The Effect of Systematic Structural Modifications on the Antibacterial Activity of Novel Oxazolidinones

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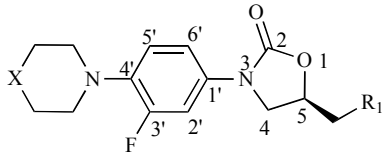
Abstract: A novel series of tetraethylene glycol (TEG) triazolyl and squaramide containing oxazolidinones were synthesized and tested for their antibacterial activity against a selected panel of Gram-positive and Gram-negative bacteria. The 4-TEG-triazolyl derivatives were prepared by 'click reaction'. The introduction of the TEG and squaramide groups did not favor antibacterial activity. The three nucleoside-containing oxazolidinones were also prepared by 'click' methodology resulted in weak antibacterial activity.

Keywords: Antibacterial agents, 'click' reaction, linezolid, oxazolidinone, squaramide, tetraethylene glycol (TEG).

Dedicated to Professor Ferenc Fülöp on the occasion of his 60th birthday

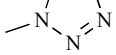
INTRODUCTION

Antimicrobial resistance is a growing health problem that threatens human health globally. Oxazolidinones represent a novel class of antibacterial agents with significant activity against multidrug-resistant Gram-positive pathogenic bacteria. Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) could be life threatening especially for patients with compromised immune systems due to HIV, surgery or other illness. Linezolid (**1**, Fig. **1**), the only clinically used member of this class, displays potent antibacterial activity as an inhibitor of bacterial ribosomal protein biosynthesis [1, 2].



1 R^1 = -NHCOCH$_3$ X = O

2 R^1 = -NHCOCH$_3$ X = N-CO-CH$_2$-OH

PH027 R^1 =  X = O

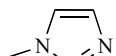
PH series R^1 =  X = N-CO-H; N-CO-CH$_3$ N-CO-Ph

Fig. (1). Chemical structures of oxazolidinone-type antibacterial agents.

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A large number of oxazolidinone derivatives with diverse structural modifications have been reported in various patent reviews and scientific literature [3-6]. The main target of these efforts is to extend the spectrum of activity and reduction and/or elimination of toxic effects and potential drug-drug interactions associated with monoamine oxidase (MAO) inhibition [4, 6]. In light of these facts we and others reported the preparation of 5-triazolylmethyl oxazolidinones based on the replacement of the 5-acetamido methyl substituents with the *N*-linked triazolyl moiety, exemplified by **PH-027** (Fig. **1**) and also showed that di-substitution and substitution of bulky groups on the triazole resulted in loss of antibacterial activity [7]. In addition, it was also clarified that the introduction of the *N*-4-acyl piperazinyl substituted oxazolidinone eperezolid derivatives gave rise to compounds with improved or comparable activity to **PH-027** and linezolid (**1**) against resistant Gram-positive clinical isolates [8, 9]. The mechanism of action of linezolid is based on its interaction with bacterial ribosome. Using photoaffinity label experiments it was found that photoactive probes were crosslinked to the catalytic site of the 50S ribosomal subunit of living bacteria [10]. Recently the crystal structure of the linezolid adduct of 50S ribosomal subunit of *Haloarcula marismortui* was determined, supporting the facts obtained from the photoaffinity label experiments [11]. With these data in hand a model was constructed using the known crystallographic structure of *E. coli* ribosome. According to this model, oxazolidinones prevent binding of aminoacyl-*t*RNA in the peptidyl transferase active site.

On the basis of these findings it could be postulated that nucleobases covalently conjugated with oxazolidinones could improve the binding of the antibiotic molecule to the ribosome, with the aid of hydrogen bonded base pair formation. Indeed, Franceschi *et al.* designed several thymine based linezolid analogs that proved effective against resistant bac-

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teria [12]. Therefore, we also decided to synthesize oxazolidinone derivatives bearing either a nucleobase or a nucleoside moiety, in order to enhance activity. Secondly, the incorporation of the neutrally charged amphiphile tetraethylene glycol (TEG) spacer moiety would both improve the water solubility. On the other hand, the squaramide derivatives are expected to mimic the C5 amide functionality of linezolid (**1**). Squaric acid itself is used in medicinal chemistry as a group mimicking carboxylate, phosphate mono- and di-esters and hydroxamic acid functions [13]. The ultimate aims of this study were to synthesize novel oxazolidinones as dimeric compounds as tetraethylene glycol derivatives bearing nucleobases and monomeric oxazolidinones with squaramide moiety at the oxazolidinone C-5 position. These compounds were expected to exhibit enhanced binding potency and selectivity in their interaction with receptors, affording improved antibacterial activity.

RESULTS AND DISCUSSION

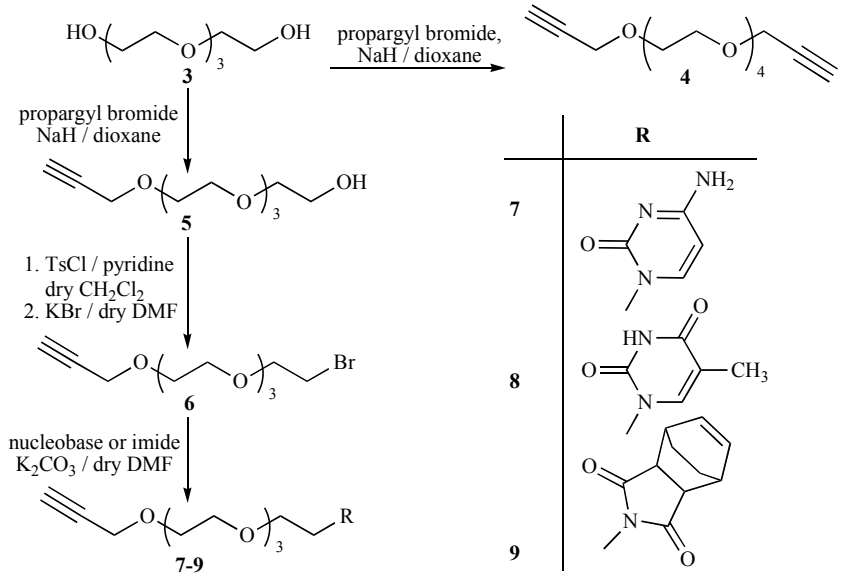
Chemistry

The syntheses of the TEG and squaramide containing oxazolidinones are presented in Schemes **1** to **3**. The tetraethylene glycol spacers were synthesized as indicated in Scheme **1**, starting from tetraethylene glycol **3**. The reaction of **3** with excess propargyl bromide in the presence of NaH in dioxane at room temperature gave rise to dipropargylated derivative **4** in 65% yield. On the other hand, the reaction of excess tetraethylene glycol **3** with propargyl bromide in the presence of NaH in dioxane at room temperature gave the monopropargylated TEG **5** in 82% yield. Conversion of the monopropargylated TEG **5** to the bromo derivative was attained by reacting **5** with tosyl chloride, followed by nucleophilic displacement of the tosyl group in the presence of KBr in DMF to give the 1-bromo-3,6,9,12-tetraoxapentadec-14-yne **6** in 66% yield. This TEG monopropargyl bromide intermediate **6** was converted to cytosine-, thymine-, 5-norbornene-2,3-dicarboximide-containing derivatives (**7-9**, respectively), as follows. Typically, this alkylation reaction involved nucleophilic displacement of the bromine of the monopropargylated bromide intermediate **6** by a nucleobase or the imide in the presence of K_2CO_3 in dry DMF to form compounds **7-9** in 42, 13 and 81% yields, respectively.

