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Synthesis of isoindole and benzoisoindole derivatives of teicoplanin pseudoaglycon with remarkable antibacterial and antiviral activities

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ABSTRACT

The primary amino function of teicoplanin pseudoaglycon has been transformed into arylthioisoindole or benzoisoindole and glycosylthioisoindole derivatives, in a reaction with o-phthalaldehyde or naphtalene-2,3-dicarbaldehyde and various thiols. All of the obtained semisynthetic antibiotics exhibited potent antibacterial activities against Gram-positive bacteria in the ng per ml concentration range. A few of them showed antiviral activity, in particular against influenza virus.

Glycopeptide antibiotics vancomycin and teicoplanin are used in the treatment of serious Gram-positive bacterial infections that are resistant to other antibiotics.[1] Due to the emergence and spread of glycopeptide-resistant enterococci (GRE) and glycopeptide intermediate-resistant *Staphylococcus aureus* (GISA), as well as teicoplanin-resistant *Staphylococcus haemolyticus*,[2] there is an urgent need for new antibiotics active against resistant bacteria.

Nagarajan et al. reported on lipophilic *N*-alkyl derivatives of glycopeptide antibiotics possessing high activity against vancomycin-resistant bacteria.[3] Later this observation led to the elaboration of the highly active subclass of lipoglycopeptides oritavancin, telavancin and dalbavancin.[4] In the past few years we have synthesized a series of new derivatives of ristocetin aglycon and of teicoplanin pseudoaglycon exhibiting high antibacterial and, in some cases, robust ant-influenza virus activity.[5] For the elucidation of the mechanism of biological action fluorescent isoindole- and benzoisoindole-fused aglycoristocetin derivatives have also prepared in this laboratory.[6] The biological evaluation of these derivatives revealed that the introduction of the isoindole and benzoisoindole moieties into the N-terminal position of the aglycoristocetin molecule resulted in remarkable increase in the antibacterial and anti-influenza virus activity.

In order to get more information about the effect of (benzo)isoindole substituents on the biological activity of glycopeptide antibiotic derivatives, we decided to prepare a series of such derivatives from teicoplanin pseudoaglycon.

The starting pseudoaglycon **2** can be prepared from teicoplanin (**1**) by treatment with anhydrous hydrogen fluoride (Scheme 1).[7]

The widely used three-component isoindole-formation reaction, involving the heteroring closure of o-phthalaldehyde (or its benzologs) with primary amine and a thiol,[8] was chosen for the derivatization of the N-terminal of the glycopeptide. Reaction of the primary amino function of **2** with o-phthalaldehyde and various thiols such as (hetero)arylthiols, as well as 1-thio-d-glucopyranose or 1-thio-*N*-acetyl-d-glucosamine derivatives afforded the corresponding arylthio- (**3**), heteroarylthio- (**4** and **5**) and glycosylthioisoindole (**9–12**) derivatives in reasonably good yields. In a similar reaction, using naphtalene-2,3-dialdehyde instead of o-phthalaldehyde the appropriate benzoisoindole derivatives **6–8** were obtained, incorporating the N-terminal nitrogen of the antibiotic into the new heteroring system (Table 1).

The new compounds **3–12** were characterized by HPLC, MALDI-TOF mass spectroscopy (key data and methods are included in the Supplementary material). The presence of the newly formed (benzo)isoindole moieties were confirmed by NMR spectroscopy.

