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Hydrophobic Derivatives of Glycopeptide Antibiotics as Inhibitors of Protein Kinases

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Abstract—As key regulators of cell signaling, protein kinases (PKs) are attractive targets for therapeutic intervention in a variety of diseases. Herein, we report for the first time the inhibitory activity of polycyclic peptides, particularly, derivatives of glycopeptide antibiotics teicoplanin and eremomycin, against a panel of 12 recombinant human protein kinases and two protein kinases (CK1 and CK2) isolated from rat liver. Several of the investigated compounds inhibited various PKs with IC_{50} values below 10 μM and caused >90% suppression of the enzyme activity at 10 μM concentration. Kinetic analysis of the protein kinase CK2α inhibition by the teicoplanin aglycon analogue **(7)** demonstrated the non-competitive mechanism of inhibition (with regard to ATP). Interestingly, the inhibitory activity of some investigated compounds correlated with the earlier described antiviral activity against HIV, HCV, and other corona- and flaviviruses.

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Keywords: polycyclic glycopeptide derivatives, protein kinases, antiviral activity

Protein kinases (PKs) are an important class of molecular targets in the treatment of many human disorders, especially oncological diseases [1, 2]. At present, studies of PKs as targets for treating microbial diseases are just in the beginning. Because viral infection activates multiple signaling pathways, virus-encoded or virus-induced cell PKs might represent new targets for antiviral therapy [3-6].

Mature virions of many highly pathogenic viruses, including human immunodeficiency virus (HIV), human hepatitis C virus (HCV), and influenza viruses, possess the so-called virus-associated PKs [7] that have been shown to play an important role in the virus entry and replication in the host cells [8, 9].

It was found that semisynthetic hydrophobic derivatives of the antibiotics vancomycin and teicoplanin (Fig.

Abbreviations: Aurora-A, serine/threonine protein kinase (PK); Aurora-B, serine/threonine PK; AXL, tyrosine PK receptor UFO; B-RAF-V600E, serine/threonine PK proto-oncogene B-Raf; CDK2/Cyclin A, cyclin-dependent serine/threonine PK 2; CEM, host cells for HIV; CK1 and CK2, serine/threonine casein kinases 1 and 2; CK2α-1, serine/threonine CK2 catalytic subunit isoform; CRFK, host cells for FIPV; DENV-2, Dengue virus (flavivirus); EC_{50}, effective compound concentration required to inhibit host cell proliferation by 50%; FIPV, feline infectious peritonitis virus (coronavirus); HCV, hepatitis C virus (flavivirus); HIV, human immunodeficiency virus (coronavirus); Huh, host cells for HIV; IC_{50}, compound concentration required to inhibit enzyme activity by 50%; IGF1R, insulin-like growth factor 1 receptor (tyrosine PK receptor); JEV, Japanese encephalitis virus (flavivirus); MET, single pass tyrosine PK receptor; PLK1, serine/threonine polo-like PK 1; PRK1, serine/threonine PK N1; SARS-CoV, severe acute respiratory syndrome-associated coronavirus; SRC, proto-oncogene tyrosine PK c-Src (c-sarcoma); TBEV, tick-borne encephalitis virus (flavivirus); VEGFR2, vascular endothelial growth factor tyrosine PK receptor; Vero-B, host cells for SARS-CoV, DENV-2, YFV-17D, JEV, and TBEV; YFV-17D, yellow fever virus (flavivirus).