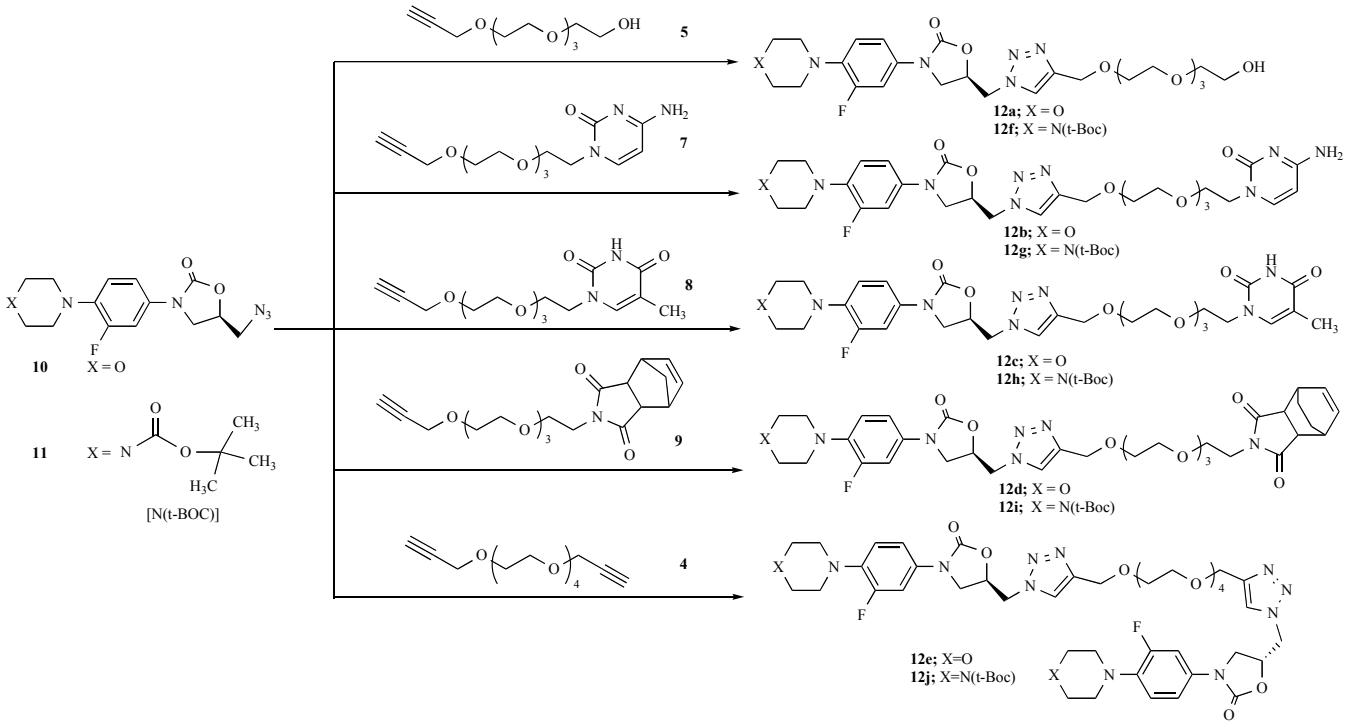
Afterwards the intermediate nucleobase- and imide-containing TEGs were converted to the desired target compounds **12a-j** by reaction with the azido oxazolidinone derivatives **10** and **11**, respectively. These azido oxazolidinones were prepared according to previously reported literature methods [14]. Since direct Huisgen-type 1,3-dipolar cycloaddition reaction without the use of catalyst is known to provide a mixture of 4- and 5-substituted triazolyl derivatives [15, 16], the covalent bonding between TEG-containing nucleobases and imide and azido oxazolidinones was generated by the copper(I) ion catalyzed 1,3-dipolar cycloaddition reactions known as "click" reaction [15, 17] to give the 4-substituted triazolyl derivatives **12a-d, f-i** (Scheme **2**). Additionally, under similar conditions, the azide analogs **10** and **11** with the TEG dipropargylated ether **4** resulted in the dimers **12e** and **12j**, respectively, in moderate to good yields.

The preparation of the squaramide derivatives were accomplished by application of the methodology for the covalent binding of two amino compounds using consecutive amidation of a squaric acid diester established by Tietze and coworkers [18]. With the use of this method we have prepared selected oxazolidinone coupled squaramide derivatives **14a-e**, as follows. Conversion of the azido derivative of linezolid **10** to the amino derivative **13** was achieved by Pd/C catalyzed hydrogenation in MeOH/AcOH [19]. As a next step reaction of **13** with squaric acid, in presence of MeOH and Et_3N at pH around 7.5 gave the squaric monoamide **14a**, which was subsequently reacted with selected amino compounds in MeOH/DCM and Et_3N to give the squaric diamides **14b-e** in good to excellent yields (Scheme **3**).

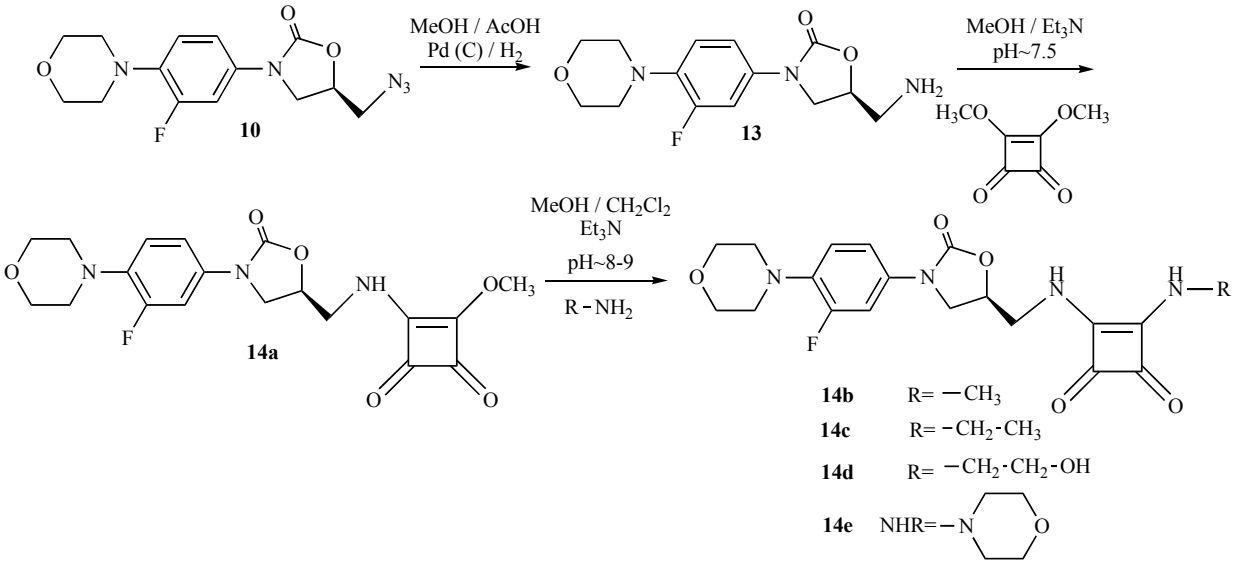
As a second approach for the formation of nucleobase-derived oxazolidinone compounds, 'click' reaction was achieved on the terminal alkyne derivative of the eperezolid backbone with the azides of nucleosides. This procedure applies only the 1,2,3-triazole as spacer between the tricyclic



Scheme 1. Synthesis of the tetraethylene spacer molecules linked to nucleobases.



Scheme 2. Synthesis of the tetraethylene spacer containing oxazolidinones **12a-j**.

