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Synthesis and In Vitro Antibacterial Activity of New C-3-Modified Carbapenems

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Abstract—New C-3 modified carbapenems have been synthesized by the Ad_NE-substitution of the enol phosphate group of 4-nitrobenzyl (4*R*)-3-[(diphenylphosphoryl)oxy]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-en-2-carboxylate by the corresponding thiols in acetonitrile in the presence of diisopropylethylamine (DIPEA). Mercaptoacetic acid methyl ester, furan-1-methylthiol, methyl N-(mercaptoacetyl)methioninate, and 2-(4-methylpiperazin-1-yl)-2-oxoethanethiol as thiols have been used. As a result, we have obtained the expected 4-nitrobenzyl esters of 3-[(2-methoxy-2-oxoethyl)thio]-, 3-[(2-furylmethyl)thio], 3-{[2-(4-methylpiperazin-1-yl)-2-oxoethyl]thio}, and 3-[((2-(S)-1-methoxy-4-methylthio-1-oxopropan-2-yl)amino)-2-oxoethyl]thio derivatives of (4*R*,5*S*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid in yields of 67–87% after purification by column chromatography. The *p*-nitrobenzyl (pNb) esters of carbapenems have been converted to the corresponding acids by hydrogenolysis over 10% Pd/C in methanol. The antibacterial activity of the resulting carbapenems and their preceding pNb-esters has been studied against microorganisms of *Escherichia coli, Pseudomonas aeruginosa*, *Candida albicans*, and *Streptococcus oralis*. The compounds that have been found exceed in their activity the known drugs Meropenem and Cilapenem.

Keywords: carbapenemenol phosphate, mercaptoacetic acid, amides, synthesis, carbapenems, antibacterial activity

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INTRODUCTION

One of the global problems in the treatment of infectious diseases with antibiotics is the development of resistant strains of microorganisms, which leads to serious consequences for patients. A recognized and effective approach to solve this problem is the creation and introduction of new or structurally modified analogs of known antibiotics [1, 2].

Despite the long history and development of beta-lactam antibiotics, this class of antibiotics is currently of particular interest for both the treatment of bacterial infections [3] and the search for new chemotherapeutic agents. The mechanism of action of beta-lactams is associated with their ability to bund covalently to enzymes involved in the construction of the bacterial cell wall and the formation of nonviable defective cells.

Among the antibiotics of the beta-lactam series, carbapenems are the most effective low-toxic representatives with a wide spectrum of action. The key structure of these compounds is 7-oxo-1-azabicyclo[3.2.0]hept-2-en-2-carboxylic acid, which provides their high resistance to beta-lactamases [4]. The presence of the 1*R*-hydroxyethyl group at the C6 position of carbapenems leads to a significant increase in antibacterial activity and protects the beta-lactam ring, thus providing stability of antibiotics against beta-lactamases. The structures of some carbapenem antibiotics used in practice (Fig. 1) chemically differ only in the SR substituent at C3 with the rest of the molecules being almost the same.

1-Methylcarbapenems (Fig. 1) are obtained by chemical synthesis. To date, a number of synthetic approaches have been proposed, a significant number of derivatives of carbapenems have been obtained, and their pharmacological properties have been studied [5–8]. Nevertheless, the search for new antibiotics of this series and the modification of the known antibiotics are still relevant because of the resistance problems. Structurally oriented analysis of the existing array of known drugs among carbapenem antibiotics allows for

Abbreviations: Ad_NE, nucleophilic addition/elimination; DIPEA, diisopropylethylamine; DMSO, dimethylsulfoxide; pNb, *p*-nitrobenzyl; MIC, minimum inhibitory concentration; PCS, positive control sample; NCS, negative control sample.

