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Antibacterial activity of cationic amphiphil conjugates with ciprofloxacin

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***Abstract*---**The aim: Design and synthesis of new compounds with antibacterial activity. Determination of the minimum inhibitory and minimum bactericidal concentration of conjugates of cationic amphiphiles based on quaternary salts of 1,4-

diazabicyclo[2.2.2]octane with ciprofloxacin. Materials and methods: The article is dedicated to the synthesis and primary assessment of antibacterial activity in vitro of conjugates of cationic amphiphiles based on quaternary salts of 1,4-diazabicyclo[2.2.2]octane with ciprofloxacin against gram-negative and gram-positive microorganisms. Results: The present work provides hybrid structures which include the widely used antibiotic ciprofloxacin and tetra-cationic DABCO derivatives. The last ones previously showed a wide spectrum of antimicrobial activity as well as primary studies of their antibacterial activity against gram-positive and gram-negative microorganisms *in vitro*. There was a high *in vitro* antibacterial activity against gram-negative pathogens, moderate against *Staphylococcus aureus* and low against *Enterococcus faecalis*. Conclusions: A range of amphiphilic polycationic compounds based on quaternary salts of 1,4-diazabicyclo[2.2.2]octane (DABCO) was synthesized and their biological activity was studied. The compounds have demonstrated high antibacterial activity. We assume that they can serve as a basis for the development of new antibacterial drugs. The results obtained indicate that the design of new antibacterial drugs based on conjugates of antibiotics with synthetic cationic amphiphiles is promising.

***Keywords*---**chemical synthesis, gram-positive bacteria, gram-negative bacteria.

Introduction

The widespread introduction of antibiotics into medical and veterinary practice leads to an increase in the number of infectious diseases caused by antibiotic-resistant bacteria throughout the world [1-2]. Thus, the issue of finding new effective both synthetic and natural antimicrobial peptides (AMP) arise. In particular, antimicrobial peptides (AMP), low molecular weight cationic peptides which are, as a rule, natural amphiphiles are being intensively studied [3-6]. Consequently, the issue of finding new effective both synthetic and natural antimicrobial agents arise. In particular, antimicrobial peptides (AMP), low molecular weight cationic peptides, which are, as a rule, natural amphiphiles, are being intensively studied [3-6]. The chief obstacle to the usage of AMPs in clinical practice is their high cost, sensitivity to the action of proteolytic enzymes as well as the hemolytic effect inherent in many AMPs [7].

Synthetic amphiphilic polycationic compounds based on quaternary salts of 1,4 - diazabicyclo [2.2.2] octane (DABCO) possess a similar spectrum of biological activity and they can serve as a basis for new antibacterial drugs that are an alternative to modern antibiotics [8-9].

The impact of an active substance directly on the bacterial walls makes it less likely that pathogens develop resistance to cationic amphiphiles. Nevertheless, a number of mechanisms have been found that make pathogens resistant to such effects [10, 11], including the fact of reducing the total negative surface charge

[12, 13]. One of the approaches to reducing the likelihood of developing resistance is the combined combination of several antibacterial drugs with different mechanisms of action, for example, antimicrobial peptides with antibiotics [14].

Currently, a small number of examples of modification of known antibiotics with fragments of cationic amphiphiles have been described in the literature. So, the work of Bai et al. describes the synthesis and antibacterial activity of amphiphilis whose structure includes the antibiotic linezolid [15]. There has been described the synthesis and antibacterial activity of a number of compounds based on aminoglycazide antibiotics (neomycin, kanamycin, amiksin) in which the cationic part of the amphiphile is a component of the antibiotic [16-18]. The absence of a rigid binding to the structure of the aminoacids used in combination with relatively simple methods for the synthesis of cationic amphiphiles opens up wide possibilities for obtaining hybrid structures that can potentially affect various targets of microorganisms.

The Aim

Design and synthesis of new compounds with antibacterial activity. Determination of the minimum inhibitory and minimum bactericidal concentration of conjugates of cationic amphiphiles based on quaternary salts of 1,4-diazabicyclo[2.2.2]octane with ciprofloxacin.

