prodrugs serve to improve drug physicochemical properties that in turn increase drug concentration at an active site and hence prolong the effect, while decreasing, toxicity and side effects. A prodrug should be stable in the stomach and in the small intestine, nontoxic, biodegradable and biocompatible, whether it has low molecular weight (amino acid, carbohydrate) or is a macromolecule (polymers).[10]

Prodrugs formed from quinolone acids and amino acid esters are more lipophilic than the parent drugs[11,12] and can show enhanced in vivo antibacterial properties[13–15] with pronounced therapeutic effects against *Pseudomonas aeruginosa*,[16,17] *Escherichia coli*,[18] *Staphylococcus aureus*[19] and *Salmonella typhi*.[15]

The antibiotics chosen for chemical modification have a wide range of activity. All but Metronidazole are considered broad-spectrum antibiotics with activity against Gram-(+) and Gram-(−) bacteria. Metronidazole, a nitro-imidazole derivative, acts through DNA inhibition and affects both protozoa and bacteria. Unlike other antibiotics, metronidazole is primarily active against anaerobic bacteria though there are some reports of effects on aerobic bacteria.[20] The fluoroquinolones chosen in this study include ciprofloxacin, a second-generation fluoroquinolone that inhibits topoisomerase, and norfloxacin, a second-generation

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fluoroquinolone and a synthetic chemotherapeutic antibacterial agent that targets DNA gyrase and topoisomerase IV. Pipemedic acid, a pyridopyrimidine, is a first generation quinolone that targets topoisomerase and is reportedly active against *P. aeruginosa* as well as several Gram positive pathogens.[21] Fluoroquinolones can cause adverse reactions in the central nervous system, skin and gastrointestinal tract.[22] Sulfadiazine, a sulfonamide, acts through inhibition of purine metabolism and prevents DNA and RNA synthesis. Peptide derivatives may decrease arbitrary degradation of antibiotic compounds thus increasing concentration at a target site; these derivatives may maintain or improve antibacterial activity while diminishing undesirable side effects because the initial dosage may be lowered if more of the antibiotic is reaching the target.

Staphylococcus aureus (RN4220), *Escherichia coli* (DH5**α**), and *Pseudomonas aeruginosa* (PAO1) were selected for antibiotic conjugate screening because of their physiological relevance and close relation to pathogenic strains which cause disease in humans. Additionally, we chose to evaluate their antibiotic activity on *B. subtilis*, a common gut commensal bacterium.[23]

N-Acylbenzotriazoles[19] are efficient reagents for N-, O-, S- and C-acylation[24] and when prepared from *N*-protected α-amino acids have been utilized for the synthesis of di-, tripeptides.[25]

We now report syntheses of diverse classes of antibiotics-amino acid conjugates by coupling ciprofloxacin **3**, pipemidic acid **5**, norfloxacin **7**, metronidazole **9** and sulfadiazine **11** with Cbz-*N*-(aminoacyl)benzotriazoles **2a**–**e**.

The coupling of ciprofloxacin (Cip) **3**, pipemidic acid (Pip) **5** and norfloxacin **7** with Cbz-*N*-(aminoacyl)benzotriazoles **2a**–**e** (prepared by our reported procedures frorm Cbz-protected amino acids **1a**–**e**) in aqueous MeCN in the presence of Et_3N for 3 h resulted in the formation of conjugates: amino acid–ciprofloxacin **4a**–**c** (68–77%), amino acid–pipemidic acid **6a**–**e** (51–82%) and amino acid–norfloxacin (75–86%)[26] (Scheme 1, Table 1).

Compounds **2a**–**e** were reacted with metronidazole **9** in the presence of a catalytic amount of dimethylaminopyridine (DMAP) under microwave irradiations at 60 °C and 50 W for 1 h to afford novel amino acid–metronidazole conjugates **10a**–**e** in good yields (72–85%)[27] (Scheme 2, Table 2).