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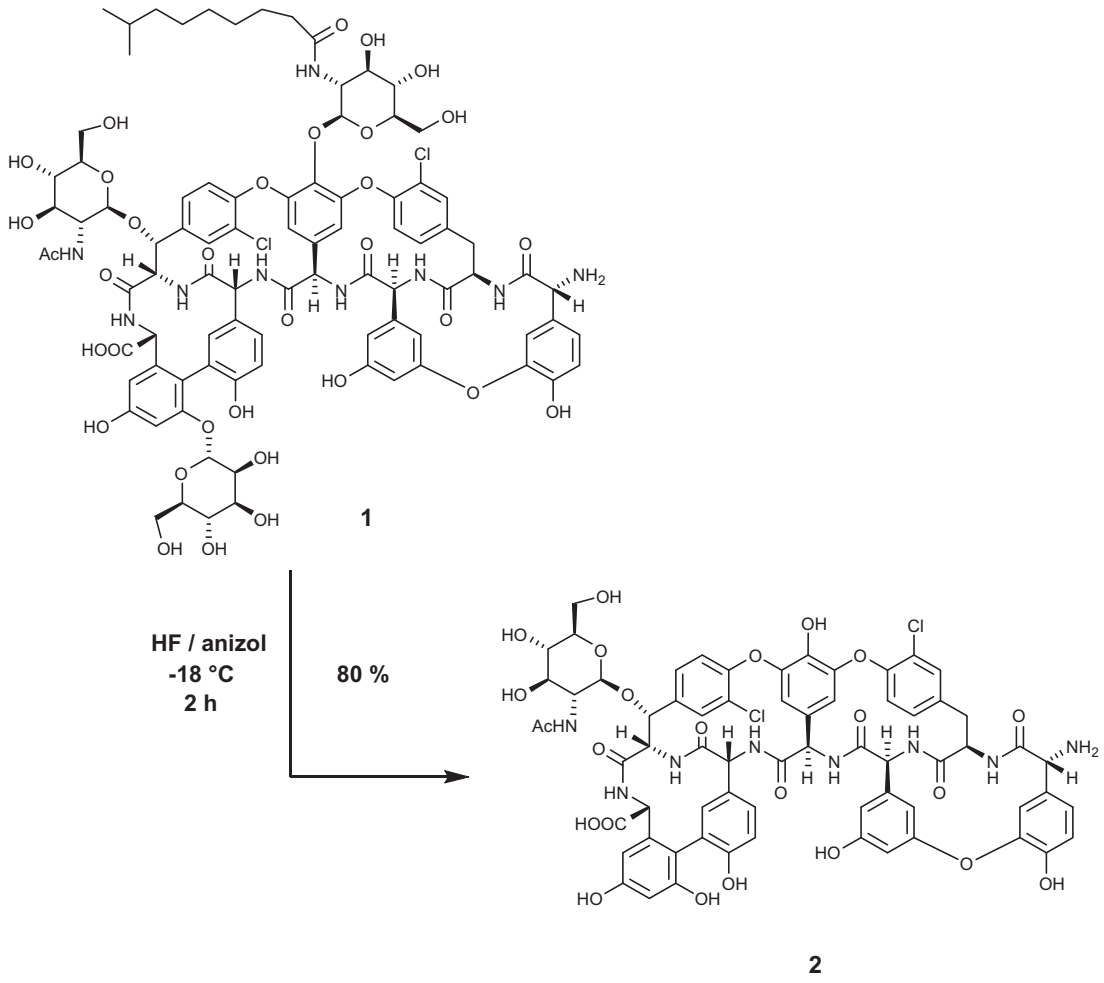
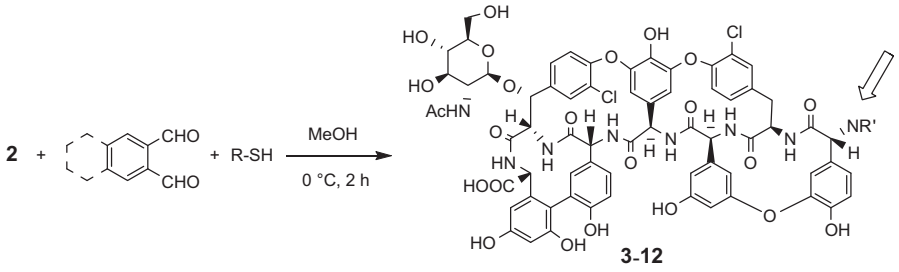
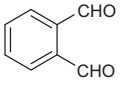
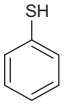
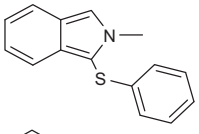
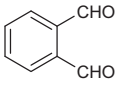
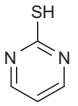
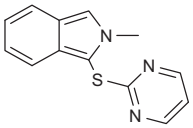


Scheme 1. Preparation of teicoplanin pseudoaglycon **2**.