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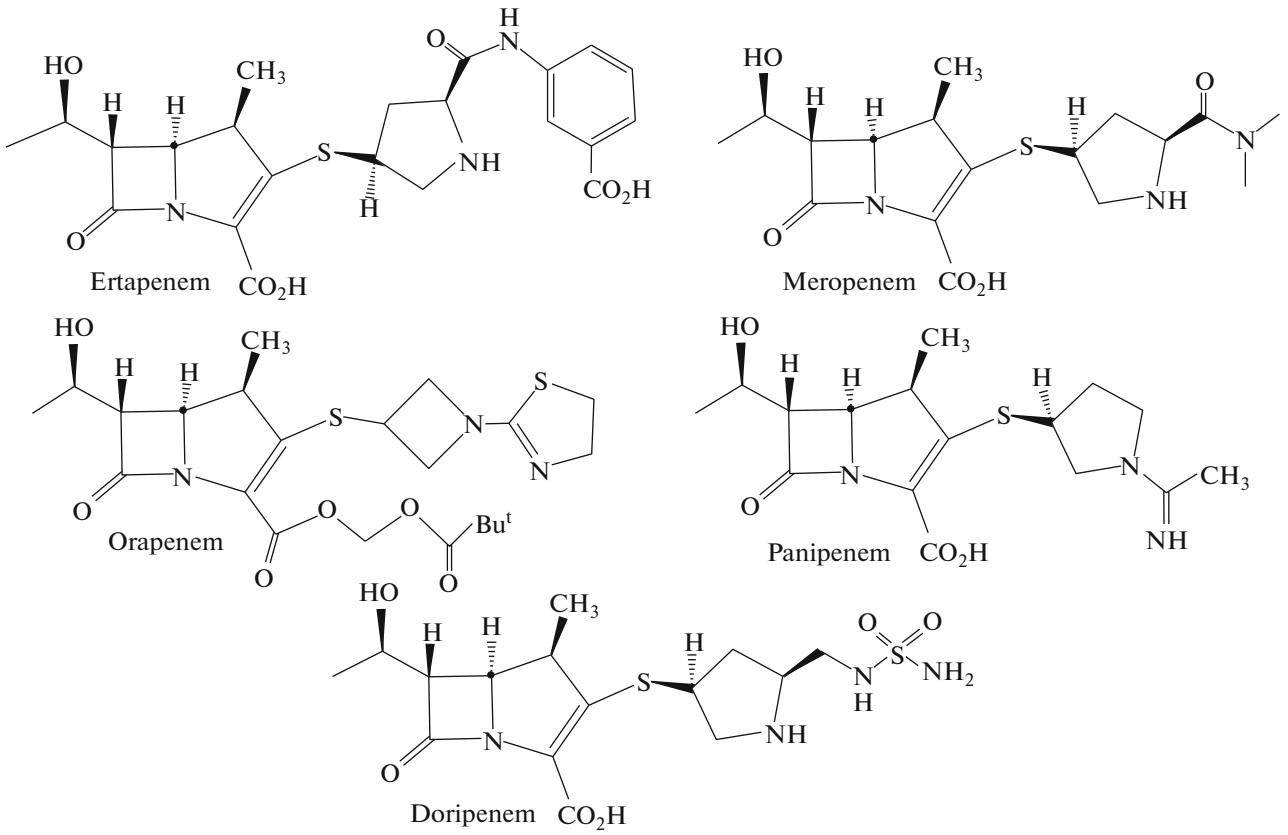


Fig. 1. Structures of some 1-methylcarbapenems used in practice and scheme of the synthesis of new C3-modified carbapenems.

the assessment of the most promising ways of their modifications. First of all, it should be noted that the beta-lactam cycle and the bicyclic part with a lateral 6-(1-hydroxyethyl) substituent, both of which are responsible for the bioactivity, remain unchanged in structurally oriented syntheses.

In the preparation of clinically balanced compounds (Fig. 1), the main structural changes were performed in the SR-fragment by the introduction of the pyrrolidine cycle that contained an *N*-containing substituent in positions 2 or 3 of the pyrrolidine ring in the carbapenem structure (except orapenem). Modifications in the carboxyl group of carbapenems are rare [9, 10] and are associated with the synthesis of esters (prodrugs), which are important in terms of increasing the lipophilicity of the drug for oral administration (see, e.g., Orapenem structure).

RESULTS AND DISCUSSION

In this work, we planned to use some derivatives of mercaptoacetic acid and furyl-2-methylthiol for the modification of the SR-part of carbapenems. This choice is caused by the desire to use readily available thiol-containing compounds, in particular, mercaptoacetamides instead of HSR compounds, which are used in the synthesis of carbapenems from phosphonate (**I**), include the pyrrolidine cycle, and are obtained in most techniques from expensive *trans*-4-hydroxy-L-proline [11].

The most practical approaches to the synthesis of carbapenem derivatives use a key compound, i.e., (4-nitrobenzyl)-(4*R*)-3-[(diphenylphosphoryl)oxy]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-en-2-carboxylate (**I**) [12, 13]. The diphenylphosphate group in this compound is replaced under mild conditions by the RS residue of RSH thiols with the formation of the carbapenem of general formula (**IIa**), which are converted to target carbapenems (**IIb**) by subsequent hydrogenolysis [14–16]. According to this scheme, we synthesized a number of carboxyl-protected carbapenems (**IIIa**)–(**VIa**) by the reaction of phosphonate (**I**) with corresponding thiols (**VII**)–(**X**) that contained no pyrrolidinone cycle. After removing the pNb ester groups, we obtained target carbapenems (**IIIb**)–(**VIb**) (Scheme 1).

pNb-Esters (**IIIa**)–(**VIa**) were isolated with a purity of ~98% by column silica gel chromatography. We did not use the recommended reverse phase chromatography on Diaion HP-20 [9] with subsequent lipophilization for the isolation of acids (**IIIb**)–(**VIb**) because of an unreasonably high loss of the target compounds during purification. We apply a simple technique, which includes filtration of hydrogenolisate to remove

Table 1. In vitro antibacterial activity of compounds **(IIIa,b)**–**(VIa,b)**

Microorganisms	MIC (μg/mL)									
	(IIIa)	**(IIIb)**	**(IVa)**	**(IVb)**	**(Va)**	**(Vb)**	**(VIa)**	**(VIb)**	Meropenem	Cilapenem
E. coli	>0.5	0.5	0.015	2.0	0.015	>32.0	0.031	0.5	1.0	0.5
P. aeruginosa	0.031	0.5	0.031	0.5	0.031	2.0	0.031	4.0	2.0	>4
S. oralis	0.015	0.125	0.031	1.0	0.031	0.125	0.015	0.125	1.0	4.0
C. albicans	0.5	0.5	0.015	0.5	0.015	0.25	0.031	0.125	1.0	4.0

the catalyst, evaporation of the solution, and the removal of side nitrotoluene under vacuum. In this case, the purity of compounds **(IIIb)**–**(VIb)** was ~95% according to the ^{1}H and ^{13}C NMR data.