Materials and Methods

All reagents and solvents were obtained from commercial sources and they did not require preliminary purification. ^{1}H, ^{13}C, ^{19}F spectra were recorded on AV 300 and AV 400, DX 500 spectrometers (Bruker, Germany). Tetramethylsilane and residual proton signals were used as an internal standard. Currently, a small number of examples of modification of known antibiotics with fragments of cationic amphiphiles have been described in the literature. So, the work of Bai et al. describes the synthesis and antibacterial activity of amphiphilis whose structure includes the antibiotic linezolid [15].

The synthesis and antibacterial activity of a number of compounds based on aminoglycazide antibiotics (neomycin, kanamycin, amiksin) in which the cationic part of the amphiphile is a component of the antibiotic has been described [16-18]. The absence of a rigid binding to the structure of the amino acids used in combination with relatively simple methods for the synthesis of cationic amphiphiles opens up wide possibilities for obtaining hybrid structures that can potentially affect various targets of microorganisms, solvents DMSO-d6, $CDCl_3$. Chemical shifts are given in the δ scale, ppm, spin - spin coupling constant J in Hz. High-resolution mass spectra were recorded on an Agilent ESI MSD XCT Ion Trap (Agilent Technologies, USA) at the Joint Center for Genomic, Proteomic and Metabolic Research (ICBFM SB RAS).

Analysis of reaction mixtures by reversed-phase chromatography was carried out on a Milichrom A-02 chromatograph (ZAO Ekonova, Russia). Column ProntoSIL-120-5-C18 AQ DB-2003, 2×75 мм, dp = 5 mkm. Analysis of reaction mixtures by reversed-phase chromatography was carried out in the solvent system: eluent A (0,01 M,

TEAAc, pH 7,0, H_2O), eluent B (0,01 M, TEAAc, pH 7.0, 90 % AcCN); Gradient A (0 % B (200 mkl), 0 % - 30 % B (1400 mkl), 30-100 % B (1800 mkl), 100% B (300 mkl)). The flow rate of the eluent is 200 ml/min. For column chromatography, silica gel 40–63 мкm/230–400 mesh (Macherey-Nagel, Germany) was used. For TLC, Alugram Kiesegel 60 $UF_{254\ plates}$ (Macherey-Nagel, Germany) were used.

Analysis of reaction mixtures by TLC was carried out in solvent systems: methylene chloride/methanol 9.5:0.5 (system A), methylene chloride/methanol 9:1 (system B).

Chemical synthesis

General procedure for the synthesis of pentafluorophenyl esters of ω-bromocarboxylic acids. ω-Bromocarboxylic acid (5.0 mmol) and pentafluorophenol (1.1 g, 6.0 mmol) were dissolved in 10 ml of methylene chloride, cooled on ice and a cooled solution of DCC in 2 ml of methylene chloride was added. The reaction mixture was stirred for 2h. The formed precipitate was filtered off, the filtrate was evaporated on a rotary evaporator. The product was purified by chromatography on silica gel using methylene chloride as eluent. The fractions containing the target compound were evaporated and dried in a vacuum.

The exit of compound (1a) - 1.49 g, 86 %. R_f 0.93 (system A). NMR ^1H spectrum (300 MHz, $CDCl_3$): δ 1.95 (m, 4H, CH_2, 3-4), 2.70 (t, J = 6.8 Hz, 2H, CH_2, 2), 3.44 (t, J = 6.0 Hz, 2H, CH_2, 5). NMR spectrum ^{19}F (282 MHz, $CDCl_3$): δ 2.50 (t, J = 19.4 Hz, 2F, m-CF), 6.85 (t, J = 21.8 Hz, 1F, p-CF), 11.94 (d, J = 17.2 Hz, 2F, o-CF).

The exit of compound (1s) - 1.74 g, 81 %. R_f 0.95 (system A). NMR spectrum ^1H (300 MHz, $CDCl_3$): δ 1.28 (m, 12H, CH_2, 5-9), 1.38 (m, 2H, CH_2, 4), 1.72-1.88 (m, 4H, CH_2, 3,10), 2.63 (t, J = 7.4 Hz, 2H, CH_2, 2), 3.38 (t, J = 6.8 Hz, 2H, CH_2, 11). NMR spectrum ^{19}F (282 MHz, $CDCl_3$): δ -0.73 (t, J = 19.2 Hz, 2F, m-CF), 3.46 (t, J = 21.6 Hz, 1F, p-CF), 8.90 (d, J = 17.2 Hz, 2F, o-CF).