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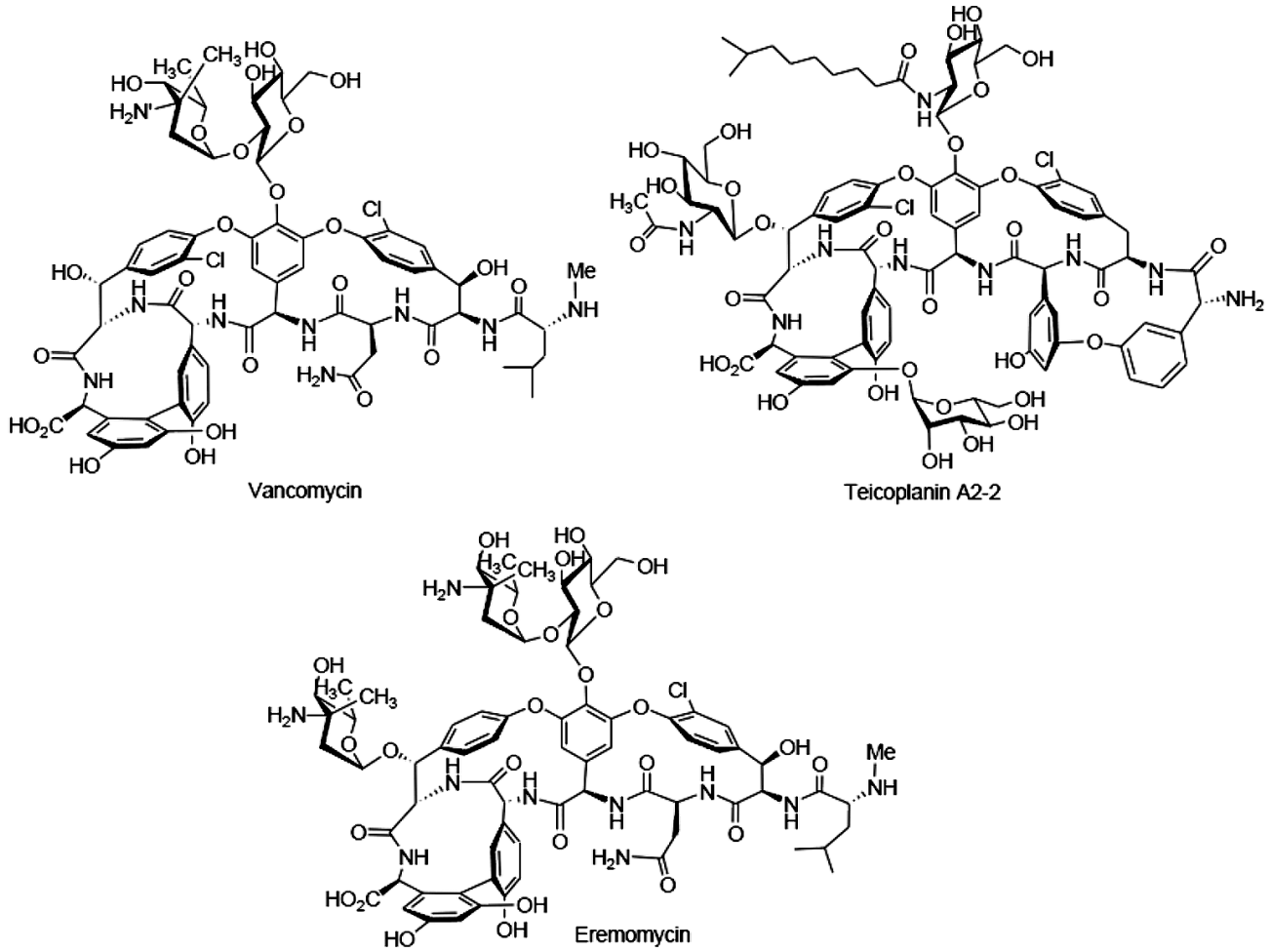


Fig. 1. Structures of vancomycin, teicoplanin A2-2, and eremomycin.

1) are able to inhibit several types of viruses, such as HIV, HCV, influenza viruses (A/H1N1, A/H3N2 and B), coronaviruses, and flaviviruses *in vitro* [10-18]. Although natural glycopeptide antibiotics do not exhibit pronounced antiviral activity in such tests, chemical removal of sugars and introduction of hydrophobic residues into resulting aglycons or pseudo-aglycons generates hydrophobic derivatives that are active against some viruses.

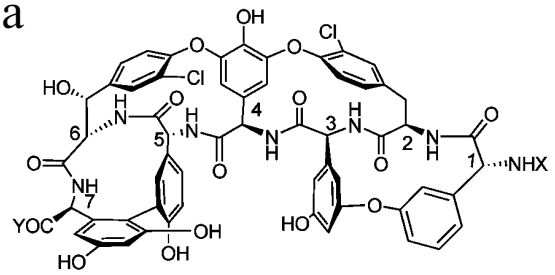
Together with vancomycin and teicoplanin, the glycopeptide antibiotic eremomycin (Fig. 1) can be used as original compounds for developing a new generation of antiviral agents [11, 12, 16] that represent non-nucleoside inhibitors of RNA replicase. Preliminary findings showed that glycopeptide aglycon derivatives act at the entry [11, 17] and post-entry [14] stages of the virus infection cycle. The processes of virus penetration into the host cell and its replication provided by specific interactions between viral structural or non-structural (co)receptors. The mechanisms of the antiviral activity of glycopeptide aglycon derivatives remain unclear. Virus entry to the cell and its life cycle depend on the use of intracellular signal transduction pathways. On these grounds, we screened several hydrophobic glycopeptide aglycon derivatives for their inhibitory activity against a panel of PKs.

MATERIALS AND METHODS

Chemicals from Sigma-Aldrich (USA) and ProQinase (Germany) were used.