The antibacterial activity in vitro was evaluated for acids **(IIIb)**–**(VIb)**, pNb-esters **(IIIa)**–**(VIa)**, and known drugs Meropenem and Cilapenem (Table 1). In the series of pNb-esters **(III)**–**(VIa)**, the most active against tested microorganisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus oralis*, and *Candida albicans*) were compounds **(IVa)** and **(Va)** that contained the furan and piperazine fragments, respectively, in the SR substituent (Table 1). Acids **(IIIb)**–**(VIb)** were in general inferior in their activity to the pNb-esters, although more active in comparison with Meropenem and Cilapenem.

Thus, the paper presents new carbapenems and their pNb-ethers **(IIIa,b)**–**(VIa,b)** that contain the SR substituents at C3, where R is the ester, sulfide, or amide groups. These compounds showed antibacterial activity against *E. coli*, *P. aeruginosa*, *S. oralis*, and *C. albicans*. The results of biotesting will be taken into account to choose promising compounds for further study.

EXPERIMENTAL (CHEMICAL)

The IR spectra (ν, cm^{-1}) were obtained on an IR Prestige-21 Shimadzu spectrophotometer for samples in a thin layer. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-300 (300.13 and 75.47 MHz) and Bruker AVANCE-500 (500.13 and 125.77 MHz) spectrometers, respectively. The chemical shift values (δ, ppm) are given using internal standards of $CDCl_3$ (δ = 7.270), acetone-d_6 (δ 2.09) (for compound **VIa**), or methanol-d_4 (δ = 3.34) (for carboxylic acids **(IIIb)**–**(VIb)**); spin-spin interaction constants (*J*) were measured in Hz. Mass spectra were recorded on a LCMS-2010EV mass spectrometer (Shimadzu) (syringe input; a solution of a sample in chloroform/acetonitrile at a flow rate of 0.1 mL/min; eluent, acetonitrile/water (95/5); the registration mode of the positive ions; the potential of needle-shaped ionizing electrode, 4.5 kV; the temperature of the interface capillary, 250°C; interface capillary voltage, 5 V). The data of the elemental analysis of the synthesized compounds were obtained using a CHNS EURO EA-2000 analyzer. The optical rotation angles were measured on a Perkin-Elmer-341 instrument. The reaction was monitored by TLC on Sorbfil plates (Russia); the compounds were visualized by wetting of the plates with a solution of anise aldehyde and sulfuric acid in ethanol, followed by heating at 120–150°C. The products were isolated by column chromatography on silica gel (30–60 g of adsorbent per 1 g of substance) using a petroleum ether/ethyl acetate mixture (from 3 : 1 to 1 : 1) or a chloroform/methanol mixture (10 : 1) as eluents. Freshly distilled solvents were used for chromatography.

We used 4-nitrobenzyl-(4*R*)-3-[(diphenylphosphoryl)oxy]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-en-2-carboxylate **(I)** (ABCR GMBH, Germany) to synthesize carbapenems. Thiols **(IX)** and **(X)** were synthesized by the interaction of 2-ethoxy-1,3-oxothiolan-5-on with *N*-methylpiperazine and methionine methyl ester. The full methods of their synthesis will be described in a separate publication.

General method for the synthesis of compounds (IIIa)–(VIa). The thiol solution (0.60 mmol) in acetonitrile (3–5 mL) was added dropwise to the stirred solution of phosphonate **(I)** (0.50 mmol) in anhydrous CH3CN (10 mL) at 0°C, followed by the addition of diisopropylethylamine (DIPEA) (0.60 mmol). The reaction mixture was stirred for 0.5–12 h at room temperature (TLC monitoring) and evaporated. The reaction products were isolated by column silica gel chromatography.

(4-Nitrobenzyl)-(4*R*,5*S*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-3-[(2-methoxy-2-oxoethyl)thio]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-en-2-carboxylate (IIIa) was obtained by the general method from phosphonate **(I)** (0.29 g, 0.50 mmol), methyl ester of 2-mercaptoacetic acid **(VII)** (0.06 mL, 0.60 mmol), and DIPEA (0.60 mmol) for 2 h (0 → 20°C). The product was purified by the column silica gel chromatography (eluent, petroleum ether–ethyl acetate, 1 : 1). Yield, 0.30 g (67%). White crystals; Tm, 160–161°C. $[\alpha]_D^{20}$ +60.1° (*c* 1.05, CH_2Cl_2). The IR spectrum: 3370, 1732, 1665, 1439, 1202, 1092. The ^{1}H NMR spectrum: 1.10 (d, 3H, *J* 7.3, C**H**$_3$), 1.13 (d, 3H, *J* 6.3, C**H**$_3$), 3.20 (dd, 1H, *J* 2.4, 6.6, **H**6), 3.38 (d, 1H, *J* 15.1, SC**H**$_2$), 3.38 (d, 1H, *J* 15.1, SC**H**$_2$), 3.53 (dq, 1H, *J* 8.8, 7.3, **H**4), 3.68 (s, 3H, OC**H**$_3$), 4.19 (dq, 1H, *J* 6.6, 6.3, C**H**–

