HYDROPHOSPHORYL DERIVATIVES OF TETRAMYCIN B: DESIGN, SYNTHESIS, BIOLOGICAL ACTIVITY AND DEVELOPMENT OF INTELLECTUAL COMPUTER SYSTEM

Valery V. Belakhov1\*, Alexander V. Garabadzhiu2a, Tamara B. Chistyakova2b

 1Schulich Faculty of Chemistry, Technion – Israel Institute of Technology, Haifa, Israel;

2aLaboratory of Molecular Pharmacology and 2bDepartment of Computer-Aided Design and Control Systems, Saint-Petersburg State Technological Institute (Technical University),

Saint-Petersburg, Russian Federation

Email: chvalery@technion.ac.il

**Supplemental Materials**

**EXPERIMENTAL CHEMICAL PART**

 Tetramycin (complex preparation) from Liaoning Wkioc Bioengineering Co., Ltd. (China), prepared *via* the microbiological synthesis by *Streptomyces noursei*, was used. The separation of tetramycin B was carried out by method described in [1, 2]. The chemicals and organic solvents from Sigma-Aldrich (USA) or Fluka (Switzerland) were used as received.

 NMR spectra (1Н, 13С NMR, COSY, DEPT, and HMQC) were recorded on a Bruker Аvance III (Germany) instrument. МеОD-*d*4 was used as solvent, and TMS was used as the internal reference. 31Р NMR spectra were recorded on a Bruker АС-200 (Germany) instrument relative to 85% Н3РО4 as the external reference. TLC analysis on Silica Gel 60 F254 (0.25 mm, Merck, Germany) plates in n-butanol-acetic acid-water 5:2:1 was used to monitor the reaction progress and to determine the purity of the derivatives **I-VII**. Melting (decomposition) points were determined using Electrothermal IA9300 (UK) instrument.

 ***N*-(**α**-Hydrophosphoryl-*п*-benzyl)tetramycin B (I-VII)** (general method). To 0.01 mole oftetramycin B in 25 ml in dry dimethylformamide with stirring 0.02 mole of the appropriate benzaldehyde was added in the presence of a catalytic amount of triethylamine. The reaction was carried out at 40°C for 3 h, then to the reaction mixture 0.015 mole of hypophosphorous acid was added and the reaction was continued at 40°C for 3-4 h. Monitoring of the reaction progress was carried out by TLC. At the end of the interaction the solution cooled to 20°C was filtered off, excessive amounts (300-400 ml) of diethyl ether were poured to the filtrate. To the precipitated oily liquid methanol (25-30 ml) was added, the resulting suspension was filtered off and the filtrate was passed through a column packed with Silica gel. The mixture of solvents CHCl3:MeOH:H2O:NH4OH, (10 : 15: 0.4 : 0.05) was used as an eluent. Eluates containing the target product were combined and subjected to 3-4 fold concentrating in a vacuum. To the concentrate 5-fold volume of diethyl ether was added, the precipitate was filtered off, washed with ether, and dried under a vacuum at 30°C for 4-5 h. The compounds **I-VII** were yielded in the form of small white or cream crystals.

 ***N*-[(**α**-Hydrophosphoryl-4-oxybenzyl)]tetramycin B (I).** Yield 60 %, mp 193–198°С(decomp.), R*f* 0.45. 1H NMR (МеОН-*d*4), δ, ppm: 0.91 (t, 3H, J29,27eq 7.0 Hz, J28,27ax 6.8 Hz, 28-CH3), 1.13 (d, 3H, 26-CH3, J26,25 6.8 Hz, 26-CH3), 1.25 (d, 1H, J10eq,11 4.8 Hz, H-10eq), 1.28 (s, 3H, H-6’-CH3), 1.37 (d, 1H, J27eq,28 7.0 Hz, H-27eq,), 1.40 (d, 1H, J6eq, 7 2.0 Hz, H-6eq,), 1.43 (dd, 1H, J27ax, 28 7.0 Hz, J27ax, 27eq 13.5 Hz, H-27ax), 1.57 (s, 1H, H-8eq), 1.65 (d, 1H, J14eq, 15 2.5 Hz, H-14Heq), 1.78 (d, 1H, J8ax, 8eq 14.2 Hz, H-8ax), 1.94 (dd, 1H, J6ax, 6eq 14.2 Hz, J6ax,7 1.0 Hz, H-6ax), 1.96 (dd, 1H, J14ax,14eq 15.0, J14ax,15 3.8 Hz, H-14ax), 2.07 (dd, 1H, J10ax,10eq 12.7 Hz, J10ax,11 4.8 Hz, H-10-ax), 2.21 (t, 1H, J12,13 10.5 Hz, H-12), 2.38 (ddd, J24,25 4.5 Hz, J24,27ax 4.5 Hz, J24,27eq 9.7 Hz, H- 24), 3.22 (d, 1H, J5’,6’Me 6.2Hz, H-5’), 3.31 (d, 1H, J4’,5’ 9.8 Hz, H-4’), 3.75 (d, 1H, J2’,3’ 3.4 Hz, H-2’), 3.78 (d, 1H, J2’,3’ 3.2 Hz, H-2’), 3.83 (dd, 1H, J5,6ax 1.8 Hz, J5,6eq 10.7 Hz, H-5), 3.82 (d, 1H, J3’,4’ 9.6 Hz, H-3’), 3.94 (d, 1H, J4,5 2.5 Hz, H-4), 4.05 (m, 1H, CH-P, H-1”), 4.23 (d, 1H, J11,12 10.5 Hz, H-11), 4.38 (d, 1H, J15,16 8.7 Hz, H-15), 4.43 (dd, 1H, J7,8ax 11.0 Hz, J7,8eq 2.0 Hz, H-7), 4.46 (d, 1H, J1’,2’ 1.2 Hz, H-1’), 4.49 (dd, 1H, J13,14ax 1.4 Hz, J13,14eq 8.5 Hz, H-13), 5.03 (d, 1H, J25,26 6.5 Hz, H-25), 5.47 (d, 1H, J23,24 9.2 Hz, H-23), 6.05 (d, 1H, J16,17 15.4 Hz, H-16), 6.07 (d, 1H, J22,23 15.0 Hz, H-22), 6.09 (d, 1H, J2,3 15.4 Hz, H-2), 6.11 (d, 1H, J17,18 10.4 Hz, H-17), 6.22 (d, 1H, J20,21 15.0 Hz, H-20), 6.25 (d, 1H, J18,19 14.4 Hz, H-18), 6.36 (d, 1H, J19,20 10.8 Hz, H-19), 6.43 (d, 1H, J21,22 10.3 Hz, H-21), 6.75 (d, 1H, J3,4 9.2 Hz, H-3), 6.95 (d, 1H, JPH 550 Hz, P-H), 6.99 (d, 2H, J 8.2 Hz, phenyl), 7.11 (d, 2H, J 8.2 Hz, phenyl), 9.91(s, 1H, Ph-*OH*). 13C NMR spectrum, δ, ppm: 12.31 (C-28), 13.72 (C-26), 17.94 (C-6’), 23.95 (C-27), 27.58 (d, *C*''H-P, *J*CP 133.5 Hz), 37.76 (C-10), 43.98 (C-6), 44.23 (C-14), 46.87 (C-8), 48.03 (C-24), 55.28 (C-3’), 57.29 (C-12), 65.13 (C-7), 65.76 (C-11), 66.57 (C-13), 69.68 (C-2’), 70.85 (C-4’), 71.90 (C-25), 73.41 (C-4), 73.67 (C-5’), 73.89 (C-5), 76.15 (C-15), 97.23 (C-1’), 97.62 (C-9), 115.77 (phenyl), 128.33 (phenyl), 131.26 (phenyl), 141.59 (phenyl), 122.09 (C-2), 129.26 (C-16), 131.24 (C-23), 131.57 (C-18), 131.99 (C-20), 132.74 (C-21), 133.11 (C-19), 135.05 (C-22), 136.17 (C-17),148.43 (C-3), 165.18 (C-1), 173.52 (C-29). 31P NMR spectrum, δ, ppm: 15.57.