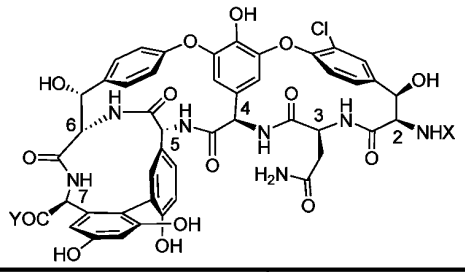
Compound library. Eremomycin was obtained from the pilot plant of Gause Institute of New Antibiotics (Moscow, Russia). Teicoplanin and teicoplanin aglycon **(1)** were kindly provided by Dr. R. Ciabatti and Dr. A. Malabarba (both from Biosearch S.p.A. Gerenzano, Italy). Teicoplanin aglycon derivatives *n*-decylamide **(2)**, 6-amino-*n*-heptyl-1-amide **(3)**, 10-amino-*n*-decylamide **(4)**, *p*-(N,N-di-*n*-butyl)benzylamide **(5)**, *p*-*n*-butylbenzyl-*n*-heptylamide **(6)**, N-1-adamantyl-oxycarbonyl derivative **(7)**, N-1-adamantyl-oxycarbonyl cyclopropylamide **(8)**, N-*t*-butyl-oxycarbonyl adamantyl-2-amide **(9)**, and N-*t*-butyl-oxycarbonyl adamantyl-1-methyl-

a



Compound	*X*	*Y*
Teicoplanin aglycon (1)	H	OH
Amides		
2	H	$C_{10}H_{21}NH$-
3	H	$NH(CH_2)_6NH$-
4	H	$NH_2(CH_2)_{10}NH$-
5	H	*p*-(NBu_2)BnNH-
6	H	*p*-Bu$BnNH(CH_2)_6NH$-
N-Acyl derivative		
7	Adoc	OH
Amides of N-acyl derivatives		
8	Adoc	Cyclopropyl
9	Boc	(Adam-2)NH-
10	Boc	(Adam-1)CH_2NH-

b



Compound	*X*	*Y*
Eremomycin aglycon (11)	*N*-Me-*D*-Leu-	OH
Des-*N*-Me-*D*-Leu-eremomycin aglycon (12)	H	OH
Amides of 11 or 12		
13	*N*-Me-*D*-Leu-	(Adam-1)CH_2NH-
14	H	*p*-FC_6H_4-$N(CH_2CH_2)_2N$-
15	*N*-Me-*D*-Leu-	$NH_2(CH_2)_{10}NH$-

Fig. 2. a) Teicoplanin aglycon **(1)** and its derivatives **(2)-(10)**. b) Eremomycin aglycon **(11)** and its derivatives **(12)-(15)**. Bu, *n*-butyl; Bn, benzyl; Adoc, 1-adamantyl-oxycarbonyl; Adam, adamantyl.

Table 1. Inhibitory activity (IC_{50}, μM) of compounds **(2)-(4)** and **(11)-(15)** against a panel of 12 human PKs

Compound	Protein kinase, IC_{50} (μM)											
	AURORA A	AURORA B	AXL	BRAF-V600E	CDK2/CycA	CK2α	IGF1R	MET	PLK1	PRK1	SRC	VEGFR2
2	1.46	2.54	2.38	4.53	2.57	8.53	1.02	6.05	5.04	2.98	2.36	4.43
3	0.54	0.78	2.63	2.66	1.5	5.13	0.85	3.85	2.07	1.00	1.13	2.37
4	0.94	0.94	3.27	3.34	3.87	18.1	0.91	2.80	4.01	0.60	2.08	4.27
11	9.87	12.0	33.4	55.0	41.2	>100	22.1	75.5	50.4	33.5	47.0	59.0
12	14.8	28.8	27.5	88.3	46.8	51.4	13.4	>100	49.99	85.5	1.2	63.2
13	2.75	0.82	11.7	16.5	24.8	14.5	4.74	10.1	24.7	3.93	6.90	17.5
14	3.64	3.85	12.7	15.2	30.4	75.4	2.14	12.2	39.3	7.36	6.87	18.7
15	8.07	10.1	11.5	25.4	22.0	81.6	8.76	33.6	25.7	8.30	34.0	30.1

amide **(10)** (Fig. 2a) and eremomycin aglycon **(11)** and its derivatives de-(N-Me-D-Leu)-eremomycin aglycon (hexapeptide) **(12)**, adamantyl-1-amide **(13)**, de-(N-Me-D-Leu)-*p*-fluorophenyl-1-N-peperazino-4-amide **(14)**, and 10-amino-*n*-decyl-1-amide **(15)** (Fig. 2b) were described in [11, 14, 16, 19]. The purity (>95%) and identity of used compounds were assessed by HPLC and spectral analysis.