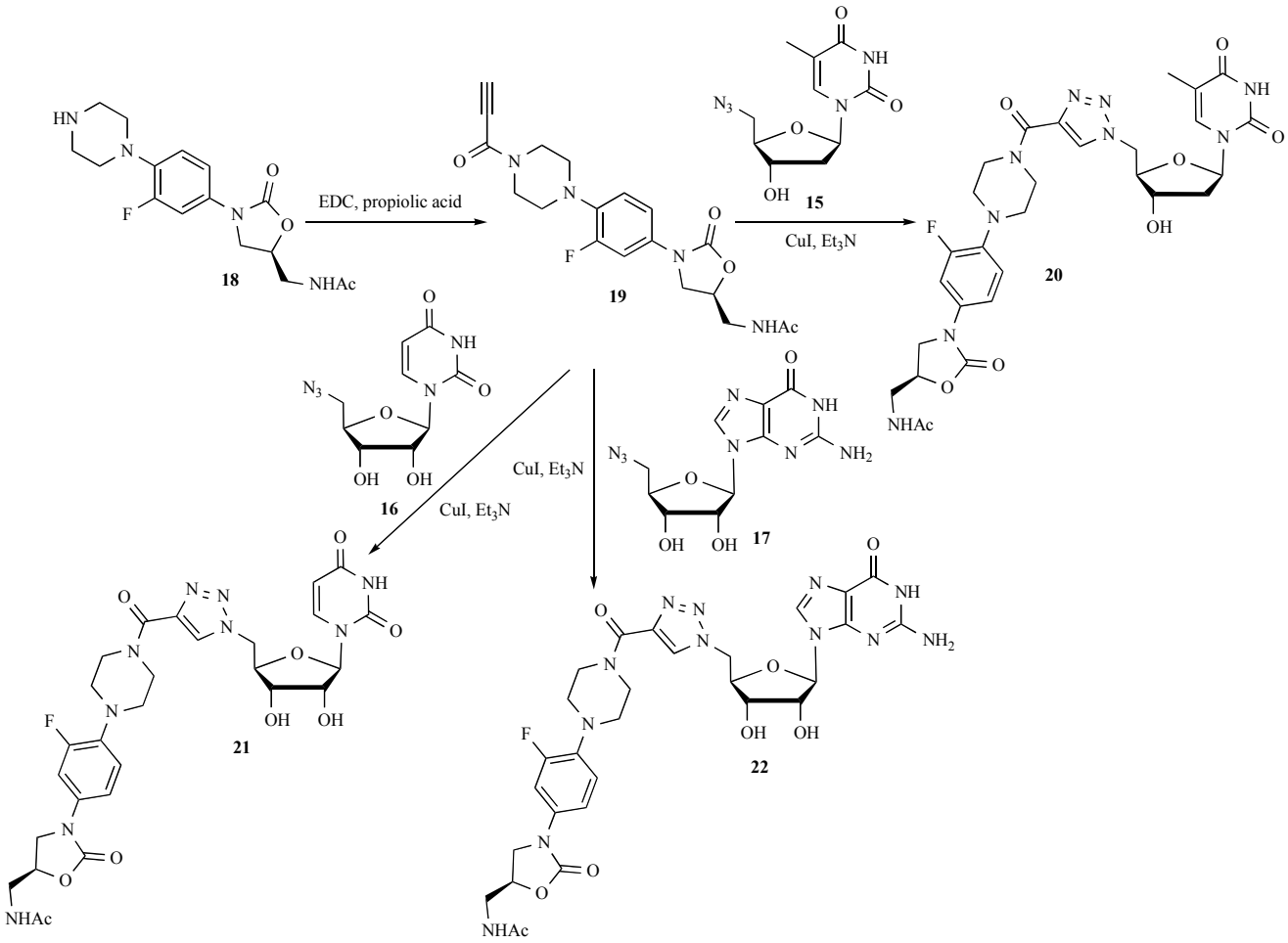


Scheme 3. Synthesis of squaramide containing oxazolidinones derivatives **14a-e**.

eperezolid residue and the nucleoside (Scheme **4**). The synthesis of 5'-azido-5'-deoxy derivatives of uridine (**15**), thymidine (**16**) and guanosine (**17**) was performed on the basis of literature procedures [20, 21 and 22, respectively]. The deprotected piperazine derivative of eperezolid (**18**) [23] was reacted with propyolic acid to form the necessary terminal alkyne derivative. The 'click' reaction was performed in the presence of copper(I) iodide with the same protocol as for compounds **12a-j** and resulted in the target nucleoside-containing oxazolidinones in average to good yields.

Antibacterial Activity

The antibacterial activity of the newly synthesized oxazolidinone derivatives were tested against a panel of standard reference strains and clinical isolates using the Mueller Hinton agar dilution method or the broth microdilution method and the antibacterial activity reported as the MIC (mg/ml) are shown in Table **1**. The activity was compared with that of linezolid (**1**), vancomycin and the triazolyl derivatives (PH027, PH041, PH075, PH084, PH120 and PH121) previously reported by our group [7-9, 24]. As can be seen from the data none of these newly synthesized compounds exhibited significant antibacterial activity against all Gram-positive bacteria tested, no activity was observed at the highest level tested. Although the ClogP values for these compounds, estimated by Symyx Draw 3.1 with bioavailability add-in (formerly ADL Draw) software [25], were within the range of linezolid (**1**) and most of the active triazolyl deriva-



Scheme 4. Synthesis of nucleoside-containing oxazolidinones derivatives **20-22**.

tives, the TEG-containing derivative showed no antibacterial activity at the highest level tested.

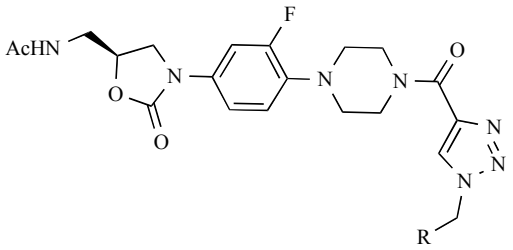
Dimeric anticancer agents, anti-HIV agents, steroids, antibiotics showing biological activities have been prepared tethering the monomers with linker chains of various lengths [26, 27]. On the basis of these results it was assumed that the dimeric ligand may enhance binding potency and selectivity at the receptors, resulting in enhanced biological activity. Unfortunately, both dimeric compounds reported in this study had weak antibacterial activity against Gram-positive cocci. In addition, all the compounds were devoid of antibacterial activity against the selected Gram-negative bacterial strains tested.

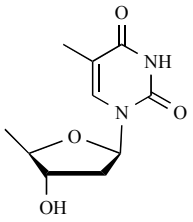
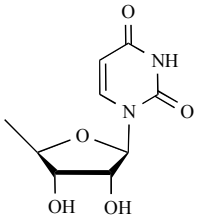
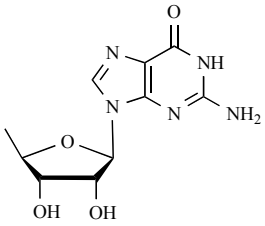
Nucleoside-containing oxazolidinones (**20-22**) were tested in a second set for a panel of standard reference strains and clinical isolates. These derivatives showed somewhat improved, but rather weak antibacterial activity than previously discussed congeners (**12**, **14**), however they were found to be much less active than the reference compounds (Table **1**). The CLogP calculation revealed for **20-22** that these compounds have a significantly differing hydrophilic profile in comparison to TEG- and squaric acid-linked derivatives (**12**, **14**).

It is known that the C-4 morpholino moiety of linezolid does not appear to make noteworthy interactions with the ribosome, thus allowing the substitution of a variety of different functionalities at phenyl C-4 position in place of the morpholine without a significant loss of activity [28]. Therefore, from a drug-design perspective, this observation allows for varied substitution at this position that can be fine tuned to adjust the antibacterial and pharmacological properties of the molecule without compromising the binding that is necessary for activity. In this light piperazine ring at this position provides a very suitable motif for further structural modifications for the introduction of spacers to improve ribosomal binding and enhance antibacterial activity. However, the introduction of the nucleoside groups *via* the triazolylcarbonyl H-acceptor did not seem to improve antibacterial activity as seen in the derivatives **20-22**, suggesting that these groups do not effectively bind to the nucleotide groups in the ribosome. This fact probably can be attributed to the relatively long distance between the pharmacophoric group and the nucleobase moiety of these molecules.

The *N*-acetamidomethyl substituent at the C-5 position of the oxazolidinone ring plays an important role in the antibacterial action of linezolid and analogs [29]. In addition, the substitution of other carbonyl containing functional groups, such as the urea, *N*-cinnamoyl, fluoro- and difluoroacetamido and thiocarbamate groups at the oxazolidinone C-5 position have been shown to favor antibacterial activity. Based on this, we postulated that a squaric acid diamide moiety could mimic the *N*-acetamido group as potential hydrogen bond forming part of the molecule [18]. While the incorporation of

Table 1. ClogP and Antibacterial Activity of Compounds 20-22 (MIC Ranges; μg/ml) Against Clinical Isolates



Compd.	-R	ClogP	Minimum Inhibitory Concentration (MIC, μg/ml) Against							
			I.[a]	II.[b]	III.[c]	IV.[d]	V.[e]	VI.[f]	VII.[g]	VIII.[h]
20		-3.2264	128	128	128	32	256	64	256	128
21		-3.4603	64	64	64	32	128	32	128	128
22		-2.3010	256	256	256	256	256	256	256	256
LZD(1)	-	0.5321	2	2	2	1	8	2	2	4

[a]I. = *Bacillus subtilis* [b]II.= Methicillin-susceptible *Staphylococcus aureus* 29213 [c]III. = Methicillin-resistant *Staphylococcus aureus* 33591 [d]IV. = *Staphylococcus epidermidis* biofilm. [e]V. = *Staphylococcus epidermidis mecA.* [f]VI. = *Enterococcus faecalis* 29212 [g]VII. = *Enterococcus faecalis* 15376 [h]VIII. = *Enterococcus faecalis* 51299.

the neutral-charge amphiphilic tetraethyleneglycol (TEG) spacer was expected to improve the water solubility, facilitate entry into bacteria and may initiate perturbation and disruption of the cytoplasmic membrane [30]. Although these compounds were anticipated to exhibit enhanced binding potency and afford improved antibacterial activity unfortunately, this was not realized.