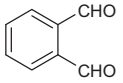
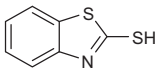
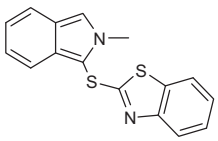
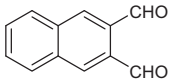
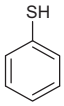
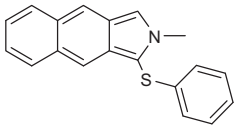
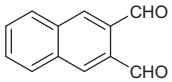
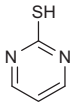
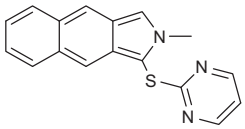
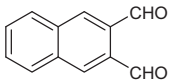
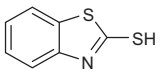
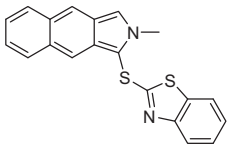
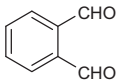
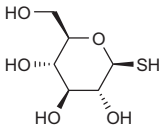
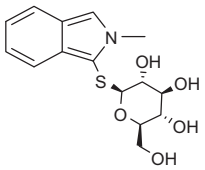
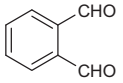
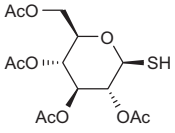
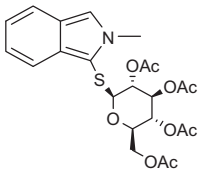
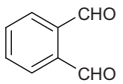
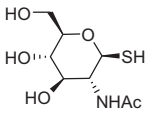
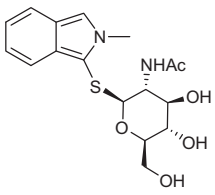
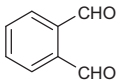
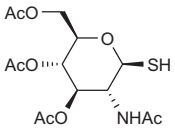
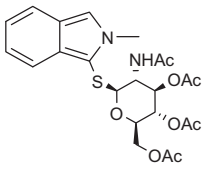
Table 1
Synthesis and structure of novel teicoplanin pseudoaglycon derivatives **3**–**12**



Product	Starting dialdehyde	Thiol reagent (R-SH)	Formed isoindole moiety (R'N-)[a]	Yield (%)	log*P*[b]
3				76	7.21
4				65	5.24

(*continued on next page*)

Table 1 (*continued*)

Product	Starting dialdehyde	Thiol reagent (R-SH)	Formed isoindole moiety (R'N-)[a]	Yield (%)	log*P*[b]
5				49	7.95
6				31	8.44
7				62	6.47
8				58	9.18
9				51	2.20
10				72	6.25
11				49	3.53
12				52	5.03

[a] General procedure: 0.11 mmol dialdehyde, 0.11 mmol thiol in 3 mL MeOH (protected from light, under argon) stirred for 20 min at 0 °C. 0.05 mmol **2** in 3 mL MeOH added and stirred for 2 h at RT.

[b] Log*P* values were calculated with ChemSketch log*P* add-on.[9]

Determination of the antibacterial activity of this series of compounds against a panel of Gram positive bacteria[10] gave excellent results, as summarized in Table 2. Almost all of the new derivatives exhibited better MIC and MBC values than the parent antibiotic teicoplanin.

The database of the European Committee on Antibacterial Susceptibility Testing (EUCAST)[11] contains the MIC values for a wide range of bacteria and antimicrobial agents. These EUCAST data were aggregated from extended time periods and many countries. According to the data of EUCAST for 6105 *Enterococcus faecalis* strains, teicoplanin is effective in the MIC range of 0.06–2 μg/mL. For our newly synthesized derivatives, the MIC values against *Enterococcus faecalis* were in the range of 0.0075–4 μg/mL, either tested against the teicoplanin susceptible wild-type strain, or two teicoplanin resistant *Enterococcus faecalis* strains containing the *vanA* or *vanB* resistance genes. In comparison, the MIC values of teicoplanin against these three genotypes were 2 μg/mL (wild-type), 256 (*vanA* genotype) and 4 μg/mL (*vanB* genotype), respectively. EUCAST reported 566 observed cases of *Staphylococcus aureus* MRSA infections with successful teicoplanin treatment having the MIC values mostly between 0.25 and 2 μg/mL. Against our *Staphylococcus aureus* MRSA strain, we found teicoplanin effective with an MIC value of 0.5 μg/mL. Notably the new isoindole derivatives showed efficacy at much lower MIC values, that is 0.0075–1 μg/mL. Against *Staphylococcus aureus* MSSA strains, teicoplanin was effective in the MIC range of 0.5–2 μg/mL (analysis from 571 reported cases). In our studies, the reference teicoplanin (**1**) was found to be active against an MSSA strain with an MIC of