***In vitro* PK activity assay with human recombinant PKs.** The activity of recombinant human PKs was assessed by the radiometric method (Table 1). The reaction mixture contained 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 μM sodium orthovanadate, 1.2 mM dithiothreitol (DTT), 50 μg/ml PEG20000, and 1 μM [γ-^{33}P]ATP (~$5 \cdot 10^5$ Ci; PerkinElmer, USA) in a final volume of 50 μl. The enzyme concentrations in the reaction mixture were the following: Aurora-A, 2 ng/μl; Aurora-B, 2 ng/μl; AXL, 2 ng/μl; B-RAF-V600E, 0.4 ng/μl; CDK2/Cyclin A, 2 ng/μl; CK2α-1, 4 ng/μl; IGF1R, 0.4 ng/μl; MET, 2 ng/μl; PLK1, 1 ng/μl; PRK1, 1 ng/μl; SRC, 0.2 ng/μl; and VEGFR2, 0.5 ng/μl. Incorporation of radioactive phosphate into the following substrates was measured: RBER-CHKtide (PLK1); tetra(LRRWSLG) (Aurora-A, Aurora-B, PRK); casein (CK2-α-1); MEK1-KM (BRAF-V600E); histone H1 (CDK2/Cyclin A); poly(Ala, Glu, Lys, Tyr) 6 : 2 : 5 : 1 (MET); poly(Glu, Tyr) 4 : 1 (AXL, IGF1R, SRC, VEGFR2).

In vitro PK activity was assayed with a Beckman Coulter/Sagian Multipette Automated Liquid Handler robotic system (USA) at the ATP concentration of 1 μM. The assay conditions have been optimized for each kinase, as well as for each lot of the recombinant proteins. To verify the quality of the recombinant proteins, we determined apparent K_m (ATP) values under the same conditions that were used for compound *in vitro* testing.

For each PK lot used, the IC_{50} values with three reference inhibitors (10 concentrations each) were determined at 1 μM ATP to confirm the reproducibility of the obtained results, as well as their correspondence to the earlier published data. Z′ factors have been determined in each assay; all of them were at least 0.41 and, in most cases, exceeded 0.6, thereby indicating good to excellent assay reproducibility.

The reactions were performed at 30°C for 80 min and then stopped by adding 50 μl of 2% H_3PO_4 (v/v). After washing off unreacted γ-^{33}P, label incorporation was measured with a Wallac Microbeta Trilux microplate scintillation counter (PerkinElmer).

***In vitro* PK activity assay with tissue-derived CK1 and CK2.** Native CK2 (α2β2) and CK1 were purified from rat liver as previously described [20]. Human recombinant CK2 α subunits were expressed in *Escherichia coli* and used in kinetic analysis after purification (see below) [20]. CK2 and CK1 phosphorylation was carried out at 37°C in the presence of increasing amounts of each inhibitor in a final volume of 25 μl containing 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 12 mM $MgCl_2$, and 0.02 mM [γ-^{33}P]ATP (500-1000 cpm/pmol), unless indicated otherwise. The phosphorylation substrates were synthetic peptides RRRADDSDDDDD (100 μM) and RRKHAAIGDDDDAYSITA (200 μM) for CK2 and CK1, respectively. The reaction was started by adding kinase to the reaction mixture and stopped after 10 min by adding 50 μl of 0.5 M H_3PO_4. Aliquots of the reaction mixture were spotted onto phosphocellulose filters (MANN Filter, Germany); the filters were washed four times with 5-10 ml of 75 mM H_3PO_4 and once with methanol (20 ml) and then dried at 25°C before counting. Each measurement was performed in triplicate; the results were calculated as mean ± SEM (SEM < 15%).

Kinetic analysis. Kinetic data were obtained using CK2α as described above. Initial reaction rates were determined at different substrate and ATP concentrations as described in [21, 22]. K_m values were calculated in the absence and presence of increasing inhibitor concentrations using Lineweaver–Burk double-reciprocal plots.

RESULTS AND DISCUSSION

The inhibitory activity of nine teicoplanin derivatives **(2)-(10)**, eremomycin aglycon **(11)**, and its derivatives **(12)-(15)** against CK2α and 11 human PKs (Aurora A, Aurora B, AXL, BRAF-V600E, CDK2/CycA, IGF1-R, MET, PLK1, PRK1, SRC, VEGFR2) were evaluated *in vitro* by either determining IC_{50} values (Table 1) or measuring enzyme residual activity (%) incubation with the tested compounds (10 and 1 μM) (Table 2).