***N*-[(**α**-Hydrophosphoryl-4-methoxybenzyl)]tetramycin B (II).** Yield 62 %, mp 188–193°С(decomp.), R*f* 0.47. 1H NMR (МеОН-*d*4), δ, ppm: 0.93 (t, 3H, J29,27eq 7.0 Hz, J28,27ax 6.8 Hz, 28-CH3), 1.14 (d, 3H, 26-CH3, J26,25 6.8 Hz, 26-CH3), 1.27 (d, 1H, J10eq,11 4.8 Hz, H-10eq), 1.30 (s, 3H, H-6’-CH3), 1.36 (d, 1H, J27eq,28 7.0 Hz, H-27eq,), 1.40 (d, 1H, J6eq, 7 2.0 Hz, H-6eq,), 1.46 (dd, 1H, J27ax, 28 7.0 Hz, J27ax, 27eq 13.5 Hz, H-27ax), 1.55 (s, 1H, H-8eq), 1.67 (d, 1H, J14eq, 15 2.5 Hz, H-14Heq), 1.80 (d, 1H, J8ax, 8eq 14.2 Hz, H-8ax), 1.92 (dd, 1H, J6ax, 6eq 14.2 Hz, J6ax,7 1.0 Hz, H-6ax), 1.97 (dd, 1H, J14ax,14eq 15.0, J14ax,15 3.8 Hz, H-14ax), 2.08 (dd, 1H, J10ax,10eq 12.7 Hz, J10ax,11 4.8 Hz, H-10-ax), 2.24 (t, 1H, J12,13 10.5 Hz, H-12), 2.39 (ddd, J24,25 4.5 Hz, J24,27ax 4.5 Hz, J24,27eq 9.7 Hz, H- 24), 3.20 (d, 1H, J5’,6’Me 6.2Hz, H-5’), 3.34 (d, 1H, J4’,5’ 9.8 Hz, H-4’), 3.69 (s, 3H, Ph-O-CH3), 3.73 (d, 1H, J2’,3’ 3.4 Hz, H-2’), 3.79 (d, 1H, J2’,3’ 3.2 Hz, H-2’), 3.83 (dd, 1H, J5,6ax 1.8 Hz, J5,6eq 10.7 Hz, H-5), 3.89 (d, 1H, J3’,4’ 9.6 Hz, H-3’), 4.01 (d, 1H, J4,5 2.5 Hz, H-4), 4.12 (m, 1H, CH-P, H-1”), 4.25 (d, 1H, J11,12 10.5 Hz, H-11), 4.35 (d, 1H, J15,16 8.7 Hz, H-15), 4.41 (dd, 1H, J7,8ax 11.0 Hz, J7,8eq 2.0 Hz, H-7), 4.45 (d, 1H, J1’,2’ 1.2 Hz, H-1’), 4.53 (dd, 1H, J13,14ax 1.4 Hz, J13,14eq 8.5 Hz, H-13), 5.07 (d, 1H, J25,26 6.5 Hz, H-25), 5.44 (d, 1H, J23,24 9.2 Hz, H-23), 6.00 (d, 1H, J16,17 15.4 Hz, H-16), 6.05 (d, 1H, J22,23 15.0 Hz, H-22), 6.09 (d, 1H, J2,3 15.4 Hz, H-2), 6.13 (d, 1H, J17,18 10.4 Hz, H-17), 6.20 (d, 1H, J20,21 15.0 Hz, H-20), 6.28 (d, 1H, J18,19 14.4 Hz, H-18), 6.37 (d, 1H, J19,20 10.8 Hz, H-19), 6.45 (d, 1H, J21,22 10.3 Hz, H-21), 6.77 (d, 1H, J3,4 9.2 Hz, H-3), 6.69 (d, 2H, J 8.2 Hz, phenyl), 7.05 (d, 1H, JPH 552 Hz, P-H), 7.21 (d, 2H, J 8.2 Hz, phenyl), 9.94 (s, 1H, Ph-*OH*). 13C NMR spectrum, δ, ppm: 12.34 (C-28), 13.76 (C-26), 17.98 (C-6’), 23.91 (C-27), 27.44 (d, *C*''H-P, *J*CP 132.6 Hz), 37.79 (C-10), 44.02 (C-6), 44.23 (C-14), 46.89 (C-8), 48.09 (C-24), 55.30 (C-3’), 55.92 (Ph-O-*C*H3), 57.32 (C-12), 65.15 (C-7), 65.76 (C-11), 66.60 (C-13), 69.64 (C-2’), 70.89 (C-4’), 71.93 (C-25), 73.44 (C-4), 73.70 (C-5’), 73.93 (C-5), 76.18 (C-15), 97.22 (C-1’), 97.60 (C-9), 115.75 (phenyl), 128.30 (phenyl), 131.31 (phenyl), 141.53 (phenyl), 122.02 (C-2), 129.31 (C-16), 131.28 (C-23), 131.60 (C-18), 132.05 (C-20), 132.79 (C-21), 133.15 (C-19), 135.10 (C-22), 136.19 (C-17), 148.47 (C-3), 165.20 (C-1), 173.56 (C-29). 31P NMR spectrum, δ, ppm: 15.65.