Table 2
Antibacterial activity of compounds **3–12**

Strains	MIC/MBC (μg/mL)										Teicoplanin
	3	4	5	6	7	8	9	10	11	12	
Bacillus subtilis ATCC 6633	1/1	0.0625/0.5	½	2/4	0.0313/2	0.0313/4	0.125/1	0.25/64	0.125/1	0.25/1	0.5–1/16
Staphylococcus aureus MSSA ATCC 29213	0.125/128	0.0313/0.5–2	0.0075/0.5	0.0313/1	0.015/0.5	0.015/0.5	0.0313/1	0.0625/16–32	0.5/1	0.125/1	0.5/2
Staphylococcus aureus MRSA ATCC 33591	0.0625/2	0.0313/8	0.0075/0.5	0.25–0.5/0.5	0.015/0.5	0.015/4	0.0075/1	0.0313–1/128	1/1–2	0.0625/2	0.5/2–4
Staphylococcus epidermidis biofilm ATCC 35984	0.0165/0.5	0.0165/1	0.0075/0.5	0.0313/0.5	0.015/0.5	0.015/0.5	0.0075/0.5	2/8	0.0313/0.5	0.0075/2-4	2/16–32
Enterococcus faecalis ATCC 29212	0.0075/256	0.0313/256	2–4/256	1/256	0.015/256	0.0313/256	2/256	1/256	1/256	2-4/256	2/64
Staphylococcus epidermidis mecA	2/32	4/64–128	4/256	0.0313/256	0.015/256	0.0313/256	0.015/32	2–4/256	0.0075–1/64	2/32	8–16/32
Enterococcus faecalis 15376 vanA	2/32–64	0.0625/256	0.0313/256	0.125/32	0.5/256	0.0313/32	0.0625/256	4/256	1/256	0.0625/256	256/256
Enterococcus faecalis ATCC 51299 vanB	2/256	1/256	0.0625/256	2/256	1/256	1/256	1/256	4/256	4/256	2/256	4/256

MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, ATCC: American Type Culture Collection, MSSA: Methicillin Sensitive *Staphylococcus aureus*, MRSA: Methicillin Resistant *Staphylococcus aureus*, mecA: mecA gene expression in *Staphylococcus*, vanA +: vanA gene positive, vanB +: vanB gene positive.

0.5 μg/mL, whereas the isoindole derivatives had lower MIC values in the range of 0.0075–0.5 μg/mL. With regard to *Staphylococcus epidermidis*, MIC values for teicoplanin were typically between 0.25 and 16 μg/mL, in the EUCAST database for 8578 cases. We determined the MIC values of teicoplanin and the isoindole derivatives against two *S. epidermidis* strains to be 2–8 μg/mL and 0.0075–4 μg/mL, respectively. Compounds **3–12** proved to be very active against vancomycin and teicoplanin resistant enterococci, with some compounds displaying MIC values in the ng/ml range. Overall, the most active compounds of the series were **6** and **7** benzoisoindole derivatives**.** Compound **9**, containing d-glucosyl substituent with free hydroxyl groups displayed better activity than its acetylated counterpart. Although for obtaining an exact structure–activity relationship a detailed study involving a higher number of compounds is required, here it can be concluded that isoindole derivatisation of **2** led to much more potent antibacterials than the parent teicoplanin.

The anti-influenza virus activities and cytotoxic concentrations[12] of our products **3–12**, in comparison to four antiviral reference agents, are summarized in Table 3.

With the exception of compounds **7** and **8**, the new teicoplanin pseudoaglycon derivatives showed no anti-influenza virus activity at subtoxic concentrations. Compared to unsubstituted teicoplanin pseudoaglycon **2**, which was found to possess weak anti-influenza A virus activity and no activity against B virus, the new heteroaromatic moiety was found to have crucial impact on the activity. For compound **7**, a favorable selectivity index was noted, since its ratio of MCC to EC_{50} was 24. The 2-*S*-heteroarylbenzoisoindole function at the N-terminus of compounds **7** and **8** appeared to be responsible for this effect. From a comparison with compound **6**, which has a 2-*S*-homoarylbenzoisoindole at the N-terminus, it can be concluded that the presence of heteroatoms in the 2-*S*-substituent may be advantageous. Most of our new derivatives displayed relatively high cytotoxicity in this assay in Madin Darby canine kidney cells, since the compound concentrations causing minimal alterations in cell morphology were 20 μM or lower.