OH), 4.21 (dd, 1H, *J* 2.4, 8.8, **H**5), 5.16 (d, 1H, *J* 14.2, OC**H**$_2$Ph), 5.43 (d, 1H, *J* 14.2, OC**H**$_2$Ph), 7.65 (d, 2H, *J* 8.6, **H**$_{Ar}$), 8.10 (d, 2H, *J* 8.6, **H**$_{Ar}$). The ^{13}C NMR spectrum: 16.57 (**C**H$_3$), 21.82 (**C**H$_3$), 33.07 (S**C**H$_2$), 43.19 (**C**4), 52.89 (O**C**H$_3$), 56.12 (**C**6), 59.60 (**C**5), 65.34 (O**C**H$_2$Ph), 65.87 (**C**H–OH), 123.71 (**C**H$_{Ar}$), 124.93 (**C**2), 128.14 (**C**H$_{Ar}$), 142.83 (**C**$^i_{Ar}$), 147.59 (**C**3), 149.87 (**C**$^i_{Ar}$), 160.23 (**C**ON), 169.28 (**C**O$_2$), 172.56 (**C**O$_2$). Mass spectrum, *m/z* (I_{rel}, %): 451 (100) [*M* + H]$^+$, 365 (17).

(4-Nitrobenzyl)-(4*R*,5*S*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-3-[(2-furylmethyl)thio]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (IVa) was synthesized by the general method from phosphonate (**I**) (0.29 g, 0.50 mmol), furyl-2-methylthiol (**VIII**) (0.06 mL, 0.60 mmol), and DIPEA (0.1 mL, 0.60 mmol) for 12 h (0→20°C). The product was purified by the column silica gel chromatography (eluent, petroleum ether–ethyl acetate, 3 : 1 → 1 : 1). Yield, 0.16 g (78%). White crystals; Tm, 134–135°C. $[\alpha]_D^{20}$ +40° (*c* 1.02, $CHCl_3$). The IR spectrum: 3360, 1759, 1670, 1274, 1092, 985. The ^{1}H NMR spectrum: 1.25 (d, 3H, *J* 7.3, C**H**$_3$), 1.47 (d, 3H, *J* 6.3, C**H**$_3$), 3.28 (dd, 1H, *J* 2.4 and 6.7, **H**6), 3.55 (1H, dq, *J* 9.2, 7.3, **H**4), 3.98 (d, 1H, *J* 14.9, SC**H**$_2$), 4.16 (d, 1H, *J* 14.9, SC**H**$_2$), 4.20 (dd, 1H, *J* 2.4, 9.2, **H**5), 4.27 (dq, 1H, *J* 6.7, *J* 6.3, C**H**–OH), 5.23 (d, 1H, *J* 13.8, C**H**$_2$Ph), 5.00 (d, 1H, *J* 13.8, C**H**$_2$Ph), 6.21 (d, 1H, *J* 3.2, **H**3$_{fur}$), 6.33 (dd, 1H, *J* 1.6, 3.2, **H**4 $_{fur}$), 7.35 (d, 1H, *J* 1.6, **H**5-$_{fur}$), 7.65 (d, 2H, *J* 8.6, **H**$_{Ar}$), 8.20 (d, 2H, *J* 8.6, **H**$_{Ar}$). The ^{13}C NMR spectrum: 16.17 (**C**H$_3$), 21.94 (**C**H$_3$), 28.59 (S**C**H$_2$), 43.28 (**C**4), 56.10 (**C**6), 59.61 (**C**5), 65.30 (O**C**H$_2$Ph), 66.02 (**C**H–OH), 108.36, 110.79 (**C**3$_{fur}$ and **C**4$_{fur}$), 123.75 (**C**H$_{Ar}$), 124.34 (**C**2), 128.17 (**C**H$_{Ar}$), 142.62 (**C**5$_{fur}$), 142.99 (**C**2$_{fur}$), 147.58 (**C**3), 150.04 (**C**$^i_{Ar}$), 151.40 (**C**$^i_{Ar}$), 160.30 (**C**ON), 172.0 (**C**O$_2$). Mass spectrum, *m/z* (I_{rel}, %): 459 (100) [M + H]$^+$, 373 (28). Found, %: C, 57.54; H, 4.91; N, 6.01; S, 7.06. $C_{20}H_{22}N_2O_8S$. Calc., %: C, 57.63; H, 4.84; N, 6.11; S, 6.99.