General methodology of compound synthesis 2a-c

To a suspension of ciprofloxacin hydrochloride (368 mg, 1 mmol) in 9 ml of methylene chloride, DIPEA (256 mkl, 1,5 mmol) and a solution of ω-bromocarboxylic acid pentafluorophenyl ester (1 mmol) in 1 ml of methylene chloride were added with stirring. The mixture was stirred for 2h at t = 22 °C until the starting reagents completely disappeared. The progress of the 7th reaction was monitored by TLC (system A). The product was precipitated from methylene chloride with a 10-fold volume of diethyl ether, dried in a vacuum.

The exit of compound (2a) – 476 mg, 96%. ^1H-NMR (400 MHz, $CDCl_3$, δ): 8.72 (c, 1H, H-2), 7.98 (d, J = 13.0 Hz, 1H, H-5), 7.34 (d, J = 7.7 Hz, 1H, H-8), 3.84 (m, 2H, H-15), 3.70 (m, 2H, H-15), 3.54 (m, 1H, H-11), 3.43 (t, J = 6.5 Hz, 2H, -CH_2Br), 3.34 (m, 2H, H-16), 3.27 (m, 2H, H-16), 2.40 (t, 2H, J = 7.3 Hz, -CH_2C(O)), 1.92 (m, 2H, -CH_2CH_2Br), 1.82 (m, 2H, -CH_2-), 1.47 (m, 2H, H-12), 1.20 (m, 2H, H-13). NMR spectrum ^{13}C (126 MHz, $CDCl_3$): δ 8.1, 23.5, 32.0, 33.3, 35.2, 41.0, 41.9, 45.1, 105.0, 107.9, 112.2, 120.0, 138.8, 145.1, 147.3, 152.4, 154.4, 166.7, 170.8, 176.8. ESI-MS m/z: $[M+H]^+$ 494.1091 (estimated for $C_{22}H_{25}BrFN_3O_4^+$: 493.10).

The exit of compound (2b) – 677 мг, 98%. ^{1}H-NMR (CDCl3, δ): 8.67 (c, 1H, H-2), 7.91 (d, J = 12.5 Hz, 1H, H-5), 7.30 (d, J = 6.9 Hz, 1H, H-8), 3.75 (m, 2H, H-15), 3.64 (m, 2H, H-15), 3.47 (m, 1H, H-11), 3.34 (t, J = 6.7 Hz, 2H, -CH_2Br), 3.26 (m, 2H, H-16), 3.21 (m, 2H, H-16), 2.33 (t, 2H, J = 7.5 Hz, -CH_2C(O)), 1.81 (m, 2H, -CH_2CH_2Br), 1.59 (m, 2H, -CH_2CH_2C(O)), 1.42 (m, 2H, -$CH_2CH_2CH_2$-), 1.32 (m, 2H, H-12), 1.12 (m, 2H, H-13). NMR spectrum ^{13}C (126 MHz, $CDCl_3$): δ 7.9, 23.8, 27.5, 32.1, 32.4, 33.5, 35.1, 40.0, 44.8, 49.1, 105.0, 107.5, 111.9, 119.6, 138.7, 145.0, 147.2, 152.1, 154.1, 166.4, 170.9, 176.6. ESI-MS m/z: $[M+H]^+$ 509.70 (counted for $C_{23}H_{27}BrFN_3O_4^+$: 508.39).

The exit of compound (2c) – 526 mg, 91%. ^{1}H-NMR($CDCl_3$, δ): 8.71 (c, 1H, H-2), 7.99 (d, J = 12.7 Hz, 1H, H-5), 7.34 (d, J = 7.0 Hz, 1H, H-8), 3.85 (m, 2H, H-15), 3.70 (m, 2H, H-15), 3.52 (m, 1H, H-11), 3.38 (t, J = 6.9 Hz, 2H, -CH_2Br), 3.34 (m, 2H, H-16), 3.27 (m, 2H, H-16), 2.36 (t, 2H, J = 7.6 Hz, -CH_2C(O)), 1.82 (m, 2H, -CH_2CH_2Br), 1.64 (m, 4H, 2CH_2), 1.43-1.15 (m, 14H, H-12, H-13, 5 CH_2-). NMR spectrum ^{13}C (126 MHz, $CDCl_3$): δ 7.8, 24.8, 27.7, 28.3, 28.9, 32.3, 32.8, 33.7, 34.9, 40.6, 44.9, 48.9, 49.8, 104.6, 107.6, 112.1, 119.7, 138.5, 145.0, 147.1, 152.1, 154.1, 166.4, 171.4, 176.5. ESI-MS m/z: $[M+H]^+$ 578.20 (counted for $C_{28}H_{37}BrFN_3O_4^+$: 577.20).