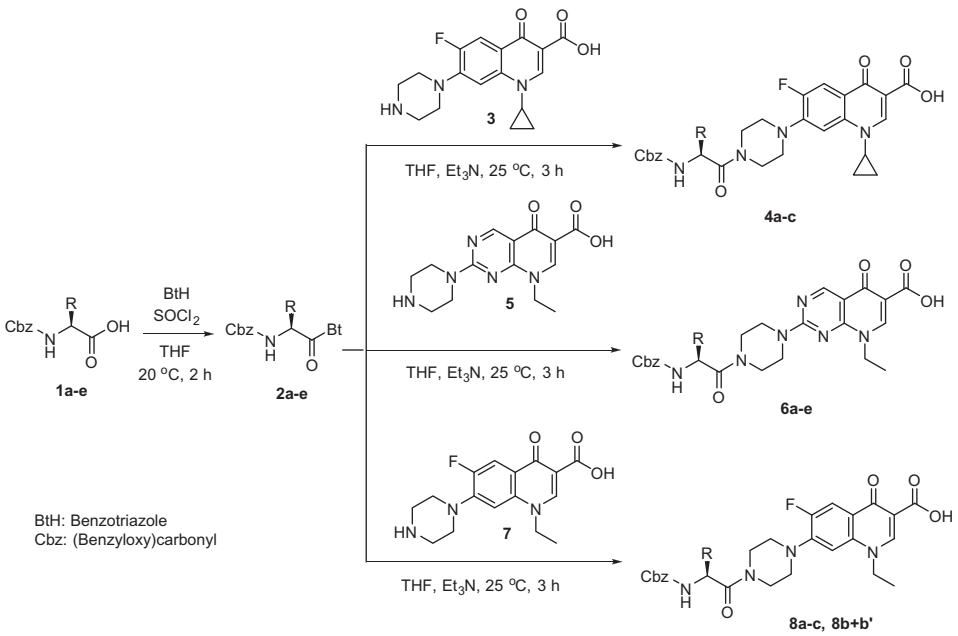
The coupling of sulfadiazine (Sul) **11** with Cbz-protected amino acids in THF in the presence of *N*-methylmorpholine and isobutyl chloroformate at room temperature for 2 h resulted in the formation of amino acid–sulfadiazine conjugates (**12a**–**c**)[28] (Scheme 3, Table 3).

It is believed that the strong lipophilic character of a drug plays an essential role in producing an antimicrobial effect. This property is related to membrane permeation in biological systems. Many of the processes of drug disposition depend on the ability to cross cellular membranes and hence there is a high correlation with lipophilicity. Hydrophobic drugs with high partition coefficients are preferentially distributed to hydrophobic compartments such as lipid bilayers of cells while hydrophilic drugs (low partition coefficients) preferentially are found in hydrophilic compartments such as blood serum.

Hydrophobicity/lipophilicity plays a major role in determining where drugs are distributed within the body after adsorption and as a consequence, in how rapidly they are metabolized and excreted. In this context, the presence of a hydrophobic moiety is important for activity. Moreover, many of the proteins involved in drug disposition have hydrophobic binding sites thus adding to the importance of lipophilicity.[29,30]

The lipophilicity of the compounds, expressed as log*P*, is the main predictor of the activity. The octanol/water partition coefficient *C*log*P* is a measure of hydrophobicity/lipophilicity and was calculated using ChemDraw Ultra 13.0 software integrated with Cambridgesoft Software (Cambridgesoft Corporation). The results are given in Table 4. The calculated values of log*P* for conjugates are higher than for the corresponding parent antibiotic.

Growth inhibition was determined by comparing treated cell cultures to untreated control cultures. The cell density of the samples which were treated with the parent antibiotic or conjugate antibiotics along with the control cultures were determined by analyzing 300 μL samples in a spectrophotometer. The OD600 of the control cultures was considered to be maximum cell growth. The optical density of treated cultures was compared to control cultures to determine percent growth inhibition using the following equation:



Scheme 1. Synthesis of amino acid–ciprofloxacin (**4a**–**c**), amino acid–pipemidic acid (**6a**–**e**) and amino acid–norfloxacin conjugates (**8a**–**c**, **8b+b′**).