The inhibitory activity of teicoplanin aglycon derivatives **(2)-(7)**, **(9)**, and **(10)**, teicoplanin aglycon **(1)**, teicoplanin, and eremomycin was evaluated *in vitro* against native CK2 and CK1 isolated from rat liver (Table 3). We also studied the kinetics of CK2α inhibition by the teicoplanin analogue **(7)** that exhibited the highest inhibitory activity in our tests (Fig. 3, a and b).

We compared the inhibitory activity of studied glycopeptide derivatives against PKs and discussed the relationship between activity, structure, and antiviral activities of the tested compounds.

Teicoplanin aglycon derivatives. Hydrophobic teicoplanin aglycon carboxamides **(2)-(4)** containing various hydrophobic residues inhibited all tested human PKs. The IC_{50} values for these compounds were in low micromolar range (Table 1), except for CK2α inhibition by **(4)** (IC_{50}, 18 μM). However, this compound was also inefficient against rat liver protein kinases CK1 and CK2 (IC_{50}, 34.76 and >40 μM, respectively) (Table 3).

Human CK2α was also less sensitive to the teicoplanin aglycon derivatives **(6)** and **(9)** (100 and 83% residual activity at 10 μM concentration, respectively) (Table 2). Compound **(6)** showed no or weak inhibitory activity (>57% residual activity at 10 μM) toward all investigated human PKs.

Teicoplanin derivatives **(2)** and **(7)-(10)** demonstrated significant inhibitory activity against tested PKs (residual activities, <10% at 10 μM concentrations). Among them, compounds **(2)** (IGF1R, SRC), **(7)** (Aurora B, IGF1R, SRC), and **(9)** (IGF1R) were strong inhibitors that suppressed enzymatic activity by more than 50% when used at 1 μM concentration (Table 2).

Compounds **(2)**, **(5)**, **(7)**, **(9)**, and **(10)** efficiently inhibited isolated rat liver CK2 (IC_{50}, ~ 2.70-4.00 μM), while compounds **(1)**, **(4)**, and **(6)** were almost inactive against this enzyme ($IC_{50} \geq 40$ μM) (Table 3).

Unlike native CK2, CK1 was less sensitive to most of the investigated compounds. The IC_{50} values for CK1 inhibition were <10 μM only for **(5)** and **(7)**, while for compounds **(1)**, **(2)**, **(4)**, **(6)**, **(9)**, and **(10)** they were much higher (~20.29-40.00 μM).

It is important to mention that the inhibitory activities of **(2)**, **(4)**, **(6)**, **(7)**, **(9)**, and **(10)** against CK2 were evaluated using both recombinant and native proteins (Tables 1-3) and correlated with the activities of these compounds in other tests. This holds true for the low inhibitory activity of **(6)**, as well as for higher inhibitory activities of compounds **(2)**, **(7)**, **(9)**, and **(10)**.

Kinetic analysis of CK2α inhibition by the teicoplanin aglycon analogue **(7)** showed that this compound suppressed the enzyme activity by the non-competitive mechanism (with respect to ATP) (Fig. 3a). To further investigate the action mechanism of compound **(7)**, CK2α inhibition was studied at constant ATP concentration in the presence of varying peptide substrate concentrations. As shown in Fig. 3b, compound **(7)** also showed a non-competitive inhibition with respect to the peptide substrate.

This is a remarkable result because the majority of kinase inhibitors compete with ATP for binding in the same active site [23]. CK2 inhibitors that are non-competitive with respect to ATP have been rarely reported.

Eremomycin aglycon derivatives. Eremomycin aglycon **(11)** and hexapeptide **(12)** showed moderate or low inhibitory activities (IC_{50}, ~12 and >100 μM, respectively) against the tested human PKs (Table 1). Eremomycin aglycon amides **(13)** and **(14)** inhibited five human PKs (Aurora A, Aurora B, IGR1R, PRK1, SRC) within the micromolar concentration range (IC_{50}, 0.8-10.1 μM). Their activities against other PKs (AXL, BRAFV600E,

Table 2. Inhibitory activity of compounds **(2)** and **(6)-(10)** against a panel of 10 human PKs expressed as enzyme residual (% of control) after its incubation with the tested compound at 10 or 1 μM concentration

Compound	AURORA B	AXL	BRAF-V600E	CK2α	IGF1R	MET	PLK1	PRK1	SRC	VEGFR2
2	2; 84	17; 105	25; 80	59; 85	5; 46	9; 75	12; 85	34; 71	16; 48	15; 107
6	84; 97	99; 113	101; 100	100; 104	57; 103	91; 104	109; 108	112; 120	63; 100	77; 113
7	2; 45	13; 89	9; 90	42; 108	3; 30	10; 61	18; 83	33; 86	8; 31	8; 108
8	2; 81	13; 96	16; 80	46; 91	6; 52	12; 94	8; 76	14; 92	12; 62	9; 85
9	2; 70	22; 97	41; 88	83; 89	4; 47	22; 92	46; 93	57; 93	17; 58	19; 82
10	3; 66	12; 113	21; 80	18; 96	2; 67	2; 91	0; 87	13; 93	9; 89	2; 78