CONCLUSION

We have hereby presented three novel methodologies for the systematic structural modification of oxazolidinone antibiotic derivatives incorporating nucleobases and tetraethylene glycol units with 1,2,3-triazole linkage, diversely substituted squaric acid units and nucleosides attached also with triazoles to the tricyclic backbone. Despite varied substitution patterns introduced to the linezolid (**1**) and eperezolid (**2**) no active antibacterial compound was identified. However, the presented results are considered useful from the point of further drug development approaches and the formation of broader structure-activity relationships in the oxazolidinones class of compounds.

EXPERIMENTAL

Materials and Methods

Unless otherwise stated the starting materials and solvents were purchased from commercial sources (Sigma-Aldrich or Fluka) and used as received. ^{1}H and ^{13}C NMR spectra were recorded at 360.13 and 90.55 MHz, respectively, with a Bruker WP-360 SY spectrometer, or at 500 MHz and 125 MHz, respectively, with a Bruker DRX-500 spectrometer using $CDCl_3$, or d_6-DMSO as solvents and tetramethylsilane (TMS) as internal standard. Multiplicities of the ^{1}H NMR signals are reported as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; dt, double triplet; q, quartet; m, multiplet; bs, broad singlet. Mass spectra were recorded with Bruker Biflex-III MALDI TOF MS mass

spectrometer. For column chromatography Merck silica gel (Kieselgel 60), 0.040-0.063 mm (230-400 mesh) was used. Thin layer chromatography (TLC) was performed on Kieselgel 60 F_{254} (Merck), using the corresponding developing system/eluent. Spots were visualized by irradiation under UV lamp, and/or by spraying with an ammonium-molibdenate/sulfuric acid solution and heating. Evaporations were carried out under diminished pressure at 35-40 °C (bath temperature).

Syntheses

3,6,9,12-Tetraoxapentadec-14-yn-1-ol (5)

To a stirred suspension of sodium hydride (4.0 g, 80 mmol, 50% oil dispersion) in dioxane (40 mL) was added the solution of tetraethylene glycol (40.0 g, 200 mmol) in small portions. The solution of propargyl bromide (7.5 mL, 50 mmol 80 w/v% in toluene) was diluted with dioxane (60 mL) and was added dropwise to the mixture in one hour. The reaction mixture was stirred at room temperature for additional two hours. Then H_2O (20 mL) was added and stirred vigorously for 10 minutes, the solvents were evaporated in vacuum. The residue was dissolved in CH_2Cl_2 (400 mL) and washed with brine (3 x 100 mL), the organic layer was dried (Na_2SO_4), filtrated and concentrated. The residual oil was purified by flash chromatography (CH_2Cl_2 / acetone, 9:1) to give **5** (9.58 g, 82% yield) as a colorless oil. MALDI-TOF MS 255.11 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 2.49 (t, 1 H), 3.59-3.73 (m, 16 H), 4.21 (d, 2 H).

1-Bromo-3,6,9,12-tetraoxapentadec-14-yne (6)

To a solution of **5** (9.28 g, 40 mmol) in CH_2Cl_2 (100 mL) and dry pyridine (20 mL, 240 mmol) was added dropwise a solution of tosyl chloride (22.8 g, 120 mmol) in CH_2Cl_2 (100 mL) at 0 °C. The mixture was kept at room temperature for 24 hours and monitored by TLC, hexane - acetone 6:4. The CH_2Cl_2 was removed under reduced pressure, H_2O (20 mL) was added to the residue and stirred vigorously for 1 h and extracted with CH_2Cl_2 (200 mL). The DCM layer was washed with 10% $NaHSO_4$ solution (2 x 70 mL), saturated $NaHCO_3$ solution (2 x 70 mL), and the organic layer was dried (Na_2SO_4) and concentrated to give 14.99 g of a yellow oil, that was dissolved (without further purification) in dry DMF, and treated with finely powdered KBr (15.0 g, 126 mmol) and stirred intensively overnight at 100 °C. The DMF was removed under reduced pressure and the residue was dissolved in EtOAc (250 mL) and washed with brine (100 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated, and the residue was purified by flash chromatography (hexane / ethyl acetate, 8:2) to give **6** (7.50 g, 66% yield) as a pale yellow oil. MALDI-TOF MS 317.12 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 2.47 (t, 1 H), 3.48 (t, 2 H), 3.67-3.70 (m, 12 H), 3.81 (t, 2 H), 4.21 (d, 2 H).

4-Amino-1-(3,6,9,12-tetraoxapentadec-14-ynyl)pyrimidin-2(1H)-one (7)

Compound **6** (732 mg, 2.49 mmol) was dissolved in dry DMF (6 mL), and treated with cytosine (333 mg, 3.0 mmol) and K_2CO_3 (745 mg, 5.4 mmol), and the reaction mixture was stirred vigorously overnight at 100 °C. After removal of the solvent the residue was dissolved in brine (10 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The organic layer was dried (Na_2SO_4), concentrated and purified by flash chromatography (CH_2Cl_2 / MeOH, 9:1) to give **7** (330 mg, 42% yield) as slightly yellow amorphous substance. MALDI-TOF MS 348.30 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 2.46 (t, 1 H), 3.58-3.73 (m, 16 H), 4.20 (d, 2 H), 5.81 (d, 1 H), 6.18 (bs, 2 H), 7.39 (d, 1H)

5-Methyl-1-(3,6,9,12-tetraoxapentadec-14-ynyl)pyrimidine-2,4(1H,3H)-dione (8)

Compound **6** (1.77 g, 6.0 mmol) in dry DMF (10 mL), reacted with thymine (756 mg, 6.0 mmol) in presence of K_2CO_3 (1.49 g, 10.8 mmol) overnight at 100 °C, and worked up as reported for compound **8**. Purification by flash chromatography (CH_2Cl_2 / acetone, 9:1 → 8:2) and then by C-18 reverse phase chromatography (MeOH / H_2O, 1:1) gave **11** (258 mg, 13% yield) as a slightly yellow oil. MALDI-TOF MS 363.50 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 1.90 (s, 3 H), 2.49 (t, 1 H), 3.63-3.73 (m, 16 H), 4.20 (d, 2 H), 7.22 (d, 1 H), 10.04 (bs, 1 H)

N-(3,6,9,12-tetraoxapentadec-14-ynyl)2,3,3a,4,7,7a-hexahydro-1,3-dioxo-4,7-methano-isoindol (9)

A solution of compound **6** (308 mg, 1.05 mmol) in dry MeCN (4 mL) was treated with 5-norbornene-2,3-dicarboximide (160 mg, 0.98 mmol) and K_2CO_3 (200 mg, 1.68 mmol) and the mixture was refluxed in oil bath for 2 hours. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (100 mL), washed with brine (1 x 50 mL), and the organic layer was dried (Na_2SO_4) and concentrated. Purification by flash chromatography (hexane / acetone, 8:2) gave **9** (301 mg, 81% yield) as a colorless oil. MALDI-TOF MS 400.14 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 1.55 (d, 1 H), 1.71-1.74 (dt, 1 H), 2.47 (t, 1H), 3.26-3.28 (m, 2 H), 3.35-3.38 (m, 2 H), 3.50-3.75 (m, 16 H), 4.21 (d, 2 H), 6.08 (t, 2 H).