***N*-[(**α**-Hydrophosphoryl-4-nitrobenzyl)]tetramycin B (III).** Yield 65 %, mp 182–187°С(decomp.), R*f* 0.49. 1H NMR (МеОН-*d*4), δ, ppm: 0.92 (t, 3H, J29,27eq 7.0 Hz, J28,27ax 6.8 Hz, 28-CH3), 1.12 (d, 3H, 26-CH3, J26,25 6.8 Hz, 26-CH3), 1.26 (d, 1H, J10eq,11 4.8 Hz, H-10eq), 1.31 (s, 3H, H-6’-CH3), 1.35 (d, 1H, J27eq,28 7.0 Hz, H-27eq,), 1.41 (d, 1H, J6eq, 7 2.0 Hz, H-6eq,), 1.45 (dd, 1H, J27ax, 28 7.0 Hz, J27ax, 27eq 13.5 Hz, H-27ax), 1.53 (s, 1H, H-8eq), 1.69 (d, 1H, J14eq, 15 2.5 Hz, H-14Heq), 1.78 (d, 1H, J8ax, 8eq 14.2 Hz, H-8ax), 1.90 (dd, 1H, J6ax, 6eq 14.2 Hz, J6ax,7 1.0 Hz, H-6ax), 1.99 (dd, 1H, J14ax,14eq 15.0, J14ax,15 3.8 Hz, H-14ax), 2.06 (dd, 1H, J10ax,10eq 12.7 Hz, J10ax,11 4.8 Hz, H-10-ax), 2.21 (t, 1H, J12,13 10.5 Hz, H-12), 2.35 (ddd, J24,25 4.5 Hz, J24,27ax 4.5 Hz, J24,27eq 9.7 Hz, H- 24), 3.18 (d, 1H, J5’,6’Me 6.2Hz, H-5’), 3.37 (d, 1H, J4’,5’ 9.8 Hz, H-4’), 3.70 (d, 1H, J2’,3’ 3.4 Hz, H-2’), 3.79 (d, 1H, J2’,3’ 3.2 Hz, H-2’), 3.81 (dd, 1H, J5,6ax 1.8 Hz, J5,6eq 10.7 Hz, H-5), 3.87 (d, 1H, J3’,4’ 9.6 Hz, H-3’), 4.00 (d, 1H, J4,5 2.5 Hz, H-4), 4.14 (m, 1H, CH-P, H-1”), 4.23 (d, 1H, J11,12 10.5 Hz, H-11), 4.36 (d, 1H, J15,16 8.7 Hz, H-15), 4.40 (dd, 1H, J7,8ax 11.0 Hz, J7,8eq 2.0 Hz, H-7), 4.47 (d, 1H, J1’,2’ 1.2 Hz, H-1’), 4.55 (dd, 1H, J13,14ax 1.4 Hz, J13,14eq 8.5 Hz, H-13), 5.10 (d, 1H, J25,26 6.5 Hz, H-25), 5.42 (d, 1H, J23,24 9.2 Hz, H-23), 5.95 (d, 1H, J16,17 15.4 Hz, H-16), 6.03 (d, 1H, J22,23 15.0 Hz, H-22), 6.09 (d, 1H, J2,3 15.4 Hz, H-2), 6.15 (d, 1H, J17,18 10.4 Hz, H-17), 6.21 (d, 1H, J20,21 15.0 Hz, H-20), 6.29 (d, 1H, J18,19 14.4 Hz, H-18), 6.34 (d, 1H, J19,20 10.8 Hz, H-19), 6.47 (d, 1H, J21,22 10.3 Hz, H-21), 6.79 (d, 1H, J3,4 9.2 Hz, H-3), 6.64 (d, 2H, J 8.2 Hz, phenyl), 7.00 (d, 1H, JPH 554 Hz, P-H), 7.23 (d, 2H, J 8.2 Hz, phenyl), 9.96 (s, 1H, Ph-*OH*). 13C NMR spectrum, δ, ppm: 12.37 (C-28), 13.76 (C-26), 18.04 (C-6’), 23.87 (C-27), 28.17 (d, *C*''H-P, *J*CP 133.8 Hz), 37.81 (C-10), 44.02 (C-6), 44.26 (C-14), 46.85 (C-8), 48.11 (C-24), 55.34 (C-3’), 57.36 (C-12), 65.12 (C-7), 65.74 (C-11), 66.57 (C-13), 69.69 (C-2’), 70.91 (C-4’), 71.98 (C-25), 73.40 (C-4), 73.72 (C-5’), 73.98 (C-5), 76.21 (C-15), 97.20 (C-1’), 97.57 (C-9), 115.80 (phenyl), 128.33 (phenyl), 131.34 (phenyl), 141.58 (phenyl), 122.06 (C-2), 129.28 (C-16), 131.32 (C-23), 131.64 (C-18), 132.09 (C-20), 132.76 (C-21), 133.17 (C-19), 135.13 (C-22), 136.22 (C-17), 148.49 (C-3), 165.24 (C-1), 173.60 (C-29). 31P NMR spectrum, δ, ppm: 15.73.