Besides this anti-influenza virus testing, compounds **2–13** were evaluated against a selected panel of DNA viruses as well as one RNA virus (vesicular stomatitis virus). The antiviral activity and cytotoxicity of **2–12** in human embryonic lung (HEL) fibroblasts[13] are summarized in Table 4.

Table 3
Anti-influenza virus activity and cytotoxicity of compounds **2–12** in MDCK cell cultures

Compounds	Antiviral EC_{50} (μM)			MCC(μM)
	Influenza A/H1N1 (A/PR/8/34)	Influenza A/H3N2 (A/HK/7/87)	Influenza B (B/HK/5/72)	
2	23	17	>100	⩾100
3	>100	>100	>100	4
4	>100	>100	>100	20
5	>100	>100	>100	4
6	>100	>100	>100	<0.8
7	0.54	0.54	>50	13
8	<4.9	1.6	>100	20
9	>100	>100	>100	<0.8
10	>100	>100	>100	20
11	>100	>100	>100	<0.8
12	>100	>100	>100	20
Zanamivir	1.6	4.8	23	>100
Ribavirin	6.1	6.0	4.6	100
Amantadine	212	0.45	>500	⩾500
Rimantadine	14	0.051	>500	500

EC_{50}: 50% effective concentration, or concentration producing 50% inhibition of virus-inducedcytopathic effect, as determined by visual scoring. MCC: Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology. MDCK cells: Madin Darby canine kidney cells.

Table 4
Antiviral activity and cytotoxicity of compounds **2–12** in HEL cell cultures

Compounds	Antiviral EC_{50} (μM)					MCC (μM)
	HSV-1 KOS	HSV-2 G	Vaccinia virus	Vesicular stomatitis virus	HSV-1 TK^- KOS ACV^r	
2	>100	>100	>100	>100	>100	>100
3	9	9	>20	>20	⩾20	⩾20
4	47	45	⩾58	>100	50	>100
5	45	45	47	>100	45	⩾100
6	>20	>20	>20	>20	>20	100
7	40	27	10	>100	45	⩾100
8	>100	>100	>100	>100	>100	>100
9	45	40	45	52	⩾45	⩾100
10	40	11	45	48	40	⩾100
11	47	45	47	52	45	⩾100
12	40	40	45	>100	45	>100
Brivudin	0.024	198	10	>250	50	>250
Cidofovir	1.5	0.9	21	>250	1.5	>250
Acyclovir	0.14	0.14	⩾250	>250	44	>250
Ganciclovir	0.015	0.013	>100	>100	4	>100

HEL: human embryonic lung fibroblasts; HSV-1 KOS: herpes simplex virus type 1 strain KOS; HSV-2 G: herpes simplex virus type 2 strain G; TK^-: thymidine kinase-deficient HSV-1 KOS strain resistant to acyclovir (ACV^r); EC_{50}: 50% effective concentration, or concentration required for 50% decrease of virus-induced cytopathogenicity. MCC: minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

Whereas teicoplanin pseudoaglycon (**2**) was found to be inactive against these viruses, several of the isoindole and benzoisoindole derivatives **3–12** showed weak inhibitory effect, although their antiviral EC_{50} values were relatively high and in the same order of magnitude as the compound concentrations causing cytotoxicity. The 2-*S*-phenylisoindole derivatized glycopeptide **3** was the most active, in particular against herpes simplex virus. It is worthwhile to mention that the compound cytotoxicity in the HEL cells (Table 4) was much less pronounced compared to MDCK cells (Table 3).

In conclusion, we have extended the series of isoindole- and benzoisoindole-fused glycopeptides with the synthesis of a set of teicoplanin pseudoaglycon derivatives. These compounds were found to possess remarkably high antibacterial activity 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 antibiotic teicoplanin. The antiviral tests of these (benzo)isoindoles showed mixed results. We were able to identify two active anti-influenza virus agents, but other RNA and DNA viruses were only weakly inhibited. On the basis of these promising antibacterial results further examinations will be performed in order to identify the mechanism of action.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.079.

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