CDK2/CycA, CK2α-1, VEGFR2) were moderate or low (IC_{50}, 11.6-75.4 μM). Compound **(15)** showed remarkably high inhibitory activity (IC_{50}, ≤ 10.1 μM) against only four PKs (Aurora A, Aurora B, IGF1R, PRK1). Human CK2α was most resistant to all eremomycin derivatives (**(11)-(15)**) (IC_{50}, 14.5 to >100 μM) and teicoplanin aglycon derivative **(6)** (IC_{50}, 18 μM).

The relationship between structure, inhibitory activity against PKs, and antiviral activity. Natural antibiotics eremomycin and teicoplanin (Fig. 1) did not inhibit rat liver CK1 and CK2 in our experiments (IC_{50}, ≥ 40 μM). Aglycon derivatives **(1)**, **(11)**, and **(12)** showed moderate to low inhibitory activities against the tested PKs (IC_{50}, ~12 to >40 μM) (Tables 1 and 3).

However, aglycon derivatives bearing one or two hydrophobic residues (Fig. 2, a and b) inhibited different PKs in micromolar concentrations (Tables 1-3). In all the cases, hydrophobic substituent in teicoplanin aglycon amides **(2)-(6)**, N-acyl derivative **(7)**, amides of N-acyl derivative **(8)-(10)**, as well as in eremomycin aglycon or eremomycin hexapeptide amides **(13)-(15)** was essential for the inhibitory activity of these compounds against PKs.

Comparison of inhibitory efficiencies of teicoplanin and eremomycin aglycon carboxamides bearing the same residue X = $NH(CH_2)_{10}NH_2$ showed that teicoplanin derivative **(4)** was significantly more active against the tested human PKs (Aurora A, Aurora B, AXL, BRAFV600E, CDK2/Cyclin A, IGF1R, MET, PLK1, PRK1, SRC, VEGFR2) than eremomycin derivative **(15)** (IC_{50}, ~ 0.78-4.00 μM vs. 8.3-33.6 μM, respectively) due to the differences in their structures. Teicoplanin, in comparison with eremomycin, has an aromatic hydrophobic amino acid at position 3 instead of asparagine (Asn) and additional chlorine atom in the amino acid 6 of the peptide core.

It have been found earlier that several compounds (**(2)-(5), (7)-(10)**) inhibiting different PKs in our experiments suppress some coronaviruses, such as HIV-1, HIV-2, feline infectious peritonitis virus (FIPV), severe acute respiratory syndrome-associated coronavirus (SARS-CoV), and flaviviruses, including hepatitis C virus (HCV), Dengue virus (DENV), yellow fever virus (YFV), tick-borne encephalitis virus (TBEV), and Japanese encephalitis virus (JEV) in micromolar concentrations in *in vitro* tests [11, 14, 16-18]. These compounds exhibit no cytotoxicity against host cells (Vero B cells for SARS-CoV, DENV-2, YFV-17D, JEV, and TBEV; CRFK cells for FIPV; CEM cells for HIV) with the selectivity index $CC_{50}/EC_{50} > 10$ (where CC_{50} is compound concentration required to inhibit host cell proliferation by 50%).

There are several publications about known specific PKs inhibitors suppressing virus replication. For example, 2-(2-chlorophenyl)-5,7-dihydroxy-8-[(3S,4R)-3-hydroxy-1-methylpiperidin-4-yl]chromen-4-one (flavopiridol) is the first CDK inhibitor in human clinical trials that blocks HIV replication [5]. Also, the CK2 inhibitor 2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole (DMAT) prevents HCV production in the host cell [24].

Table 3. Inhibitory activity (IC_{50}, μM) of teicoplanin, eremomycin, teicoplanin aglycon **(1)** and its derivatives **(2)**, **(4)-(7)**, **(9)**, and **(10)** against serine/threonine kinases CK1 and CK2 from rat liver

Compound	Protein kinase, IC_{50} (μM)	
	CK1	CK2
Teicoplanin	>40.00	>40.00
Eremomycin	>40.00	>40.00
1	>40.00	>40.00
2	20.29	3.60
4	34.76	>40.00
5	8.58	4.00
6	20.71	40.00
7	7.85	2.70
9	23.36	3.40
10	21.43	3.50

The relationship between inhibitory and antiviral activities of glycopeptide derivatives may be of great interest for the development of antiviral drugs, since it has been shown that human cyclin-dependent kinases (CDKs) and CK2 are required for HIV-1 replication [5, 6, 25, 26], whereas CK1, CK2, PLK1, and SRC are involved in HCV replication [9, 27-29].