***N*-[(**α**-Hydrophosphoryl-4-*N*-dimethylaminobenzyl)]tetramycin B (IV).** Yield 69 %, mp 178–183°С(decomp.), R*f* 0.42. 1H NMR (МеОН-*d*4), δ, ppm: 0.90 (t, 3H, J29,27eq 7.0 Hz, J28,27ax 6.8 Hz, 28-CH3), 1.14 (d, 3H, 26-CH3, J26,25 6.8 Hz, 26-CH3), 1.22 (d, 1H, J10eq,11 4.8 Hz, H-10eq), 1.29 (s, 3H, H-6’-CH3), 1.36 (d, 1H, J27eq,28 7.0 Hz, H-27eq,), 1.42 (d, 1H, J6eq, 7 2.0 Hz, H-6eq,), 1.47 (dd, 1H, J27ax, 28 7.0 Hz, J27ax, 27eq 13.5 Hz, H-27ax), 1.55 (s, 1H, H-8eq), 1.67 (d, 1H, J14eq, 15 2.5 Hz, H-14Heq), 1.77 (d, 1H, J8ax, 8eq 14.2 Hz, H-8ax), 1.88 (dd, 1H, J6ax, 6eq 14.2 Hz, J6ax,7 1.0 Hz, H-6ax), 1.94 (dd, 1H, J14ax,14eq 15.0, J14ax,15 3.8 Hz, H-14ax), 2.09 (dd, 1H, J10ax,10eq 12.7 Hz, J10ax,11 4.8 Hz, H-10-ax), 2.20 (t, 1H, J12,13 10.5 Hz, H-12), 2.33 (ddd, J24,25 4.5 Hz, J24,27ax 4.5 Hz, J24,27eq 9.7 Hz, H- 24), 2.83 [c, 6H, N(CH3)2], 3.20 (d, 1H, J5’,6’Me 6.2Hz, H-5’), 3.32 (d, 1H, J4’,5’ 9.8 Hz, H-4’), 3.68 (d, 1H, J2’,3’ 3.4 Hz, H-2’), 3.75 (d, 1H, J2’,3’ 3.2 Hz, H-2’), 3.80 (dd, 1H, J5,6ax 1.8 Hz, J5,6eq 10.7 Hz, H-5), 3.89 (d, 1H, J3’,4’ 9.6 Hz, H-3’), 3.98 (d, 1H, J4,5 2.5 Hz, H-4), 4.21 (m, 1H, CH-P, H-1”), 4.27 (d, 1H, J11,12 10.5 Hz, H-11), 4.34 (d, 1H, J15,16 8.7 Hz, H-15), 4.42 (dd, 1H, J7,8ax 11.0 Hz, J7,8eq 2.0 Hz, H-7), 4.49 (d, 1H, J1’,2’ 1.2 Hz, H-1’), 4.56 (dd, 1H, J13,14ax 1.4 Hz, J13,14eq 8.5 Hz, H-13), 5.12 (d, 1H, J25,26 6.5 Hz, H-25), 5.40 (d, 1H, J23,24 9.2 Hz, H-23), 5.93 (d, 1H, J16,17 15.4 Hz, H-16), 6.01 (d, 1H, J22,23 15.0 Hz, H-22), 6.08 (d, 1H, J2,3 15.4 Hz, H-2), 6.17 (d, 1H, J17,18 10.4 Hz, H-17), 6.25 (d, 1H, J20,21 15.0 Hz, H-20), 6.29 (d, 1H, J18,19 14.4 Hz, H-18), 6.37 (d, 1H, J19,20 10.8 Hz, H-19), 6.52 (d, 1H, J21,22 10.3 Hz, H-21), 6.78 (d, 1H, J3,4 9.2 Hz, H-3), 6.68 (d, 2H, J 8.2 Hz, phenyl), 7.09 (d, 1H, JPH 557 Hz, P-H), 7.25 (d, 2H, J 8.2 Hz, phenyl), 9.98 (s, 1H, Ph-*OH*). 13C NMR spectrum, δ, ppm: 12.34 (C-28), 13.73 (C-26), 18.09 (C-6’), 19.25 [N(CH3)2], 23.82 (C-27), 27.64 (d, *C*''H-P, *J*CP 134.9 Hz), 37.81 (C-10), 44.02 (C-6), 44.26 (C-14), 46.85 (C-8), 48.11 (C-24), 55.34 (C-3’), 57.36 (C-12), 65.12 (C-7), 65.74 (C-11), 66.57 (C-13), 69.69 (C-2’), 70.91 (C-4’), 71.98 (C-25), 73.40 (C-4), 73.72 (C-5’), 73.98 (C-5), 76.21 (C-15), 97.20 (C-1’), 97.57 (C-9), 115.80 (phenyl), 128.33 (phenyl), 131.34 (phenyl), 141.58 (phenyl), 122.06 (C-2), 129.28 (C-16), 131.32 (C-23), 131.64 (C-18), 132.09 (C-20), 132.76 (C-21), 133.17 (C-19), 135.13 (C-22), 136.22 (C-17), 148.49 (C-3), 165.24 (C-1), 173.60 (C-29). 31P NMR spectrum, δ, ppm: 15.94.