The general methodology of compound synthesis 3 a-c

The bromo derivative of ciprofloxacin (0.5 mmol) was dissolved in 5 ml of chloroform, 1,4-diazabicyclo [2.2.2] octane (0.5 mmol) was added and the reaction mixture was refluxed until the starting compound disappeared (4-10h). The progress of the reaction was monitored by TLC (system A), HPLC. The solvent was removed on a rotary evaporator. The product was precipitated from methyl alcohol with a 10-fold volume of diethyl ether and dried in a vacuum.

The exit of a compound (3a) – 199 mg, 67%. ^{1}H-NMR (DMCO-d6, δ): 15.16 (c, 1H, COOH), 8.67 (c, 1H, H-2), 7.94 (d, J = 13.0 Hz, 1H, H-5), 7.58 (d, J = 7.3 Hz, 1H, H-8), 3.83 (m, 1H, H-11), 3.70 (m, 4H, H-15), 3.36 (m, 6 H, DABCO), 3.26 (m, 2H, -CH_2-N^{DABCO}), 3.03 (m, 10H, H-16, CH_2^{DABCO}), 2.47 (t, 2H, J = 6.9 Hz, -CH_2C(O)), 1.71 (m, 2H, -CH_2-), 1.55 (m, 2H, -CH_2-), 1.32 (m, 2H, H-12), 1.19 (m, 2H, H-13). ^{13}C-NMR (DMCO-d6, δ): 176.3, 170.3, 165.9, 153.9, 151.9, 148.0, 144.8, 139.1, 118.7, 106.7, 106.6, 63.0, 51.5, 44.7, 43.9, 35.9, 31.5, 21.6, 20.8, 7.6. ESI-MS m/z: $[M+H]^+$ 526.2810 (counted for $C_{28}H_{37}FN_5O_4^+$: 526.28).

The exit of a compound (3b) – 288 mg, 93 %. ^{1}H-NMR (DMCO-d6, δ): 15.16 (c, 1H, COOH), 8.61 (c, 1H, H-2), 7.86 (d, J = 13.0 Hz, 1H, H-5), 7.53 (d, J = 7.4 Hz, 1H, H-8), 3.78 (m, 1H, H-11), 3.65 (m, 4H, H-15), 3.24 (m, 6 H, DABCO), 3.16 (m, 2H, -CH_2-N^{DABCO}), 3.12 (m, 4H, H-16), 2.98 (m, 6 H, CH_2^{DABCO}), 2.38 (t, 2H, J = 7.3 Hz, -CH_2C(O)), 1.65 (m, 2H, -CH_2-), 1.54 (m, 2H, -CH_2-), 1.28 (m, 4H, -$CH_2CH_2CH_2$-, H-12), 1.14 (m, 2H, H-13). ^{13}C-NMR (DMCO-d6, δ): 176.4, 170.6, 166.0, 154.0, 152.0, 148.1, 144.9, 139.2, 118.8, 110.9, 106.7, 106.7, 63.1, 51.5, 49.8, 44.7, 36.0, 31.9, 25.7, 24.3, 21.0, 7.7. ESI-MS m/z: $[M+H]^+$ 540.00 (counted for $C_{29}H_{39}FN_5O_4^+$: 540.30).

The exit of a compound (3c) – 335 mg, 97 %. ^{1}H-NMR (DMCO-d6, δ): 15.18 (c, 1H, COOH), 8.65 (c, 1H, H-2), 7.89 (d, J = 13.1 Hz, 1H, H-5), 7.56 (d, J = 7.4 Hz, 1H, H-8), 3.82 (m, 1H, H-11), 3.67 (m, 4H, H-15), 3.36 (m, 4H, H-16), 3.27 (m, 6 H,

DABCO), 3.17 (m, 2H, -CH_2-N^{DABCO}), 3.01 (м, 6 H, DABCO), 2.36 (т, 2H, J = 7.4 Hz, -$CH_2C(O)$), 1.64 (m, 2H, -CH_2-), 1.51 (m, 2H, -CH_2-), 1.27 (m, 14H, -$(CH_2)_6$-, H-12), 1.18 (m, 2H, H-13). ^{13}C-NMR (DMCO-d6, δ): 176.3, 170.8, 166.0, 153.9, 151.9, 148.0, 144.9, 139.1, 118.7, 111.9, 106.7, 106.6, 63.2, 51.5, 49.7, 44.7, 35.9, 32.2, 28.9, 28.8, 25.9, 24.7, 21.0, 7.6. ESI-MS m/z: $[M+H]^+$ 610.37 (counted for $C_{34}H_{49}FN_5O_4^+$: 610.38).