Eremomycin aglycon derivatives **(13)-(15)** containing hydrophobic residues have been investigated as anti-HIV-1 agents [16]. The antiviral activity was high for **(13)** (EC_{50}, 1.6 μM) and moderate for **(14)** and **(15)** (EC_{50}, 12 and 15 μM, respectively) (Table 1). Hydrophobic teicoplanin aglycon derivatives (amides **(2)-(5)**, **(7)**) efficiently inhibited human PKs and CK2 (IC_{50}, ⩽ 10 μM) (Tables 1-3), as well as exhibited anti-HIV-1 activity with EC_{50} values between 2.6 and 15 μM [11]. This demonstrates that the inhibitory activity of several investigated compounds against PKs correlates with their antiviral activity displayed at micromolar concentrations.

Hydrophobic teicoplanin aglycon derivatives **(7)-(10)** strongly inhibiting human PKs (Aurora B, AXL, IGF1R, MET, PLK1, SRC, VEGFR2) (⩽22% control when used at 10 μM concentration) and CK2 (IC_{50}, ⩽ 10 μM) (Tables 2 and 3) selectively suppressed HCV replication in model subgenomic HCV replicon systems and cultured cells (EC_{50}, 5.7, 7.0, 4.9, and 3.6 μM, respectively, in Huh 5-2 host cells) [14].

The most interesting data have been obtained for the teicoplanin aglycon derivative N-*t*-butyl-oxycarbonyl

