SYNTHESIS, ANTIBACTERIAL ACTIVITY AND DOCKING STUDIES OF NEW THIOSEMICARBAZONE CONJUGATES OF MACROLIDE ANTIBiotics

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Introduction

One of the 20th century’s significant achievements is a discovery of azithromycin (8) and owing to its exceptional therapeutic properties, it has come to be one of the most successful antibiotics worldwide. Nowadays, an increasing prevalence of antibiotic-resistant pathogens suggests that we are entering into a “Post-Antibiotic Era”. Investment in new anti-infective platforms is essential and urgent in order to achieve significant progress in our understanding of bacterial resistance and to exploit new approaches to control it.

In order to overcome the resistance problems, significant efforts have been made to search for novel agents with all of the desirable features of the earlier generation of macrolides. The discovery of highly potent 3-O-deacetylated derivatives, ketolides, was a step forward to tackle the resistance problems. However, some serious drawbacks have been observed for those compounds classes: the emergence of resistance developed shortly after their introduction and serious side effects which lead to their restrictions as seen with teicoplanin.

Thus, in order to prepare active compounds less prone to the above mentioned difficulties we developed a strategy aiming at the synthesis of azithromycin analogues involving thiosemicarbazone moiety. Strategies which involved macroside conjugates incorporating heteroaromatic rings[1,2] and, as well as macroside-nucleoside and macroside-nucleobase conjugates[3−9] have already been described and these compounds showed an increased affinity for the ribosome. Similarly, we expect that by introducing thiosemicarbazone moiety, as a new interactive group that interacts with the azide backbone could further improve the activity[10,11]. As a continuation of our research in this field, we describe here the synthesis of a small library of novel hybrid compounds, conjugates of 15-membered azides and thiosemicarbazones as well as in vitro activity against key respiratory Gram-positive and Gram-negative pathogens.

Synthesis of new thiosemicarbazone-azithromycin conjugates

The synthesis of thiosemicarbazone-azithromycin conjugates is shown in Scheme 1. The key intermediate, 9a-(y-aminopropyl) derivative 7 was prepared by two-step synthesis in high yields (>85%). The synthesis involved Michael addition of acrylonitrile with 9-deoxo-9-dihydro-9a-aza-9a-homoerithromycin A (4) (which is the last intermediate in the synthesis of azithromycin (5)), gave 9a-(y-aminopropyl) derivative 6 and subsequent catalytic hydrosylation with Pd/C, yielded 9a-(y-aminopropyl) derivative 7. Thiosemicarbazides (3) were prepared by reaction of 4-formyloxalic acid (1) with hydrazine hydrate followed by addition of various isothiocyanates to hydrazide (2).

Thiosemicarbazone-azithromycin conjugates 8a−e were obtained by coupling of corresponding thiosemicarbazides (3) with (y-aminopropyl) derivative 7 in the presence of HATU and diisopropylethylamine in dichloromethane at room temperature in moderate yield.

Assignments of proton and carbon chemical shifts were made by the combined use of one-dimensional (1H and 13C) and two-dimensional (gHGCOSY, gHQC and gHMBC) NMR spectra.

Scheme 1. Route of synthesis for macroside thiosemicarbazones conjugates

Biological results

Representatives of 5 novel thiosemicarbazuone-azithromycin conjugates 8a−e and three standard macrolide antibiotics were tested against several bacterial species. Among bacterial species tested, macroside resistant strains were included for both clinically relevant mechanisms of resistance to macrolides, ribosomal methylation and efflux pumps.

The methods used for antimicrobial testing were both micro-dilution protocols performed according to Clinical Laboratory Standards Institute (CLSI) guidelines. Read outs were MIC values, where MIC stands for minimum inhibitory concentration and is defined as last tested concentration of compound at which there is no visible growth of bacteria.

The in vitro MICS of novel class of the thiosemicarbazone-azithromycin conjugates 8a−e against a panel of erythromycin-susceptible and erythromycin-resistant Gram-positive and Gram-negative bacterial strains in comparison to azithromycin (5), erythromycin and thiosemicarbazine as standards are also presented in Table 1. All synthesized conjugates 8a−e showed the highest activity against erythromycin-resistant S. pneumoniae (<0.125 µg/mL), the same activity as azithromycin (5), erythromycin and thiosemicarbazone. Most of the synthesized conjugates showed high activity against sensitive S. pneumoniae (0.5 µg/mL). Only moderate activity against efflux-mediated resistant S. pneumoniae was observed. Tested compounds showed moderate activity against sensitive S. aureus (8−16 µg/mL) two times lower in comparison to azithromycin (5). Among Gram negative strains, activity of thiosemicarbazone-azithromycin conjugates 8a−e was observed only against H. influenzae (8−16 µg/mL).

Table 1. Antibacterial activity (MIC in µg/mL) of thiosemicarbazone-azithromycin conjugates (8a−e) in comparison to azithromycin (5), erythromycin and thiosemicarbazone.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MIC (µg/mL)</th>
<th>S. pneumoniae</th>
<th>H. influenzae</th>
<th>S. aureus</th>
<th>E. coli</th>
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Conclusion

We presented here the synthesis, docking studies and antibacterial activity of the novel class of thiosemicarbazone-azithromycin conjugates. According to docking studies, coupling of a thiosemicarbazone moiety with aromatic rings(s) to the 9a position of the 15-membered azide scaffold via amide linker allowed the formation of π-interactions between the macrolide and the nucleotides present in the 23S RNA of the prokaryotic ribosome. Also, based on the conformations of macrolides in the PTC future attempts in macrolide design should be directed at increasing the specific binding of macrolide benzene ring to 23S RNA nucleotides by adding polar groups which could stabilize interactions which could be a promising method to tackle the resistance problems. Newly prepared conjugates 8a−e displayed moderate antibacterial activity against efflux-mediated resistant S. pneumoniae strain. Although the limited number of compounds studied here can not allow for a comprehensive SAR analysis, these compounds serve as a good platform to explore the nature of bacterial resistance. This can be a basis for further modifications and development of compounds with improved activity against resistant bacterial strains.

Docking studies

Relatively small binding energy of Compound 8b to a peptide transferase center (PTC) of Escherichia coli ribosome calculated by molecular docking shows effective binding of mentioned compound to targeted inhibitory region of prokaryotic ribosomes. Position of macrolactone ring of Compound 8b inside of PTC corresponds to conformation of Azithromycin inside PTC while terminal benzene ring of thiosemicarbazone substitute is forming hydrophobic interactions with nitrogen bases 23S RNA. During dimethylation of AZ2058 23S RNA efficacy of binding of Compound 8b increases which indicates potential antimicrobial activity of other potential structures. On the other hand, Compound 8a has relatively high binding energy calculated by molecular docking which indicates poor binding of Compound 8a to a PTC of Escherichia coli. However, it is important to notice that macrolactone ring of more stable conformations of Compound 8a is located to narrow part of PTC exit channel which can explain why Compound 8a effectively prevents translation in prokaryotic ribosomes.

Figure 1. Compound 8b bound to PTC in its minimal energy state (left) and with 2.4 kJ/mol greater energy than minimal (right)

References