The general methodology of compound synthesis 4 a-c

Dichloro-p-xylene (15.4 mg, 0.088 mmol) and compound 3 a-c (0.193 mmol) were dissolved in 1 ml of methanol. The reaction mixture was stirred at t =50 °C 48 h. The formed precipitate was separated by centrifugation and washed 2 times with MeOH and then dried in a vacuum.

The exit of a compound (4a) – 25.2 mg, 23 %. ^{1}H-NMR (DMCO-d6, δ): 15.07 (c, 2H, COOH), 8.58 (c, 2H, H-2), 7.75 (c, 4H, $H^{xilocol}$), 7.73 (m, 2H, H-5), 7.50 (d, J = 7.4 Hz, 2H, H-8), 5.02 (c, 4H, CH_2^{xylol}), 4.14-3.87 (m, 24H, DABCO), 3.78 (m, 2H, H-11), 3.67 (m, 8H, H-15), 3.56 (m, 4H, -CH_2-N^{DABCO}), 3.39 (m, 8H, H-16), 2.47 (m, 4H, -$CH_2C(O)$), 1.70 (m, 4H, -CH_2-), 1.55 (m, 4H, -CH_2-), 1.31 (m, 4H, H-12), 1.16 (m, 4H, H-13). ^{13}C-NMR (ДМСО-d6, δ): 176.4, 170.4, 166.0, 154.0, 152.0, 148.1, 145.0, 139.2, 134.0, 129.4, 118.9, 111.0, 107.0, 107.0, 63.6, 50.8, 49.9, 44.7, 36.1, 31.7, 21.4, 7.8. ESI-MS m/z: $[M+2Br]^{2+}$ 658.40 (counted for $C_{64}H_{82}Br_2F_2N_{10}O_8^{2+}$: 658.23), $[M+Br]^{3+}$ 411.90 (counted for $C_{64}H_{82}BrF_2N_{10}O_8^{3+}$: 411.85).

The exit of a compound (4b) – 61.3 mg, 49%. ^{1}H-NMR (DMCO-d6, δ): 15.18 (c, 2H, COOH), 8.66 (c, 2H, H-2), 7.92 (d, J = 13.1 Hz, 2H, H-5), 7.75 (c, 4H, H^{xylol}), 7.57 (d, J = 7.2 Hz, 2H, H-8), 4.98 (c, 4H, CH_2^{xylol}), 4.07-3.88 (m, 24H, DABCO), 3.82 (m, 2H, H-11), 3.68 (m, 8H, H-15), 3.49 (m, 4H, -CH_2-N^{DABCO}), 3.29 (m, 8H, H-16), 2.40 (t, 4H, J = 7.1 Hz, -$CH_2C(O)$), 1.69 (m, 4H, -CH_2-), 1.56 (m, 4H, -CH_2-), 1.31 (m, 8H, -CH_2CH_2-, H-12), 1.19 (m, 4H, H-13). ^{13}C-NMR (DMCO-d6, δ): 176.3, 170.5, 166.9, 153.7, 152.0, 148.0, 144.9, 139.1, 133.7, 129.1, 118.8, 111.0, 106.7, 106.6, 65.5, 63.4, 50.4, 49.7, 44.5, 35.9, 31.8, 25.2, 24.1, 21.2, 7.6. ESI-MS m/z: $[M-2H]^{2+}$ 591.00 (counted for $C_{66}H_{84}F_2N_{10}O_8^{2+}$: 591.30), $[M-H]^{3+}$ 394.30 (counted for $C_{66}H_{85}F_2N_{10}O_8^{3+}$: 394.55).