(4-Nitrobenzyl)-(4*R*,5*S*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-4-methyl-3-{[2-(4-methylpiperazin-1-yl)-2-oxoethyl]thio}-7-oxo-1-azabicyclo[3.2.0]hept-2-en-2-carboxylate (Va) was obtained by the general procedure from phosphonate (**I**) (0.29 g, 0.50 mmol), 2-(4-methylpiperazin-1-yl)-2-oxoethanthiol (**IX**) (0.11 u, 0.60 mmol), and DIPEA (0.1 mL, 0.57 mmol) for 3 h at 0°C. The product was purified by silica gel chromatography (chloroform–methanol, 10 : 1). Yield, 0.187 g (78%). Yellow viscous oil. $[\alpha]_D^{20}$ +39.0° (*c* 1.00, CH_2Cl_2). The IR spectrum, ν, cm^{-1}: 3358, 1767, 17015, 1635, 1521, 1459, 1345, 1210, 1139, 1048. The ^{1}H NMR spectrum: (aceton-d_6, 300 MHz): 1.22 (d, 3H, *J* 7.3, C**H**$_3$), 1.30 (d, 3H, *J* 6.2, C**H**$_3$), 2.20 (s, 3H, C**H**$_3$), 2.28–2.40 (m, 4H, 2NC**H**$_2$), 2.80 (br.s, 1H, O**H**), 3.30 (dd, 1H, *J* 2.5, 6.5, **H**6), 3.40–3.60 (m, 4H, 2NC**H**$_2$), 3.78 (dq, 1H, *J* 9.3, 7.3, **H**4), 3.83 (d, 1H, *J* 14.6, SC**H**$_2$) and 3.95 (d, 1H, *J* 14.6, SC**H**$_2$), 4.15 (dq, 1H, *J* 6.5, 6.2, C**H**–OH), 4.25 (dd, 1H, *J* 2.5, 9.3, **H**5), 5.30 (d, 1H, *J* 14.1, C**H**$_2$Ph) and 5.55 (d, 1H, *J* 14.1, C**H**$_2$Ph), 7.80 (d, 2H, *J* 8.5, **H**$_{Ar}$) and 8.25 (d, 2H, *J* 8.6, **H**$_{Ar}$). The ^{13}C NMR spectrum: 16.28 (**C**H$_3$), 21.82 (**C**H$_3$), 32.87 (S**C**H$_2$), 41.74, 46.12, 54.35, 54.66 (N**C**H$_2$), 43.22 (**C**4), 45.69 (N**C**H$_3$), 56.06 (**C**6), 59.72 (**C**5), 65.35 (**C**H$_2$Ph), 65.69 (**C**H–OH), 123.77 (**C**H$_{Ar}$), 125.17 (**C**2), 128.17 (**C**H$_{Ar}$), 142.89 (**C**$^i_{Ar}$), 147.59 (**C**3), 150.13 (**C**$^i_{Ar}$), 160.28 (**C**ON), 166.43 (**C**ON), 172.94 (**C**O$_2$). Mass spectrum, *m/z* (I_{rel}, %): 519 (100) [M + H]$^+$, 269 (35). Found, %: C, 55.41; H, 5.69; N, 10.93; S, 6.27. $C_{20}H_{22}N_2O_8S$. Calc., %: C, 55.59; H, 5.83; N, 10.80; S, 6.18.

(4-Nitrobenzyl)-(4*R*,5*S*,6*S*)-6-((1*R*)-1-hydroxyethyl)-3-[(2-((*S*)-1-methoxycarbonyl-3-methylthio-1-propyl)amino)-2-oxoethyl]thio-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-en-2-the carboxylate (VIa) was obtained by the general procedure from phosphonate (**I**) (0.29 g, 0.50 mmol), methyl-*N*-(mercaptoethyl)methioninate (**X**) (0.14 u, 0.60 mmol), and DIPEA (0.1 mL, 0.50 mmol) for 0.5 h at 0°C. The product was purified by silica gel chromatography (chloroform–methanol, 10 : 1). Yield, 0.25 g (87%). Light yellow oily substance. $[\alpha]_D^{20}$ +87.5° (c 1.00, CH_2Cl_2). The IR spectrum: 3353, 3324, 1757, 1739, 1676, 1518, 1437, 1347, 1203, 1134. The ^{1}H NMR spectrum: 1.23 (d, 3H, *J* 7.3, C**H**$_3$,), 1.35 (d, 3H, *J* 6.3, C**H**$_3$), 1.70 (br.s, 1H, O**H**), 1.95–2.40 (m, 1H, C**H**$_{2\ Met}$), 2.08 (s, 3H, SC**H**$_3$), 2.10–2.18 (m, 1H, C**H**$_{2\ Met}$), 2.45–2.55 (m, 2H, C**H**$_{2\ Met}$), 3.28 (dd, 1H, *J* 2.7, 6.5, **H**6), 3.42 (d, 1H, *J* 16.9, SC**H**$_2$), 3.44 (dq, 1H, *J* 9.2, 7.3, **H**4), 3.68 (d, 1H, *J* 16.9, SC**H**$_2$), 3.72 (s, 3H, OC**H**$_3$), 4.25 (dq, 1H, *J* 6.5, 6.3, C**H**–OH), 4.32 (dd, 1H, *J* 2.7, 9.3, **H**5), 4.68 (ddd, 1H, *J* 4.8, 8.0, 12.5, C**H**–NH), 5.23 (d, 1H, *J* 13.8, C**H**$_2$Ph) and 5.52 (d, 1H, *J* 13.8, C**H**$_2$Ph), 7.20 (d, 1H, *J* 8.0, N**H**), 7.67 (dd, 2H, *J* 8.8, **H**$_{Ar}$), 8.22 (d, 2H, *J* 8.8, **H**$_{Ar}$). The ^{13}C NMR spectrum: 15.39 (**C**H$_3$), 16.60 (**C**H$_3$), 21.73 (**C**H$_3$), 29.96 (**C**H$_2$), 30.50 (**C**H$_2$), 34.76 (**C**H$_2$), 42.76 (**C**4), 51.96 (O**C**H$_3$), 52.66 (**C**HNH), 56.05 (**C**6), 59.70 (**C**5), 65.42 (**C**H$_2$Ph), 65.71 (**C**H–OH), 123.75 (**C**H$_{Ar}$), 125.86 (**C**2), 128.11 (**C**H$_{Ar}$), 142.76 (**C**$^i_{Ar}$), 147.58 (**C**3), 148.70 (**C**$^i_{Ar}$), 160.40 (**C**ON), 167.48 (**C**ON), 171.65 (**C**O$_2$), 172.69 (**C**O$_2$). Mass spectrum, *m/z* (I_{rel}, %): 582 (100) [*M* + H]$^+$, 496 (80) [*M*H–CO$_2$Me]$^+$, 238 (50). Found, %: C, 51.52; H, 5.49; N, 7.38; S, 10.94. $C_{25}H_{31}N_3O_9S_2$. Calc., %: C, 51.73; H, 5.37; N, 7.22; S, 11.03.