***N*-[(**α**-Hydrophosphoryl-4-fluorobenzyl)]tetramycin B (V).** Yield 60 %, mp 173–178°С(decomp.), R*f* 0.38. 1H NMR (МеОН-*d*4), δ, ppm: 0.94 (t, 3H, J29,27eq 7.0 Hz, J28,27ax 6.8 Hz, 28-CH3), 1.10 (d, 3H, 26-CH3, J26,25 6.8 Hz, 26-CH3), 1.21 (d, 1H, J10eq,11 4.8 Hz, H-10eq), 1.28 (s, 3H, H-6’-CH3), 1.35 (d, 1H, J27eq,28 7.0 Hz, H-27eq,), 1.39 (d, 1H, J6eq, 7 2.0 Hz, H-6eq,), 1.47 (dd, 1H, J27ax, 28 7.0 Hz, J27ax, 27eq 13.5 Hz, H-27ax), 1.55 (s, 1H, H-8eq), 1.67 (d, 1H, J14eq, 15 2.5 Hz, H-14Heq), 1.82 (d, 1H, J8ax, 8eq 14.2 Hz, H-8ax), 1.90 (dd, 1H, J6ax, 6eq 14.2 Hz, J6ax,7 1.0 Hz, H-6ax), 1.96 (dd, 1H, J14ax,14eq 15.0, J14ax,15 3.8 Hz, H-14ax), 2.09 (dd, 1H, J10ax,10eq 12.7 Hz, J10ax,11 4.8 Hz, H-10-ax), 2.19 (t, 1H, J12,13 10.5 Hz, H-12), 2.41 (ddd, J24,25 4.5 Hz, J24,27ax 4.5 Hz, J24,27eq 9.7 Hz, H- 24), 3.20 (d, 1H, J5’,6’Me 6.2Hz, H-5’), 3.36 (d, 1H, J4’,5’ 9.8 Hz, H-4’), 3.70 (d, 1H, J2’,3’ 3.4 Hz, H-2’), 3.78 (d, 1H, J2’,3’ 3.2 Hz, H-2’), 3.83 (dd, 1H, J5,6ax 1.8 Hz, J5,6eq 10.7 Hz, H-5), 3.89 (d, 1H, J3’,4’ 9.6 Hz, H-3’), 4.00 (d, 1H, J4,5 2.5 Hz, H-4), 4.08 (m, 1H, CH-P, H-1”), 4.27 (d, 1H, J11,12 10.5 Hz, H-11), 4.35 (d, 1H, J15,16 8.7 Hz, H-15), 4.40 (dd, 1H, J7,8ax 11.0 Hz, J7,8eq 2.0 Hz, H-7), 4.45 (d, 1H, J1’,2’ 1.2 Hz, H-1’), 4.53 (dd, 1H, J13,14ax 1.4 Hz, J13,14eq 8.5 Hz, H-13), 5.07 (d, 1H, J25,26 6.5 Hz, H-25), 5.43 (d, 1H, J23,24 9.2 Hz, H-23), 6.00 (d, 1H, J16,17 15.4 Hz, H-16), 6.06 (d, 1H, J22,23 15.0 Hz, H-22), 6.09 (d, 1H, J2,3 15.4 Hz, H-2), 6.15 (d, 1H, J17,18 10.4 Hz, H-17), 6.21 (d, 1H, J20,21 15.0 Hz, H-20), 6.29 (d, 1H, J18,19 14.4 Hz, H-18), 6.34 (d, 1H, J19,20 10.8 Hz, H-19), 6.49 (d, 1H, J21,22 10.3 Hz, H-21), 6.75 (d, 1H, J3,4 9.2 Hz, H-3), 6.92 (d, 1H, JPH 555 Hz, P-H), 7.02 (d, 2H, J 8.2 Hz, phenyl), 7.14 (d, 2H, J 8.2 Hz, phenyl). 13C NMR spectrum, δ, ppm: 12.35 (C-28), 13.69 (C-26), 17.94 (C-6’), 24.01 (C-27), 27.53 (d, *C*''H-P, *J*CP 132.9 Hz), 37.74 (C-10), 43.93 (C-6), 44.28 (C-14), 46.90 (C-8), 48.09 (C-24), 55.31 (C-3’), 57.22 (C-12), 65.10 (C-7), 65.79 (C-11), 66.52 (C-13), 69.70 (C-2’), 70.82 (C-4’), 71.95 (C-25), 73.38 (C-4), 73.69 (C-5’), 73.82 (C-5), 76.11 (C-15), 97.28 (C-1’), 97.60 (C-9), 115.69 (phenyl), 128.38 (phenyl), 131.29 (phenyl), 141.62 (phenyl), 122.11 (C-2), 129.29 (C-16), 131.21 (C-23), 131.50 (C-18), 132.06 (C-20), 132.70 (C-21), 133.16 (C-19), 135.11 (C-22), 136.13 (C-17),148.47 (C-3), 165.21 (C-1), 173.56 (C-29). 31P NMR spectrum, δ, ppm: 15.80.