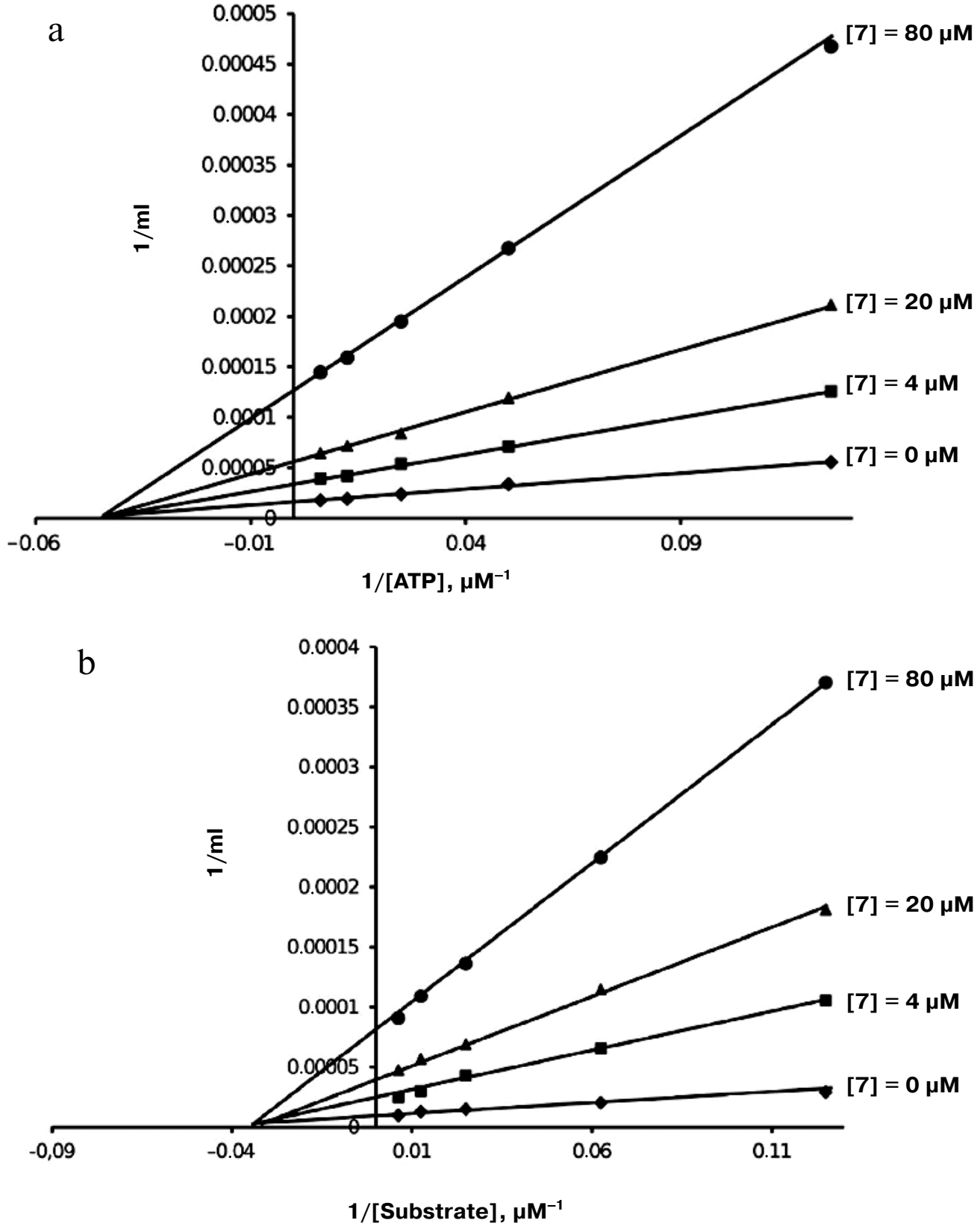


Fig. 3. Kinetic analysis of protein kinase CK2α inhibition by compound **(7)**. a) The concentration of the peptide substrate was constant (100 μM); ATP concentration varied from 4 to 160 μM; b) ATP concentration was constant (80 μM); peptide substrate concentration varied from 4 to 160 μM.

adamantyl-2-amide **(9)**. It was found that this compound targets the early stage (binding/entry) of the DENV-2 infection [17]. The fact that **(9)** prevents DENV-2 entry into the host cell and HCV replication does not imply the same mechanism of action. Indeed, it was demonstrated that **(9)** inhibits HCV replication at the post-entry stage (in Huh 9-13 cells) but its target has not been established yet [14]. However, considering that DENV-2 and HCV belong to the same family (Flaviviridae), it is remarkable that the same molecule inhibits replication of such closely related viruses by affecting two entirely different stages of the replication cycle.

In this manuscript, we have for the first time evaluated the inhibitory activities of 15 polycyclic peptide derivatives (teicoplanin and eremomycin aglycons and their derivatives) against a panel of 12 human protein kinases and rat liver protein kinases CK1 and CK2.

Teicoplanin aglycon analogue **(7)** was a rare example of non-competitive inhibitor of protein kinase activity with respect to both ATP and peptide substrate, as demonstrated by kinetic analysis of its inhibitory action on recombinant CK2α.

Several investigated hydrophobic analogues of polycyclic glycopeptide antibiotics (**(2)-(5)**, **(8)-(10)**, **(12)**) that have been earlier found to suppress replication of HIV, HCV, and the other corona- and flaviviruses [11, 14, 16-18] inhibited different PKs in the micromolar concentration range (Tables 1-3).

The patterns common for PK inhibition and antiviral activity of the investigated compounds were established: (i) both inhibitory and antiviral activities are determined by the base aglycon structure; (ii) hydrophobic residue in aglycon is essential for the inhibitory activity; (iii) the position of this hydrophobic residue in aglycon is not important; (iv) the number of hydrophobic residues (one or two) is not important.

Glycopeptide aglycon derivatives are promiscuous inhibitors of PKs, and their inhibitory activity depends on structure. The fact that they inhibit different types of PKs may explain their activity against unrelated viruses (HIV, HCV, etc.) Moreover, the levels and activities of PKs in virus-infected cells, as well as the inhibitory potential of glycopeptide aglycon derivatives and their ability to permeate the host cell membrane, can modulate the antiviral activity of these compounds.

The role of PK inhibition in the antiviral activity of hydrophobic glycopeptide aglycon derivatives remains to be investigated; the ability of these compounds to affect cellular protein kinases *in vivo* still has to be verified. Since ATP concentrations used in the *in vitro* assays are significantly below average intracellular ATP concentrations, the potency of kinase inhibitors *in vitro* does not necessarily reflect their potency in cells. Therefore, further optimization of glycopeptide derivatives will aim to improve their potency, as well to demonstrate the ability of these compounds to inhibit intracellular protein kinases.

The search for antiviral compounds among protein kinase inhibitors seems to be very promising. These novel approaches may pave the way to new prospects in drug discovery, while simultaneously providing valuable tools for studying signal transduction and viral replication.

Moreover, such studies may be useful for understanding the mechanism of the antiviral activity of hydrophobic glycopeptide aglycons and promote the development of a novel class of PKs inhibitors based on glycopeptides antibiotics and related compounds.

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Conflict of Interests

The authors declare no conflict of interests in financial or any other area.

Ethical Approval

All procedures with animals were conducted in accordance with the legal requirements adopted by the Russian Federation and international organizations (EU Directive 2010/63/EU from September 22, 2010; Article 27).

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