4,7,10,13,16-Pentaoxanonadeca-1,18-diyne (4)

To a stirred suspension of sodium hydride (3.0 g, 60 mmol, 50 % oil suspension) in dioxane (80 mL) was added a solution of tetraethylene glycol (4.0 g, 20 mmol) in small portions. Then a solution of propargyl bromide (8.0 mL, 53 mmol, 80 w/v % in toluene) was added and the reaction mixture was stirred at room temperature for two hours. Water (20 mL) was added and stirred vigorously for 10 minutes, and the solvents were evaporated in vacuum. The residue was dissolved in CH_2Cl_2 (400 mL) and washed with brine (3 x 100 mL), the organic layer was dried (Na_2SO_4) and concentrated. The residual oil was purified by flash chromatography (hexane / acetone, 8:2 → 7:3) to give **7** (3.8 g, 65% yield) as a pale yellow oil. MALDI-TOF MS 293.13 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 2.49 (t, 2 H), 3.59-3.73 (m, 16 H), 4.21 (d, 4 H).

General Method for Preparation of 1,2,3-triazol Derivatives 12a-j

In a solution of the corresponding propargyl derivatives and one (**12a-d, f-i**) or two (**12e, j**) equivalent **13** or **14** in acetonitrile argon was bubbled for 10 minutes and then equivalent Et_3N and 10% equivalent CuI were added. The reaction mixture was stirred for 2 hours or overnight (**12d, e, i, j**) at room temperature. After removal of the solvent under

reduced pressure, the residue was purified by flash chromatography.

(R)-3-(3-fluoro-4-morpholinophenyl)-5-((4-(13-hydroxy-2,5,8,11-tetraoxatridecyl)-1H-1,2,3-triazol-1-yl)methyl)oxazolidin-2-one (12a)

Purification by flash chromatography (CH_2Cl_2 / acetone, 1:1) and then C-18 reverse phase chromatography (MeOH / H_2O, 1:1) gave **12a** (110 mg, 54% yield) as white amorphous substance. MALDI-TOF MS 576.20 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 3.03 (t, 4 H), 3.57-3.71 (m, 16 H), 3.85 (t, 4 H), 3.91 (q, 1 H), 4.13 (t, 1 H), 4.66 (s, 2 H), 4.75 (t, 2 H), 5.03-5.10 (m, 1 H), 6.89 (t, 1 H), 6.99-7.02 (dd, 1 H), 7.32-7.37 (dd, 1 H), 7.87 (s, 1 H). ^{13}C NMR ($CDCl_3$, 90.55 MHz) δ: 47.5, 50.8, 51.9, 61.5, 64.4, 66.8, 69.7, 70.1, 70.3, 70.4, 72.5, 107.5, 107.8, 114.1, 118.7, 124.3, 132.2, 136.7, 136.7, 145.7, 153.4, 153.9, 156.7.

(R)-tert-butyl 4-(2-fluoro-4-(5-((4-(13-hydroxy-2,5,8,11-tetraoxatridecyl)-1H-1,2,3-triazol-1-yl)methyl)-2-oxooxazolidin-3-yl)phenyl)piperazine-1-carboxylate (12f)

Purification by flash chromatography (CH_2Cl_2 / acetone, 1:1 → 4:6 → 3:7) gave **12f** (50 mg, 26% yield) as white amorphous substance. MALDI-TOF MS 675.24 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 1.48 (s, 9 H), 2.98 (t, 4 H), 3.57-3.71 (m, 20 H), 3.91-3.95 (q, 1 H), 4.12 (t, 1 H), 4.68 (s, 2 H), 4.76 (t, 2 H), 5.06-5.11 (m, 1 H), 6.89 (t, 1 H), 6.99-7.02 (dd, 1 H), 7.33-7.37 (dd, 1 H), 7.89 (s, 1 H). ^{13}C NMR ($CDCl_3$, 90.55 MHz) δ: 28.3, 47.4, 50.4, 52.0, 61.3, 64.2, 69.6, 70.3, 70.4, 79.7, 107.3, 107.6, 114.0, 119.2, 124.3, 132.4, 132.5, 136.5, 136.6, 145.5, 153.4, 153.9, 154.5, 156.6.

(R)-5-((4-(13-(4-amino-2-oxopyrimidin-1(2H)-yl)-2,5,8,11-tetraoxatridecyl)-1H-1,2,3-triazol-1-yl)methyl)-3-(3-fluoro-4-morpholinophenyl)oxazolidin-2-one (12b)

Purification by flash chromatography (CH_2Cl_2 / hexane / MeOH, 7:2:1 → CH_2Cl_2 / MeOH, 9:1) gave **12b** (150 mg, 46% yield) as pale yellow crystals, m.p. 51-54 °C. MALDI-TOF MS 669.39 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 3.02 (t, 4 H), 3.57-3.69 (m, 16 H), 3.85 (t, 4 H), 3.90-3.94 (m, 3 H), 4.16 (1 H), 4.63 (s, 2 H), 4.73-4.86 (m, 2 H), 5.07-5.11 (m, 1 H), 5.79 (d, 1 H), 6.88 (t, 1 H), 6.98 (d, 1 H), 7.33 (d, 1 H), 7.40 (d, 1 H), 7.91 (s, 1 H). ^{13}C NMR ($CDCl_3$, 90.55 MHz) δ: 47.4, 49.3, 50.8, 52.1, 64.1, 66.8, 68.9, 69.6, 70.2, 70.3, 70.4, 70.5, 93.7, 107.4, 107.7, 114.1, 118.6, 124.5, 132.2, 132.3, 136.5, 136.6, 145.2, 146.9, 153.5, 153.8, 156.5, 156.6, 166.1.

(R)-tert-butyl 4-(4-(5-((4-(13-(4-amino-2-oxopyrimidin-1(2H)-yl)-2,5,8,11-tetraoxatridecyl)-1H-1,2,3-triazol-1-yl)methyl)-2-oxooxazolidin-3-yl)-2-fluoro phenyl)piperazine-1-carboxylate (12g)

Purification by flash chromatography (CH_2Cl_2 / MeOH, 95:5) gave **12g** (176 mg, 52% yield) as pale yellow crystals. m.p. 127-131 °C. MALDI-TOF MS 768.31 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 1.48 (s, 9 H), 2.97 (t, 4 H), 3.57-3.71 (m, 20 H), 3.91-3.95 (m, 3 H), 4.17 (t, 1 H), 4.64 (s, 2 H), 4.74-4.87 (m, 2 H), 5.07-5.13 (m, 1 H), 5.79 (d, 1 H), 6.88 (t, 1 H), 6.97-7.00 (dd, 1 H), 7.31-7.36 (dd, 1 H), 7.44 (d, 1 H), 7.91 (s, 1 H). ^{13}C NMR ($CDCl_3$, 90.55 MHz) δ: 28.3, 47.5, 49.3, 50.5, 52.2, 64.0, 68.8, 69.6, 70.3, 70.7, 79.8, 94.1, 107.3, 114.1, 119.2, 119.2, 124.7, 132.6, 132.7, 136.4, 136.5, 145.0, 146.8, 153.6, 153.9, 154.6, 156.5, 165.9.

(R)-1-(1-(1-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)-1H-1,2,3-triazol-4-yl)-2,5,8,11-tetraoxatridecan-13-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (12c)

Purification by flash chromatography (CH_2Cl_2 / acetone, 1:1 → 4:6 → 3:7 → 1:9) and then C-18 reverse phase chromatography (MeOH / H_2O, 1:1) gave **12c** (100 mg, 41% yield) as white amorphous solid substance. MALDI-TOF MS 684.23 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 1.87 (s, 3 H), 3.03 (t, 3 H), 3.61-3.71 (m, 16 H), 3.86 (t, 4 H), 3.89-3.93 (q, 1 H), 4.15 (t, 1 H), 4.66 (s, 2 H), 4.72-4.82 (m, 2 H), 5.04-5.11 (m, 1 H), 6.88 (t, 1 H), 6.99-7.02 (dd, 1 H), 7.20 (s, 1 H) 7.32-7.36 (dd, 1 H), 7.86 (s, 1 H), 9.94 (s, 1 H). ^{13}C NMR ($CDCl_3$, 90.55 MHz) δ: 12.2, 47.4, 48.0, 50.9, 52.1, 64.4, 66.8, 68.9, 69.8, 70.3, 70.4, 70.5, 107.5, 107.8, 109.5, 114.1, 118.7, 118.8, 124.2, 132.2, 132.3, 136.7, 136.8, 141.9, 145.7, 150.9, 153.4, 153.9, 156.7, 164.4.