General method for hydrogenation of compounds (IIIa)–(VIa). 10% Pd/C (30 mg) was added to the stirred solution of one of compounds (**IIIa)–(VIa**) (0.1 g), and the reaction mixture was stirred for 2 h in the atmosphere of hydrogen (control by TLC). The catalyst was removed by filtration, the filtrate was evaporated and vacuumed.

(4*R*,5*S*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-3-[(2-methoxy-2-oxoethyl)tio]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-en-2-carboxylic acid (IIIb) was obtained by the general procedure from compound (**IIIa**) (0.1 g, 0.22 mmol) and 10% Pd/C (30 mg). Yield, 56 mg (82%). The IR spectrum: 3430, 1728, 1713, 1609, 1520, 1455, 1436, 1408, 1347, 1300, 1278, 1136. The ^{1}H NMR spectrum: 1.20 (d, 3H, *J* 7.3, C**H**$_3$), 1.28 (d, 3H, *J* 6.2, C**H**$_3$), 3.25 (dd, 1H, *J* 2.3, 7.1, **H**6), 3.55 (d, 1H, *J* 15.3, SC**H**$_2$), 3.73 (s, 3H, OC**H**$_3$), 3.76 (d, 1H, *J* 15.3, SC**H**$_2$), 4.08–4.13 (m, 2H, **H**4, C**H**–OH), 4.16 (dd, 1H, *J* 2.3, 9.1, **H**5). The ^{13}C NMR spectrum: 15.59 (**C**H$_3$), 20.45 (**C**H$_3$), 32.51 (S**C**H$_2$), 42.97 (**C**4), 51.75 (O**C**H$_3$), 56.33 (**C**6), 59.30 (**C**5), 65.53 (**C**H–OH), 126.50 (**C**2), 151.50 (**C**3), 163.00 (**C**ON), 170.15 (**C**O$_2$), 173.66 (**C**O$_2$). Mass spectrum, *m/z* (I_{rel}, %): 324 (70) $[M - H]^-$, 299 (31), 270 (75), 238 (50), 226 (18). Found, %: C, 49.40; H, 5.58; N, 4.60; S, 10.05. $C_{13}H_{17}NO_6S$. Calc., %: C, 49.51; H, 5.43; N, 4.44; S, 10.17.

(4*R*,5*S*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-4-methyl-7-oxo-3-[(2-furylmethyl)thio]-1-azabicyclo[3.2.0]hept-2-en-2-carboxylic acid (IVb) was obtained by the general procedure from compound (**IVa**) (0.09 g, 0.19 mmol) and 10% Pd/C (30 mg). Yield, 63 mg (71%). The ^{1}H NMR spectrum: 1.20 (d, 3H, *J* 7.3, C**H**$_3$), 1.29 (d, 3H, *J* 6.3, C**H**$_3$), 3.23 (dd, 1H, *J* 7.2, 2.4, **H**6), 3.58 (dq, 1H, *J* 9.1, 7.3, **H**4), 4.03 (d, 1H, *J* 14.7, SC**H**$_2$), 4.09 (dq, 1H, *J* 7.2, 6.3, C**H**–OH), 4.12 (dd, 1H, *J* 9.1, *J* 2.4, **H**5), 4.22 (d, 1H, *J* 14.7, SC**H**$_2$), 6.29 (d, 1H, *J* 3.2, **H**3$_{fur}$), 6.34 (dd, 1H, *J* 1.9, 3.2, **H**4$_{fur}$), 7.43 (d, 1H, *J* 1.9, **H**5$_{fur}$). The ^{13}C NMR spectrum: 15.68 (**C**H$_3$), 20.46 (**C**H$_3$), 27.82 (S**C**H$_2$), 43.03 (**C**4), 56.27 (**C**6), 59.33 (**C**5), 65.57 (**C**H–OH), 107.63, 110.28 (**C**3$_{fur}$ and **C**4$_{fur}$), 142.32 (**C**5$_{fur}$, 150.93 (**C**2$_{fur}$), 125.87 (**C**2), 149.34 (**C**3), 162.92 (**C**ON), 173.70 (**C**O$_2$). Mass spectrum, *m/z* ($I_{rel.}$, %): 324 (60) $[M + H]^+$, 315 (76), 279 (50), 238 (56). Found, %: C, 55.60; H, 5.44; N, 4.49; S, 9.84. $C_{15}H_{17}NO_5S$. Calc., %: C, 55.71; H, 5.30; N, 4.33; S, 9.92.