***N*-[(**α**-Hydrophosphoryl-4-chlorobenzyl)]tetramycin B (VI).** Yield 60 %, mp 180–185°С(decomp.), R*f* 0.44. 1H NMR (МеОН-*d*4), δ, ppm: 0.91 (t, 3H, J29,27eq 7.0 Hz, J28,27ax 6.8 Hz, 28-CH3), 1.08 (d, 3H, 26-CH3, J26,25 6.8 Hz, 26-CH3), 1.19 (d, 1H, J10eq,11 4.8 Hz, H-10eq), 1.26 (s, 3H, H-6’-CH3), 1.32 (d, 1H, J27eq,28 7.0 Hz, H-27eq,), 1.41 (d, 1H, J6eq, 7 2.0 Hz, H-6eq,), 1.49 (dd, 1H, J27ax, 28 7.0 Hz, J27ax, 27eq 13.5 Hz, H-27ax), 1.53 (s, 1H, H-8eq), 1.64 (d, 1H, J14eq, 15 2.5 Hz, H-14Heq), 1.80 (d, 1H, J8ax, 8eq 14.2 Hz, H-8ax), 1.87 (dd, 1H, J6ax, 6eq 14.2 Hz, J6ax,7 1.0 Hz, H-6ax), 1.99 (dd, 1H, J14ax,14eq 15.0, J14ax,15 3.8 Hz, H-14ax), 2.12 (dd, 1H, J10ax,10eq 12.7 Hz, J10ax,11 4.8 Hz, H-10-ax), 2.24 (t, 1H, J12,13 10.5 Hz, H-12), 2.48 (ddd, J24,25 4.5 Hz, J24,27ax 4.5 Hz, J24,27eq 9.7 Hz, H- 24), 3.17 (d, 1H, J5’,6’Me 6.2Hz, H-5’), 3.40 (d, 1H, J4’,5’ 9.8 Hz, H-4’), 3.66 (d, 1H, J2’,3’ 3.4 Hz, H-2’), 3.74 (d, 1H, J2’,3’ 3.2 Hz, H-2’), 3.84 (dd, 1H, J5,6ax 1.8 Hz, J5,6eq 10.7 Hz, H-5), 3.92 (d, 1H, J3’,4’ 9.6 Hz, H-3’), 4.01 (d, 1H, J4,5 2.5 Hz, H-4), 4.10 (m, 1H, CH-P, H-1”), 4.24 (d, 1H, J11,12 10.5 Hz, H-11), 4.33 (d, 1H, J15,16 8.7 Hz, H-15), 4.45 (dd, 1H, J7,8ax 11.0 Hz, J7,8eq 2.0 Hz, H-7), 4.45 (d, 1H, J1’,2’ 1.2 Hz, H-1’), 4.59 (dd, 1H, J13,14ax 1.4 Hz, J13,14eq 8.5 Hz, H-13), 5.10 (d, 1H, J25,26 6.5 Hz, H-25), 5.48 (d, 1H, J23,24 9.2 Hz, H-23), 5.95 (d, 1H, J16,17 15.4 Hz, H-16), 6.00 (d, 1H, J22,23 15.0 Hz, H-22), 6.08 (d, 1H, J2,3 15.4 Hz, H-2), 6.14 (d, 1H, J17,18 10.4 Hz, H-17), 6.22 (d, 1H, J20,21 15.0 Hz, H-20), 6.30 (d, 1H, J18,19 14.4 Hz, H-18), 6.38 (d, 1H, J19,20 10.8 Hz, H-19), 6.52 (d, 1H, J21,22 10.3 Hz, H-21), 6.71 (d, 1H, J3,4 9.2 Hz, H-3), 6.94 (d, 1H, JPH 552 Hz, P-H), 7.00 (d, 2H, J 8.2 Hz, phenyl), 7.17 (d, 2H, J 8.2 Hz, phenyl). 13C NMR spectrum, δ, ppm: 12.41 (C-28), 13.73 (C-26), 18.04 (C-6’), 24.08 (C-27), 27.45 (d, *C*''H-P, *J*CP 134.0 Hz), 37.78 (C-10), 43.99 (C-6), 44.33 (C-14), 46.96 (C-8), 48.12 (C-24), 55.37 (C-3’), 57.26 (C-12), 65.17 (C-7), 65.71 (C-11), 66.58 (C-13), 69.75 (C-2’), 70.87 (C-4’), 72.03 (C-25), 73.42 (C-4), 73.60 (C-5’), 73.88 (C-5), 76.16 (C-15), 97.34 (C-1’), 97.71 (C-9), 115.77 (phenyl), 128.13 (phenyl), 131.34 (phenyl), 141.72 (phenyl), 122.27 (C-2), 129.20 (C-16), 131.34 (C-23), 131.39 (C-18), 132.23 (C-20), 132.81 (C-21), 133.35 (C-19), 135.19 (C-22), 136.22 (C-17),148.36 (C-3), 165.30 (C-1), 173.68 (C-29). 31P NMR spectrum, δ, ppm: 15.89.

