Final report
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In the first stage of the project we have synthesized several oxazolidone type, linezolid analogs with dimeric structures linked by tetraethyleneglycol chain. Since linezolid acts on bacterial ribosomes, inhibiting the protein biosynthesis, we conjugated the oxazolidone antibiotic with nucleobases supposing that hydrogen bonded base pair formation with the ribosomal RNA bases will improve the antibacterial activity. Unfortunately, our linezolid analogs had no antibacterial activity.

Using hydrogen fluoride reagent we prepared ristocetin aglycon and teicoplanin pseudoaglycon. After transforming the primary amino group of these aglycons into azido function, we could derivatize them with lipophilic side chains in a 1,3-azide-alkyne dipolar cycloaddition reaction, in order to improve their antibacterial activities. Some of our teicoplanin pseudoaglycon derivatives proved to be very good antibacterials, even against vancomycin and teicoplanin resistant bacteria. One of the ristocetin compounds had good activity against influenza virus strains. We explained the high activities by a possible cluster formation of the antibiotic molecules in aqueous solution. DOSY NMR experiments as well as dynamic light scattering measurements corroborated this postulate: aggregates containing about 240 molecules were detected. Using saturation difference NMR technique we could study the interaction of such lipophilic conjugate with the terminal tripeptide of the bacterial peptidoglycan. We suppose that other glycopeptide antibiotic aglycons containing lipophilic side chains will exhibit high antibacterial activity based on the strong, multivalent interaction of the self assembled, multivalent antibiotic clusters with the repeating unit of the bacterial cell wall peptidoglycan. For supporting this supposal we changed our research directive to this principle.

In the three component reaction of the primary amino group of ristocetin aglycon with ortho-phthalaldehyde or naphtalene-2,3-dialdehyde and various thiols, isoindol derivatives were obtained. These derivatives exhibited very good antibacterial, as well as good antiinfluenza virus activity. The excellent fluorescent properties of these isoindols were studied. The antibiotic molecules formed 100 nanometer sized aggregates in aqueous solution. We applied the three component isoindol forming reaction for constructing fluorescent polymers.

Similar three component reactions have been performed on teicoplanin pseudoaglycon resulting in the formation of isoindole derivatives. These compounds were found to possess

remarkably high antibacterial activity in the nanogram/ml concentration range against a panel of Gram positive bacteria including some resistant strains. Almost all of the new derivatives exhibited better MIC and MBC values than the parent teicoplanin. We were able to identify two active anti-influenza virus agents.

As an extension of our studies of self-assembling, multivalent antibiotics we attached the teicoplanin pseudoaglycon molecule covalently to a fullerene molecule, since tha latter is a very lipophilic species. We constructed an appropriate fullerene carrier molecule bearing four tetraethyleneglycol moieties for enhancement the water solubility. Our antibiotic fullerene conjugate formed 200 nm sized aggregates and was active against vancomycin and teicoplanin resistant bacteria.

The antiviral action mechanism studies of one of our ristocetin aglycon derivatives carrying a lipophilic side chain revealed that it completely prevented the entry of the virus into the nucleus of the host cell.

For the sugar derivatisation of antibiotics a photocatalytic, radical thiol-ene reaction was studied on various unsaturated saccharides. Recently we have used this reaction for the derivatisation of a cephalosporin derivative.

In order to obtain new antibacterials, phosphonate analogs of the bacterial cell wall component lipid II. were synthesized. Unfortunately, they have no antibacterial activity.

Summarizing the most positive results of the project, we can conclude that cluster forming derivatives of glycopeptide antibiotics which inhibit the bacterial cell wall biosynthesis result very good antibacterials and in some cases new type of antiinfluenza viral compounds. We suppose that in these cases the basis of the bioactivity is a multivalent interaction between multimeric ligands and multimeric receptors. Hopefully, this principle could be extended to more types of antibiotics in the future.

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