(4*R*,5*S*,6*S*)-6-[(1*R*)-1-Hydroxyethyl-4-methyl-3-{[2-(4-methylpiperazin-1-yl)-2-oxoethyl]thio}-7-oxo-1-azabicyclo[3.2.0]hept-2-en]-2-carboxylic acid (Vb) was obtained by the general procedure from compound (**Va**) (0.088 g, 0.17 mmol) and 10% Pd/C (30 mg). Yield, 50 mg (75%). A number of the piperidine ring and C4-H signals are not given in the ^{1}H NMR spectrum of zwitterionic compound (**Vb**) because of a poor resolution. The ^{1}H NMR spectrum: 1.18 (d, 3H, *J* 7.3, C**H**$_3$), 1.25 (d, 3H, *J* 6.3, C**H**$_3$), 2.79 (s, 3H, C**H**$_3$), 3.20 (dd, 1H, *J* 2.6, 6.3, **H**6), 3.22 (br.s, 2H, NC**H**$_2$), 3.60 (m, 1H, **H**4), 3.61 (d, 1H, *J* 14.2, SC**H**$_2$), 3.75 (d, 1H, *J* 14.2, SC**H**$_2$), 4.10 (q, 1H, *J* 6.3, C**H**–OH), 4.15 (dd, 1H, *J* 2.6, 9.6, **H**5). The ^{13}C NMR spectrum: 15.22 (**C**H$_3$), 20.34 (**C**H$_3$), 32.36 (S**C**H$_2$), 39.24, 45.57, 52.87, 52.97 (N**C**H$_2$), 41.64 (N**C**H$_3$), 42.65 (**C**4), 55.77 (**C**6), 59.73 (**C**5), 64.96 (**C**H–OH), 125.18 (**C**2), 147.07 (**C**3), 166.18 (**C**ON), 168.02 (**C**ON), 174.78 (**C**O$_2$). Mass spectrum, *m/z* ($I_{rel.}$, %): 384 (100) $[M + H]^+$, 340 (33), 289 (26), 269 (66), 216 (26), 143 (28). Found, %: C, 53.39; H, 6.41; N, 11.08; S, 8.22. $C_{17}H_{25}N_3O_5S$. Calc., %: C, 53.25; H, 6.57; N, 10.96; S, 8.36.

(4*R*,5*S*,6*S*)-6-[((1*R*)-1-Hydroxyethyl)-3-[(2-((*S*)-1-methoxycarbonyl-3-thiomethyl-1-propyl)amino)-2-oxoethyl)thio]-4-methyl-7-oxo-1-azabicyclo-[3.2.0]hept-2-en]-2-carboxylic acid (VIb) was obtained by the general procedure from compound (**VIa**) (0.102 g, 0.18 mmol) and 10% Pd/C (30 mg). Yield, 68 mg (85%). The ^{1}H NMR spectrum: 1.20 (d, 3H, *J* 7.3, C**H**$_3$), 1.28 (d, 3H, *J* 6.2, C**H**$_3$), 1.93–2.30 (m, 1H, C**H**$_{2Met}$), 2.08 (s, 3H, SC**H**$_3$), 2.08–2.16 (m, 1H, C**H**$_{2\ Met}$), 2.47–2.62 (m, 2H, C**H**$_{2\ Met}$), 3.25 (dd, 1H, *J* 2.4, 7.1, **H**6), 3.53 (d, 1H, *J* 15.1, SC**H**$_2$), 3.56 (dq, 1H, *J* 9.2, 7.3, **H**4), 3.67 (d, 1H, *J* 15.1, SC**H**$_2$), 3.71 (s, 3H, OC**H**$_3$), 4.09 (dq, 1H, *J* 7.1, 6.2, C**H**-OH), 4.18 (dd, 1H, *J* 2.4, 9.2, **H**5), 4.53–4.6 (m, 1H, C**H**N). The ^{13}C NMR spectrum: 13.83 (**C**H$_3$), 15.64 (**C**H$_3$), 20.47 (**C**H$_3$), 29.70 (S**C**H$_2$), 30.22 (**C**H$_2$), 34.12 (**C**H$_2$), 42.93 (**C**4), 48.46 (**C**HN), 51.66 (O**C**H$_3$), 56.29 (**C**6), 59.31 (**C**5), 65.48 (**C**H–OH), 126.54 (**C**2), 148.18 (**C**3), 164.04 (**C**ON), 169.85 (**C**ON), 172.03 (**C**O$_2$), 173.77 (**C**O$_2$). Mass spectrum, *m/z* ($I_{rel.}$, %): 447 (18) $[M + H]^+$, 435 (70), 289 (26), 421 (75), 403 (25), 206 (50). Found, %: C, 48.58; H, 5.96; N, 6.14; S, 14.47. $C_{18}H_{26}N_2O_7S_2$. Calc., %: C, 48.42; H, 5.87; N, 6.27; S, 14.36.

EXPERIMENTAL (BIOLOGY)

The antibacterial activity of the synthesized carbapenems was studied using test microorganisms, i.e., the museum strains of *Escherichia coli* no. 25922, *Pseudomonas aeruginosa* (strain SS14 KC 866140), *Candida albicans* no. 24433 (all of them were from the collection of the Clinic of Bashkir State Medical University), and *Streptococcus oralis* no. 27417 (from the collection of the Department of Fundamental and Applied Microbiology of Bashkir State Medical University).