***N*-[(**α**-Hydrophosphoryl-4-bromobenzyl)]tetramycin B (VII).** Yield 60 %, mp 191–196°С(decomp.), R*f* 0.41. 1H NMR (МеОН-*d*4), δ, ppm: 0.89 (t, 3H, J29,27eq 7.0 Hz, J28,27ax 6.8 Hz, 28-CH3), 1.16 (d, 3H, 26-CH3, J26,25 6.8 Hz, 26-CH3), 1.23 (d, 1H, J10eq,11 4.8 Hz, H-10eq), 1.30 (s, 3H, H-6’-CH3), 1.36 (d, 1H, J27eq,28 7.0 Hz, H-27eq,), 1.42 (d, 1H, J6eq, 7 2.0 Hz, H-6eq,), 1.49 (dd, 1H, J27ax, 28 7.0 Hz, J27ax, 27eq 13.5 Hz, H-27ax), 1.59 (s, 1H, H-8eq), 1.68 (d, 1H, J14eq, 15 2.5 Hz, H-14Heq), 1.80 (d, 1H, J8ax, 8eq 14.2 Hz, H-8ax), 1.91 (dd, 1H, J6ax, 6eq 14.2 Hz, J6ax,7 1.0 Hz, H-6ax), 2.03 (dd, 1H, J14ax,14eq 15.0, J14ax,15 3.8 Hz, H-14ax), 2.11 (dd, 1H, J10ax,10eq 12.7 Hz, J10ax,11 4.8 Hz, H-10-ax), 2.23 (t, 1H, J12,13 10.5 Hz, H-12), 2.49 (ddd, J24,25 4.5 Hz, J24,27ax 4.5 Hz, J24,27eq 9.7 Hz, H- 24), 3.17 (d, 1H, J5’,6’Me 6.2Hz, H-5’), 3.42 (d, 1H, J4’,5’ 9.8 Hz, H-4’), 3.65 (d, 1H, J2’,3’ 3.4 Hz, H-2’), 3.74 (d, 1H, J2’,3’ 3.2 Hz, H-2’), 3.80 (dd, 1H, J5,6ax 1.8 Hz, J5,6eq 10.7 Hz, H-5), 3.92 (d, 1H, J3’,4’ 9.6 Hz, H-3’), 4.02 (d, 1H, J4,5 2.5 Hz, H-4), 4.13 (m, 1H, CH-P, H-1”), 4.29 (d, 1H, J11,12 10.5 Hz, H-11), 4.36 (d, 1H, J15,16 8.7 Hz, H-15), 4.40 (dd, 1H, J7,8ax 11.0 Hz, J7,8eq 2.0 Hz, H-7), 4.46 (d, 1H, J1’,2’ 1.2 Hz, H-1’), 4.59 (dd, 1H, J13,14ax 1.4 Hz, J13,14eq 8.5 Hz, H-13), 5.11 (d, 1H, J25,26 6.5 Hz, H-25), 5.52 (d, 1H, J23,24 9.2 Hz, H-23), 5.97 (d, 1H, J16,17 15.4 Hz, H-16), 6.09 (d, 1H, J22,23 15.0 Hz, H-22), 6.12 (d, 1H, J2,3 15.4 Hz, H-2), 6.18 (d, 1H, J17,18 10.4 Hz, H-17), 6.23 (d, 1H, J20,21 15.0 Hz, H-20), 6.31 (d, 1H, J18,19 14.4 Hz, H-18), 6.40 (d, 1H, J19,20 10.8 Hz, H-19), 6.56 (d, 1H, J21,22 10.3 Hz, H-21), 6.79 (d, 1H, J3,4 9.2 Hz, H-3), 6.94 (d, 1H, JPH 556 Hz, P-H), 7.06 (d, 2H, J 8.2 Hz, phenyl), 7.22 (d, 2H, J 8.2 Hz, phenyl). 13C NMR spectrum, δ, ppm: 12.44 (C-28), 13.61 (C-26), 18.07 (C-6’), 24.13 (C-27), 26.79 (d, *C*''H-P, *J*CP 132.9 Hz), 37.79 (C-10), 44.06 (C-6), 44.39 (C-14), 46.99 (C-8), 48.17 (C-24), 55.38 (C-3’), 57.31 (C-12), 65.01 (C-7), 65.62 (C-11), 66.40 (C-13), 69.82 (C-2’), 70.64 (C-4’), 71.78 (C-25), 73.45 (C-4), 73.74 (C-5’), 73.92 (C-5), 76.28 (C-15), 97.39 (C-1’), 97.81 (C-9), 115.83 (phenyl), 128.45 (phenyl), 131.47 (phenyl), 141.77 (phenyl), 122.26 (C-2), 129.41 (C-16), 131.09 (C-23), 131.66 (C-18), 132.23 (C-20), 132.91 (C-21), 133.28 (C-19), 135.01 (C-22), 136.33 (C-17),148.56 (C-3), 165.09 (C-1), 173.42 (C-29). 31P NMR spectrum, δ, ppm: 15.77.

**EXPERIMENTAL BIOLOGICAL PART**

 ***Pharmacological studies.*** The starting tetramycin B was used as the reference in all biological tests. The acute toxicity (LD50) of hydrophosphoryl derivatives of tetramycin B **I-VII** was tested using white mongrel mice males (18–20 g) that were maintained at room temperature on a standard ration with natural illumination and ambient temperature. The groups of 10 animal units were observed during 5 days. The tested compounds were diluted with 0.5% aqueous solution of carboxymethyl cellulose, and the suspension was administered intraperitoneally. The LD50 values of compounds **I-VII** were calculated using the Kerber method. The pharmacological experiments were performed in full accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes [3].

 ***Studies of antifungal activity.*** The antifungal activity of derivatives **I-VII** towards 11 candidiasis agents was determined according to the NCCLS М27 standard using the serial dilution method [4, 5] in liquid nutrient medium [6]. The minimal fungistatic concentration (MFC, μg/ml) was determined via visual inspection of the growth of the test culture in the experimental and reference tubes; the experiments were run in triplicate (Table 1).

 ***Studies of antiviral activity.*** The toxicity of compounds **I**-**VII** was determined before their antiviral activity was studied. The compounds were injected into the allantoic cavity or onto the chorioallantoic membrane (CAM) of 10-11-day chicken embryos. Ten embryos were used for each dilution. They were incubated in a thermostat at hatching temperature and were candled every 2 days (Table 2).

 Antiviral activity of hydrophosphoryl derivatives of tetramycin B **I**-**VII** was studied in *in ovo* tests against DNA-containing Vaccinia virus (Vv) using strain L-IBP 01.72. The working Vv titers were 10–6-10–8 pock-forming units (PFU). Virus-containing material was prepared in McIlvaine buffer. Test compounds were injected into the CAM of 10-11-day embryos in prophylactic scheme (1 h before virus injection) or therapeutic scheme (1 h after virus infection). Embryos were incubated for 48-72 h at 37°C. Counting of the pocks was performed on CAM of embryos (Table 2).