(R)-tert-butyl 4-(2-fluoro-4-(5-((4-(13-(5-methyl-2,4-dioxo-3,4-dihydro pyrimidin-1(2H)-yl)-2,5,8,11-tetraoxatridecyl)-1H-1,2,3-triazol-1-yl)methyl)-2-oxooxazolidin-3-yl)phenyl)piperazine-1-carboxylate (12h)

Purification by flash chromatography (CH_2Cl_2 / acetone, 1:1) and then C-18 reverse phase chromatography (MeOH / H_2O, 7:3) gave **12h** (115 mg, 41% yield) as white crystals, m.p. 108-111 °C. MALDI-TOF MS 783.28 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 1.48 (s, 9 H), 1.87 (s, 3 H), 2.96-2.99 (t, 4 H), 3.57-3.71 (m, 16 H), 3.86-3.94 (m, 3 H), 4.14 (t, 1 H), 4.66 (s, 2 H), 4.72-4.82 (m, 2 H), 5.04-5.10 (m, 1 H), 6.88 (t, 1 H), 6.98-7.00 (dd, 1 H), 7.19 (d, 1 H), 7.31-7.36 (dd, 1 H), 7.84 (s, 1 H), 9.76 (s, 1 H). ^{13}C NMR ($CDCl_3$, 90.55 MHz) δ: 12.2, 28.3, 47.4, 48.0, 50.5, 52.0, 64.3, 68.9, 69.7, 70.4, 70.5, 79.8, 107.4, 107.7, 109.5, 114.0, 114.1, 119.2, 124.2, 132.4, 132.5, 136.6, 136.7, 141.9, 145.7, 150.9, 153.3, 153.9, 154.6, 156.7, 164.4.

(R)-1-(1-(1-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)-1H-1,2,3-triazol-4-yl)-2,5,8,11-tetraoxatridecan-13-yl)-2,3,3a,4,7,7a-hexahydro-1,3-dioxo-4,7-methanoisoindole (12d)

Purification by flash chromatography (hexane / acetone, 95:5 → 9:1 → acetone 100 %) gave **12d** (449 mg, 75% yield) as white amorphous substance. MALDI-TOF MS 721.33 m/z $[M+Na]^+$. ^1H NMR ($CDCl_3$, 360 MHz) δ: 1.54 (d, 1 H), 1.70-1.73 (dt, 1 H), 3.04 (t, 4 H), 3.24-3.25 (m, 2 H), 3.35-3.37 (m, 2 H), 3.49-3.69 (m, 16 H), 3.86 (t, 4 H), 3.91-3.95 (q, 1 H), 4.14 (t, 1 H), 4.69 (s, 2 H), 4.72-4.82 (m, 2 H), 5.03-5.10 (m, 1 H), 6.08 (t, 2 H), 6.90 (t, 1 H), 6.99-7.02 (dd, 1 H), 7.32-7.37 (dd, 1 H), 7.85 (s, 1 H). ^{13}C NMR ($CDCl_3$, 90.55 MHz) δ: 37.1, 44.6, 45.4, 47.2, 50.6, 51.8, 51.9, 64.1, 66.6, 66.8, 69.4, 69.6, 70.2, 70.2, 107.2, 107.5, 113.8, 118.4, 118.53, 124.1, 132.1, 132.2, 134.1, 136.3, 136.4, 145.4, 153.2, 153.7, 156.4.

(R)-tert-butyl 4-(2-fluoro-4-(5-((4-(13-(2,3,3a,4,7,7a-hexahydro-1,3-dioxo-4,7-methano-isoindol -2-yl)-2,5,8,11-tetraoxatridecyl)-1H-1,2,3-triazol-1-yl)methyl)-2-oxooxazolidin-3-yl)phenyl)piperazine-1-carboxylate (12i)

Purification by flash chromatography (hexane / acetone, 1:1 → acetone 100 %) gave **12i** (430 mg, 70% yield) as whi-

te crystals, m.p. 79-82 °C. MALDI-TOF MS 820.30 m/z $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 1.48 (s, 9 H), 1.54 (d, 1 H), 1.70-1.73 (dt, 1 H), 2.98 (t, 4 H), 3.24-3.25 (m, 2 H), 3.36-3.38 (m, 2 H), 3.49-3.69 (m, 20 H), 3.90-3.95 (q, 1 H), 4.14 (t, 1 H), 4.68 (s, 2 H), 4.71-4.81 (m, 2 H), 5.03-5.10 (m, 1 H), 6.08 (t, 2 H), 6.89 (t, 1 H), 6.99-7.02 (dd, 1 H), 7.32-7.37 (dd, 1 H), 7.84 (s, 1 H). ^{13}C NMR ($CDCl_3$, 90.55 MHz) δ: 28.2, 37.2, 44.6, 45.5, 47.2, 50.3, 51.8, 51.9, 64.1, 66.9, 69.5, 69.6, 70.2, 70.3, 79.6, 107.2, 107.5, 113.8, 119.1, 124.1, 132.3, 132.5, 134.1, 136.3, 136.4, 145.4, 153.2, 153.8, 154.4, 156.5, 177.4.

Reaction of 10 with 4 to Form Click Dimer (12e)

Purification by flash chromatography (toluene / MeOH, 9:1) gave **12e** (151 mg, 47% yield) as white crystals, m.p. 130-133 °C. MALDI-TOF MS 935.50 m/z $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 3.04 (t, 8 H), 3.64-3.67 (m, 16 H), 3.86 (t, 8 H), 3.88-3.93 (q, 2 H), 4.12 (t, 2 H), 4.66 (s, 4 H), 4.68-4.78 (m, 4 H), 5.01-5.08 (m, 2 H), 6.89 (t, 2 H), 6.98-7.01 (dd, 2 H), 7.31-7.35 (dd, 2 H), 7.83 (s, 2 H). ^{13}C NMR ($CDCl_3$, 90.55 MHz) δ: 47.4, 50.8, 52.0, 64.3, 66.8, 69.6, 70.3, 70.4, 107.4, 107.7, 114.1, 118.7, 124.3, 132.3, 132.4, 136.6, 136.7, 145.6, 153.4, 153.9, 156.6.

Reaction of 11 with 4 to Form Click Dimer (12j)

Purification by flash chromatography (toluene / MeOH, 9:1 → 8:2) gave **12j** (218 mg, 79% yield) as white crystals, m.p. 161-164 °C. MALDI-TOF MS 1133.53 m/z $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 1.48 (s, 18 H), 2.98 (t, 8 H), 3.57-3.73 (m, 24 H), 3.89-3.93 (q, 2 H), 4.13 (t, 2 H), 4.66-4.79 (m, 8 H), 5.02-5.09 (m, 2 H), 6.88 (t, 2 H), 6.98-7.01 (dd, 2 H), 7.31-7.36 (dd, 2 H), 7.85 (s, 2 H). ^{13}C NMR ($CDCl_3$, 90.55 MHz) δ: 28.3, 47.4, 50.4, 52.0, 64.2, 69.6, 70.3, 70.4, 79.7, 107.3, 107.6, 114.0, 119.2, 124.3, 132.4, 132.5, 136.5, 136.6, 145.5, 153.4, 153.9, 154.5, 156.6.

(S)-5-(aminomethyl)-3-(3-fluoro-4-morpholinophenyl)oxazolidin-2-one (13)

Compound **10** (3.0 g, 9.3 mmol) was dissolved in MeOH (250 mL), acetic acid (5 mL) and Pd (C) were added and hydrogen was bubbled into the reaction flask for 2 hours. After evaporation of the solvent in vacuum, the residue was purified by flash chromatography (CH_2Cl_2 / MeOH, 9:1) to give **13** as white crystals (2.2 g, 81% yield). MALDI-TOF MS 318.38 m/z $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 2.48 (br s, 2H), 2.81-3.11 (t, 2H), 3.25-3.48 (m, 6 H), 3.72-3.80 (t, 4 H), 4.71 (m, 1H), 7.12 (t, 1 H), 7.34 (d, 1 H), 7.59 (d, 1 H). ^{13}C NMR ($CDCl_3$, 90.55 MHz) δ: 44.2, 48.1, 54.9, 66.2, 87.5, 112.7, 116.0, 127.2, 130.3, 132.7, 153.3, 157.1.