The working solutions were prepared from the solutions of the tested compounds in dimethyl sulfoxide (DMSO) by addition the Mueller–Hinton broth (HiMedia, India). Known antibiotics Meropenem (Meropeni) (Deepak international, India) and LifetimeCilapenem (Imipenem + Cilastatin (Imipenemi + Cilastatini)) (Belmedpreparaty, Republic of Belarus) were used as the reference preparations.

Minimum inhibitory concentrations (MPC) of chemical compounds **(IIIa)–(IIIa)** and **(IIIb)–(VIIIb)** were evaluated by the reference microdilution method in the broth. A certain amount of the tested compound in sterile vials was dissolved in 1 mL of DMSO, and the percentage concentration (C, %) was calculated. Then, the working solutions were prepared by two successive dilutions of the basic solutions (above and below the concentration of 1 μg/mL) in Muller–Hin-

ton broth. The diluted solutions were used on the day of preparation.

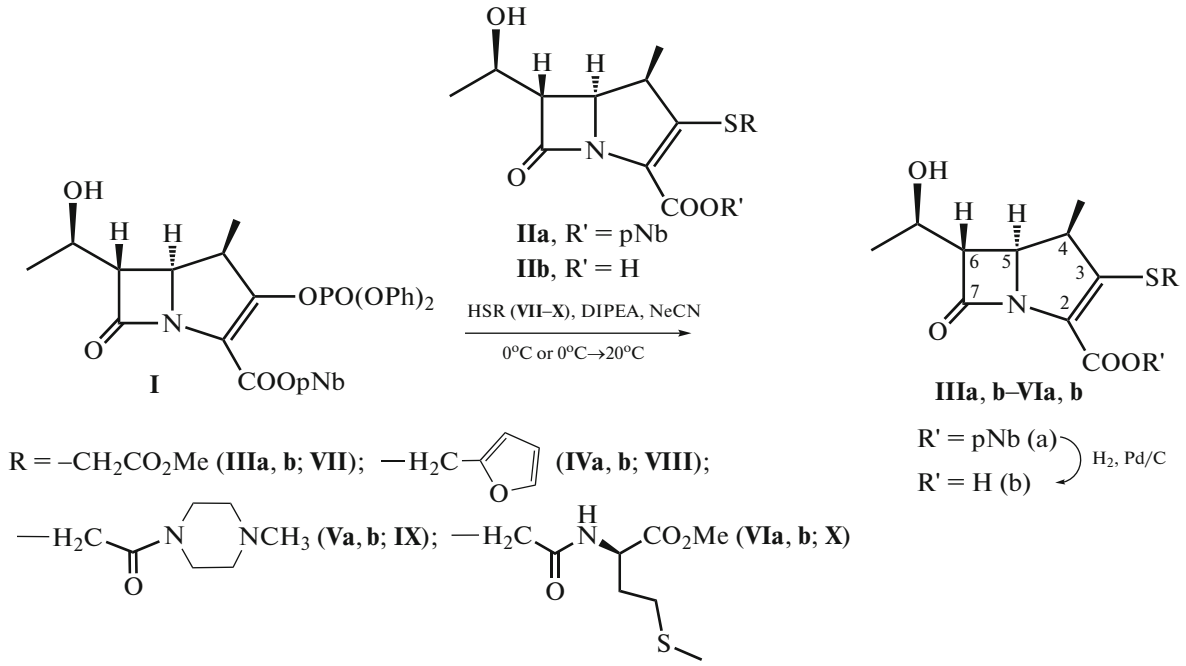
To prepare the inoculum, four–five morphologically homogeneous colonies were grown on a pure nonselective solid nutrient medium and suspended in physiological solution, followed by the incubation at 37°C for 18–24 h. The suspension was brought to turbidity, which was equivalent to 0.5 McFarland standard (1.5×10^8 CFU/mL). Further, the prepared inoculum was diluted by the Muller–Hinton broth (1 : 100 dilution) to obtain the required density of the microbial culture 5×10^6 CFU/mL. The plates were inoculated for no more than 30 min after the inoculum preparation to preserve the required number of viable cells.

To evaluate the MIC values, the working solutions of each tested chemical compound (50 μL each) were placed into separate wells in the plates, followed by the addition of the bacterial suspension (5×10^6 CFU/mL, 50 μL).

Positive and negative control samples (PCS and NCS, respectively) were necessarily used to control the growth of all tested strains of microorganisms. PCS was placed in a well that contained 50 μL of the broth and inoculum of the corresponding microorganism without a chemical compound. Similarly, NCS was placed in a well that contained 50 μL of the broth without both the inoculum and tested compound.

The plates were sealed with a transparent film before incubation and sealed in plastic bags to prevent drying. The plates were incubated in a thermostat for 16–20 h at 37°C. For more uniform heating, the plates were stacked in stacks of no more than five pieces.

Microdilutions for the evaluation of the MIC values for the tested compounds were from 0.5 to 0.015625 μg/mL.



Scheme 1. Synthesis of new C3-modified carbapenems.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies using animals or humans as objects of research.

Conflict of Interest

The authors state that there is no conflict of interests.

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