 Antiviral activity of compounds **I–VII** was examined against RNA containing viruses, oncogenic Rous sarcoma virus (RSV) [(strain RSV (RSV-1)], and infectious virus of type A ([a strain A2 Odessa 2882/82 (H3N2)] and type B ([a strain B/USSR (69)] in developing chicken embryos. After determining the toxicity of compounds 10-11-day-old, developing chicken embryos were administered in the test with the RSV on the CAM according to prophylactic and therapeutic schemes. Number of foci of neoplastic transformation in the CAM of embryos was counted on 8th day after incubation at 37°C. Determination of antiinfluenzal action were carried out by the introduction of hydrophosphoryl derivatives of tetramycin B **I-VII** in the chorioallantoic cavity of 10-11-day-old developing chick embryos by prophylactic scheme, i.e.,10-100 EID50 of virus for 1-1.5 h before incubation. The results were taken into account after 48 h of incubation of embryos at 37°C in the case of influenza virus of type A and after 72 h at 34°C in the case of influenza virus of type B (Table 2).

 The presence of virus in embryos was determined in the reaction of hemagglutination with chicken red blood cells in the allantoic fluid of experimental and control embryos. An isotonic sodium chloride solution was injected into the control embryos instead of substances under study.

 Antiviral activity of the tested compounds in experiments *in ovo* was assessed by a protection index (PI), which was calculated by the following formula:

 Amount of virus-infected embryos in the control (%) –

Amount of virus-infected embryos in the experiment (%)

PI = –––––––––––––––––––––––––––––––––––––––––––––– × 100.

 Amount of virus-infected embryos in the control (percentage)

 The hydrophosphoryl derivatives of tetramycin B (**I**-**VII**) were studied against all model viruses in at least three series of tests. Results from the virology studies were processed statistically using the Microsoft Excel 2000 program and Stat Soft Statistica v 6.0 software with parametric and non-parametric variational statistics methods. The significance of differences was determined using the Student *t*-criterion and the Pearson χ2 factor. Differences were considered significant for *p* < 0.05.

**REFERENCES**

1. Zhang, N., Song, Z., Xie, Y., Cui, P., Jiang, H., Yang, T., Ju, R., Zhao, Y., Li, J., Liu, X., *Word J. Microbiol. Biotechnol.*, **2013**, 29, 1443-1452.

2. Song, Y., He, L., Chen, L., Ren, Y., Lu, H., Geng, S., Mu, W., Liu, F., *Eur. J. Plant Pathol.*, **2016**, 146, 337-347.

3. *European Convention for the Protection of Vertebrate Animals Used for Experimental and*

 *Other Scientific Purposes. European Treaty Series,* Strasbourg (France), Mach 18, 1986.

4. *National Committee for Clinical Laboratory Standards.* *Reference Method for Broth*

*Dilution Antifungal Susceptibility Testing of Yeasts,* Approved Standard M27-A. Wayne, PA, USA, 1997.

5. Espinel-Ingroff, A., Boyle, K., and Sheehan, D.J., *Mycopathologia*, **2001**, 150, 101-115.

6. Rex, J.H., Pfaller, M.A., Galgiani, J.N., Bartlett, M.S., Espinel-Ingroff, A., Ghannoum, M.A., Lancaster, M., Odds, F.C., Rinaldi, M.G., Walsh, T.J., and Barry, A.L., *Clin. Infect. Dis.*, **1997**, 24, 235-247.

Table S 1. Antifungal Activity of Hydrophosphoryl Derivatives of Tetramycin B **I-VII**, minimal fungistatic concentration (μg/mL).

|  |  |  |
| --- | --- | --- |
| Test-culture | Compound | Tetramycin B |
| **I** | **II** | **III** | **IV** | **V** | **VI** | **VII** |
| *Candida albicans* | 15.50 | 15.50 | 15.50 | 3.90 | 1.95 | 1.95 | 1.95 | 15.50 |
| *Candida utilis* | 15.50 | 15.50 | 15.50 | 15.50 | 15.50 | 15.50 | 15.50 | 15.50 |
| *Candida tropicalis* | 15.50 | 15.50 | 15.50 | 15.50 | 3.90 | 3.90 | 1.95 | 7.75 |
| *Candida krusei* | 15.50 | 15.50 | 15.50 | 7.75 | 3.90 | 1.95 | 1.95 | 15.50 |
| *Candida parapsilosis* | 31.0 | 31.0 | 15.50 | 7.75 | 3.90 | 1.95 | 1.95 | 7.75 |
| *Candida guillermondii* | 15.50 | 15.0 | 15.0 | 15.50 | 15.50 | 15.50 | 15.50 | 15.50 |
| *Candida glabrata* | 15.50 | 15.50 | 15.50 | 15.50 | 3.90 | 3.90 | 3.90 | 15.50 |
| *Candida lusitaniae* | 31.0 | 15.50 | 15.50 | 15.50 | 15.50 | 15.50 | 15.50 | 15.50 |
| *Candida lipolytica* | 31.0 | 15.50 | 15.50 | 7.75 | 7.75 | 15.50 | 7.75 | 7.75 |
| *Candida norvegensis* | 31.0 | 31.0 | 31.0 | 15.50 | 15.50 | 15.50 | 15.50 | 31.0 |
| *Candida kefyr* | 31.0 | 31.0 | 31.0 | 15.50 | 15.50 | 15.50 | 15.50 | 15.50 |

Table S 2. Toxicity and Antiviral Activity of Hydrophosphoryl Derivatives of Tetramycin B

**I-VII**.

|  |  |  |
| --- | --- | --- |
| Compound | Toxicity,mg/embryo | Protection index, % |
| vaccinia virus | RSV | influenza virus |
| LD50 | MTD | prophylactic scheme | therapeutic scheme | prophylactic scheme | therapeutic scheme | type А | type В |
| prophylacticscheme |
| **I****II****III****IV****V****VI****VII****Tetramycin B** | 300350250450650600700200 | 200250150350550500600100 | 43.949.741.840.071.377.581.653.7 | 34.638.041.332.765.962.573.145.8 | 32.041.967.739.388.469.589.269.6 | 27.336.959.832.672.166.778.061.5 | 32.947.652.558.869.361.065.754.4 | 26.430.146.049.858.752.659.948.3 |

MTD – maximum tolerated dose.