(S)-3-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methylamino)-4-methoxycyclobut-3-ene-1,2-dione (14a)

Amino compound **13** (118 mg, 0.4 mmol) was dissolved in MeOH (4 mL) and the pH was adjusted to 7.5 with Et_3N, 3,4-dimethoxycyclobut-3-ene-1,2-dione (62 mg, 0.44 mmol) was added and the reaction mixture was stirred at room temperature. After a few minutes fine precipitate was observed. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2 / MeOH, 98:2) to give **14a** (144 mg, 88% yield) as white crystals, m.p. 206-211 °C (dec.). MALDI-TOF MS 428.23 m/z $[M+Na]^+$. ^{1}H NMR (d_6-DMSO, 360 MHz) δ: 2.96 (t, 4 H), 3.56-3.68 (m, 1 H), 3.74 (t, 4 H), 3.79-3.83 (m, 2 H), 4.13 (t, 1 H), 4.28 (s, 3 H), 4.78-4.86 (m, 1 H), 7.07 (t, 1 H), 7.21 (d, 1 H), 7.49 (d, 1 H). ^{13}C NMR (d_6-DMSO, 90.55 MHz) δ: 30.3, 45.8, 46.9, 50.7, 66.2, 71.9, 106.7, 107.0, 114.3, 119.3, 133.2, 133.3, 135.7, 135.8, 153.3, 154.0, 156.0, 167.8, 168.5.

(S)-3-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methylamino)-4-(methylamino)cyclobut-3-ene-1,2-dione (14b)

Compound **14a** (101 mg, 0.25 mmol) was dissolved in the mixture of MeOH – CH_2Cl_2 (3-3 mL) and the pH was adjusted to 8-9 with Et_3N. Methyl ammonium chloride (20 mg, 0.3 mmol) was dissolved in MeOH (1 mL) and methyl amine was liberated with equivalent Et_3N. This solution was added to the stirred reaction mixture at room temperature. After a few minutes fine precipitate was observed. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2 / MeOH, 95:5) to give **14b** (63 mg, 62% yield) as white crystals, m.p. 220-225 °C (dec.). MALDI-TOF MS 427.16 m/z $[M+Na]^+$. ^{1}H NMR (d_6-DMSO, 360 MHz) δ: 2.95 (t, 4 H), 3.10 (s, 3 H), 3,73 (t, 4 H), 3.75-3.92 (m, 3 H), 4.13 (t, 1 H), 4.77-4.89 (m, 1 H), 7.06 (t, 1 H), 7.17-7.20 (dd, 1 H), 7.45-7.50 (dd, 1 H). ^{13}C NMR (d_6-DMSO, 90.55 MHz) δ: 30.3, 45.8, 46.9, 50.7, 66.2, 71.9, 106.7, 107.0, 114.3, 119.3, 133.2, 133.3, 135.7, 135.8, 153.3, 154.0, 156.0, 167.8, 168.6.

(S)-3-(ethylamino)-4-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methylamino)cyclobut-3-ene-1,2-dione (14c)

Compound **14a** (43 mg, 0.1 mmol) was dissolved in the mixture of MeOH – CH_2Cl_2 (1.5-1.5 mL) and the solution of ethylamine (2 mL, 2 M in THF) was added to the reaction mixture and stirred at room temperature for 2 hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2 / MeOH, 95:5) to give **14c** (37 mg, 83% yield) as white crystals, m.p. 230-234 °C (dec.). MALDI-TOF MS 441.38 m/z $[M+Na]^+$. ^{1}H NMR (d_6-DMSO, 360 MHz) δ: 1.12 (t, 3 H), 2.95 (t, 4 H), 3.49-3.57 (m, 2 H), 3.73 (t, 4 H), 3.78-3.92 (m, 2 H), 4.14 (t, 1 H), 4.83-4.90 (m, 1 H), 7.06 (t, 1 H), 7.17-7.20 (dd, 1 H), 7.46-7.51 (dd, 1 H). ^{13}C NMR (d_6-DMSO, 90.55 MHz) δ: 19.3, 37.8, 45.2, 47.9, 50.1, 66.2, 76.2, 108.9, 111.0, 118.2, 120.3, 127.5, 133.4, 153.3, 154.0, 156.0, 167.8, 168.6.

(S)-3-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methylamino)-4-(2-hydroxyethylamino)cyclobut-3-ene-1, 2-dione (14d)

Compound **14a** (101 mg, 0.25 mmol) was dissolved in the mixture of MeOH / CH_2Cl_2 (3 : 3 mL) and the pH was adjusted to 8-9 with Et_3N. Ethanolamine (21 mg, 0.34 mmol) was added and the reaction mixture was stirred overnight at room temperature. Fine precipitate was observed, filtrated and washed with CH_2Cl_2 and cold MeOH to give **14d** (70 mg, 64% yield) as white crystals, m.p. 243-246 °C (dec.). MALDI-TOF MS 457.36 m/z $[M+Na]^+$. ^{1}H NMR (d_6-DMSO, 360 MHz) δ: 2.95 (t, 4 H), 3.43-3.63 (m, 3 H), 3.73 (t, 4 H), 3.75-3.92 (m, 2 H), 4.14 (t, 1 H), 4.83-4.93 (m, 1 H), 4.94-4.98 (m, 1 H), 7.06 (t, 1 H), 7.17-7.20 (dd, 1 H), 7.46-7.51 (dd, 1 H), 7.61 (br s, 1 H), 7.72 (br s, 1 H). ^{13}C NMR (d_6-DMSO, 90.55 MHz) δ: 45.7, 46.7, 50.6, 60.6, 66.1,

71.9, 106.5, 106.8, 114.1, 119.2, 133.1, 133.2, 135.5, 135.6, 153.1, 153.8, 155.8, 167.6, 168.0.

(S)-3-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methylamino)-4-morpholinocyclobut-3-ene-1,2-dione (14e)

Compound **14a** (101 mg, 0.25 mmol) was dissolved in the mixture of MeOH – CH_2Cl_2 (3-3 mL) and the pH was adjusted to 8-9 with Et_3N. Morpholine (26 µL, 0.30 mmol) was added and the reaction mixture was stirred overnight at room temperature. The precipitate of the product was filtered and washed with H_2O, MeOH and CH_2Cl_2 to give **14e** (80 mg, 70% yield) as white crystals. m.p. 245 °C (dec.). MALDI-TOF MS 483.37 m/z $[M+Na]^+$. ^{1}H NMR (d_6-DMSO, 360 MHz) δ: 2.95 (t, 4 H), 3.63 (s, 8 H), 3.72 (t, 4 H), 3.79-3.83 (q, 1 H), 3.89 (t, 2 H), 4.13 (t, 1 H), 4.77-4.84 (m, 1 H), 7.06 (t, 1 H), 7.16-7.19 (dd, 1 H), 7.44-7.48 (dd, 1 H), 7.99 (t, 1 H). ^{13}C NMR (d_6-DMSO, 90.55 MHz) δ: 46.1, 46.8, 47.0, 50.7, 65.9, 66.2, 72.0, 106.6, 107.0, 114.3, 119.3, 119.4, 133.3, 133.4, 135.6, 135.7, 153.3, 154.0, 156.0, 166.2, 167.1.

(S)-N-((3-(3-fluoro-4-(4-propioloylpiperazin-1-yl)phenyl)-oxazolidin-2-one-5-yl)methyl) acetamide (19)

To the solution of **18** (336 mg, 1 mmol) in a mixture of CH_2Cl_2 (10 ml) and DMF (3 ml), propiolic acid (400 mg, 5.7 mmol), Et_3N (415 µl, 3.0 mmol) and EDC·HCl (200 mg, 1.0 mmol) were added. After 12 hours the crude product was purified with flash column chromatography (CH_2Cl_2 / MeOH, 95:5), it resulted 186 mg (47.9 %) of **19**. MALDI-TOF MS 411.44 m/z $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.46, 7.07, 6.91 (m, 3 H); 4.76 (m, 1 H); 4.04, 3.75, 3.70, 3.60 (m, 4 H); 3.92, 3.80 (m, 4 H); 3.15 (s, 1 H); 3.08 (m, 4 H); 3.04 (m, 4 H), 2.02 (s, 3 H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 171.0, 155.6, 154.2, 151.8, 135.8, 133.6, 119.6, 113.9, 107.5, 79.5, 71.9, 53.6, 51.1, 50.2, 47.6, 42.0, 41.5, 23.1.

General Method for Coupling Reactions

The 5'-azidonucleoside (0.20 mmol) was dissolved in DMF (2 ml) and the flask was thoroughly rinsed with argon. Eperezolid derivative (**19**, 0.20 mmol), Et_3N (28 µl, 0.20 mmol) and CuI (5 mg, 26 µmol) were added and the reaction mixture was stirred for 24 h under argon atmosphere. After removal of the solvent *in vacuo* the crude was purified using flash column chromatography.

Compound 20: 5'-Azido-5'-deoxythymidine (**14**, 57 mg, 0.20 mmol) was reacted with **19** (78 mg, 0.20 mmol). Eluent for column chromatography: CH_2Cl_2 / MeOH, 95:5 → 9:1. Yield: 108 mg (82 %). MALDI-TOF MS 678.39 m/z $[M+Na]^+$. ^{1}H-NMR (d_6-DMSO, 307 K, 500 MHz): δ 11.28 (brs, 1 H), 8.53 (s, 1H), 8.19 (t, 1H), 7.50 (dd, 1H), 7.35 (d, 1H), 7.18 (dd, 1H); 7.10 (t, 1H), 6.18 (dd, 1H), 5.50 (brs, 1H), 4.78 (dd, 1H), 4.75-4.66 (m, 2H), 4.34-4.27 (m, 1H), 4.22 (s, 2H), 4.16-4.11 (m, 1H), 4.09 (t, 1H), 3.79 (brs, 2H), 3.71 (dd, 1H), 3.41 (t, 2H), 3.08-3.01 (m, 4H), 2.25-2.17 (m, 1H), 2.12 (ddd, 1H), 1.84 (s, 3H), 1.81 (d, 3H). ^{13}C-NMR (d_6-DMSO, 307 K, 125 MHz): δ 170.5, 164.1, 159.9, 155.2, 154.3, 150.9, 143.4, 136.5, 135.7, 134.2, 129.9, 120.4, 114.6, 110.4, 107.1, 84.7, 84.2, 72.0, 71.2, 51.8, 51.2, 47.8, 46.8, 42.4, 41.9, 38.3, 22.9, 12.5.

Compound 21: 5'-Azido-5'-uridine (**15**, 41 mg, 0.16 mmol) was reacted with **19** (62 mg, 0.16 mmol). Eluent for column chromatography: CH_2Cl_2 / MeOH, 9:1. Yield: 36 mg (27 %). MALDI-TOF MS 680.39 m/z $[M+Na]^+$. ^{1}H-NMR (d_6-DMSO, 307 K, 500 MHz): δ 11.33 (brs, 1H), 8.51 (s, 1H), 8.20 (t, 1H), 7.59 (d, 1H), 7.50 (dd, 1H), 7.18 (dd, 1H), 7.10 (t, 1H), 5.74 (d, 1H), 5.7-5.2 (br, 1H), 5.64 (d, 1H), 4.79 (dd, 1H), 4.76-4.67 (m, 2H), 4.26-4.14 (m, 3H), 4.12 (t, 1H), 4.09 (t, 1H), 4.02 (t, 1H), 3.79 (s, 2H), 3.71 (dd, 1H), 3.40 (t, 2H), 3.07-3.01 (m, 4H), 1.83 (s, 3H). ^{13}C-NMR (d_6-DMSO, 307 K, 125 MHz): δ 170.6, 163.4, 159.9, 155.2, 154.5, 151.1, 143.4, 141.7, 135.7, 134.2, 130.0, 120.4, 114.6, 107.1, 102.6, 89.5, 82.0, 72.5, 72.0, 71.1, 52.0, 47.8, 46.7, 46.2, 42.3, 41.9, 22.9.

Compound 22: 5'-Azido-5'-guanosine (**16**, 47 mg, 0.16 mmol) was reacted with **19** (62 mg, 0.16 mmol). Eluent for column chromatography: CH_2Cl_2 / MeOH, 6:4, containing 1 % AcOH. Yield: 28 mg (18 %). MALDI-TOF MS 719.40 m/z $[M+Na]^+$. ^{1}H-NMR (d_6-DMSO, 307 K, 500 MHz): δ 8.40 (s, 1H), 8.24 (t, 1H), 7.78 (s, 1H), 7.50 (dd, 1H), 7.17 (dd, 1H), 7.11 (t, 1H), 6.91 (s, 2H), 5.71 (d, 1H), 4.88-4.76 (m, 2H), 4.76-4.66 (m, 1H), 4.55 (t, 1H), 4.30-4.24 (m, 1H), 4.20 (t, 1H), 4.18 (s,1H), 4.08 (t, 1H), 3.77 (brs, 2H), 3.72 (dd, 1H), 3.40 (t, 2H), 3.07-2.99 (m, 4H), 1.84 (s, 3H). ^{13}C-NMR (d_6-DMSO, 307 K, 125 MHz): δ 170.5, 159.9, 155.2, 154.7, 154.5, 151.6, 143.2, 136.4, 135.8, 134.2, 129.8, 120.4, 114.6, 107.1, 120.4, 87.8, 82.7, 72.9, 72.0, 71.6, 52.2, 51.3, 47.8, 46.8, 42.6, 41.9, 22.9.

MICROBIOLOGY

Antibacterial Susceptibility Testing

The efficacy of compounds **12a-14e, PH075, PH121, PH041, PH120, PH084** was determined with the Mueller Hinton agar dilution method according to the Clinical and Laboratory Standard Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards) [31]. The "minimum inhibitory concentrations" (MIC's, µg/ml) were determined on Mueller-Hinton (MH) agar with medium containing dilutions of antibacterial agents ranging from 0.12 to 64 µg/ml. The MIC was defined as the minimum inhibitory concentration that completely inhibited growth of the bacteria. Test compounds were dissolved in 20% water in DMSO, while linezolid was dissolved in 40% water in ethanol, respectively. For all staphylococci and enterococci, tests were performed using MH agar plates, while MH agar plates supplemented with 5% sheep blood were used for *S. pneumoniae* and *M. catarrhalis*, and chocolate agar for *H. influenzae* to facilitate their growth. The Gram-positive clinical isolates utilized in this study consisted of methicillin-resistant *S. aureus* (MRSA, n=10), methicillin-susceptible *S. aureus* (MSSA, n=10), methicillin-resistant coagulase-negative staphylococci (MR-CNS, n=3), methicillin-sensitive coagulase-negative staphylococci (MS-CNS, n=6), *S. pneumoniae* (n=3), vancomycin-sensitive (VSE, n=6) and vancomycin-resistant (VRE, n=4) enterococci. The Gram-negative clinical isolates tested included *H. influenzae* (n=4) and *M. catarrhalis* (n=1). The reference strains, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and *H. influenzae* ATCC 49247 were used as controls. The final bacterial concentra-

tion for 0,1 ml inocula was 107 CFU/ml, and was incubated at 35 oC for 18 h.

The efficacy of compounds **20-22** (Table **1**) was determined with the broth micro dilution method according to the CLSI guideline 2. Stock solutions containing different concentrations of the substances were prepared in either distilled water or H_2O and methanol (1 : 1) or H_2O and DMSO (1 : 1), respectively, depending on the solubility of the given preparation. These were two-fold serially diluted from 256 to 0.5 μg/ml in cation-adjusted Mueller-Hinton broth, and 100 μl of each dilution was transferred into microplate holes. Bacterial strains were grown on 5 % bovine blood agar plates at 35 °C overnight. Appropriate numbers of colonies were suspended in physiological saline in order to reach the density of 0.5 McFarland for inoculation. Inoculation was carried out with 10 μl of each bacterial suspension. Incubation was performed at 35 °C for 18 h and determination of the minimal inhibitory concentration (MIC) was made with the naked eyes on a mirror. As a control, solvents were also tested for inhibition of bacterial growth, and none of them exerted bacteriostatic effect at the concentration used.

ACKNOWLEDGEMENTS

This work was supported by the Research Administration, Kuwait University Research Grant PC01/05 (OAP), the Instrument Grants GS01/01 and GS03/01 awarded to the Science Analytical Facilities (SAF). The Hungarian National Science Fund (OTKA Reg. No. K79126) and the Hungarian TAMOP 4.2.1/B-09/1/KONV-2010-0007.

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Received: July 26, 2010 Revised: September 28, 2010 Accepted: October 25, 2010