The exit of a compound (4c) – 57.5 мг, 42 %. ^{1}NMR (DMCO-d6, δ): 15.17 (c, 2H, COOH), 8.66 (c, 2H, H-2), 7.92 (d, J = 13.1 Hz, 2H, H-5), 7.74 (c, 4H, H^{xylol}), 7.57 (d, J = 7.2 Hz, 2H, H-8), 4.97 (c, 4H, CH_2^{xylol}), 4.07-3.87 (m, 24H, DABCO), 3.82 (m, 2H, H-11), 3.68 (m, 8H, H-15), 3.50-3.22 (m, 12H, H-16, -CH_2-N^{DABCO}), 2.35 (t, 4H, J = 7.3 Hz, -$CH_2C(O)$), 1.64 (m, 4H, -CH_2-), 1.51 (m, 4H, -CH_2-), 1.31 (m, 28H, -$(CH_2)_6$-, H-12), 1.19 (m, 4H, H-13). ^{13}C-NMR (DMCO-d6, δ): 176.3, 170.8, 165.9, 153.9, 151.9, 148.0, 145.0, 139.1, 133.7, 129.1, 118.7, 110.9, 106.7, 106.6, 63.6, 50.5, 49.7, 48.6, 44.6, 35.9, 32.2, 28.8, 28.5, 25.5, 24.8, 21.3, 7.6. ESI-MS m/z: $[M+2Br]^{2+}$ 742.60 (counted for $C_{76}H_{106}Br_2F_2N_{10}O_8^{2+}$: 742.32), $[M+Br]^{3+}$ 468.40 (counted for $C_{76}H_{106}BrF_2N_{10}O_8^{3+}$: 468.58).

The methodology of a synthesis of compounds

1,5-Dibromopentane (11 mkl, 0.078 mmol) and compound 3 b (107 mg, 0.172 mmol) were dissolved in 1 mL of methanol. The reaction mixture was stirred at T =

50°C 48 h. The product was precipitated from methyl alcohol with a 10-fold volume of acetone. The formed precipitate was separated by centrifugation, washed 2 times with acetone and dried in a vacuum.

The exit of a compound (4d) – 97 mg, 84%. ^{1}H-NMR (DMCO-d6, δ): 15.21 (c, 2H, COOH), 8.68 (c, 2H, H-2), 7.94 (d, J = 12.9 Hz, 2H, H-5), 7.58 (d, J = 7.1 Hz, 2H, H-8), 3.94 (m, 24H, DABCO), 3.83 (m, 2H, H-11), 3.74-3.27 (m, 8H, H-15, 8H, -CH_2-N^{DABCO}, 8H, H-16), 2.43 (t, 4H, J = 7.2 Hz, -CH_2C(O)), 1.77 (m, 4H, -CH_2-), 1.60 (m, 4H, -CH_2-), 1.33 (m, 8H, -CH_2CH_2-, H-12), 1.19 (m, 4H, H-13). ^{13}C-NMR (DMCO-d6, δ): 176.3, 170.5, 165.9, 153.7, 153.7, 148.1, 144.9, 139.1, 118.8, 111.1, 106.7, 106.6, 63.3, 50.4, 49.7, 44.5, 35.9, 31.8, 25.3, 24.1, 21.3, 7.6. ESI-MS m/z: $[M-2H]^{2+}$ 574.00 (counted for $C_{63}H_{86}F_2N_{10}O_8^{2+}$: 574.33), $[M-H]^{3+}$ 383.00 (counted for $C_{63}H_{87}F_2N_{10}O_8^{3+}$: 383.22).

Biological investigation

Bacterial stamps *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 51299), Pseudomonas aeruginosa (ATCC 9027), Proteus mirabilis (ATCC 6380), Citrobacter freundii (ATCC 8090), Salmonella enterica (ATCC 14028), Escherichia coli (ATCC 259220) were obtained from the collection of microorganisms Of Sybir department of the Russian academy of science (Novosybirsk, Russia). The stamps were preserved in a liquis saltless environment LB (Luria-Bertani, BD, USA) with 25% glycerine at 70 ºC. Before using the cultures were sown onto agricultural environment and incubated for days at 37 ºC.

Determination of the minimum inhibitory and minimum bactericidal concentrations

To assess the antibacterial activity strains of 2 gram-positive (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 51299) and 5 gram-negative (*Pseudomonas aeruginosa* ATCC 9027, *Proteus mirabilis* ATCC 6380, *Citrobacter freundii* ATCC 8090, *Salmonella enterica* ATCC 14028, *Escherichia coli* ATCC 259220) microbodies were chosen. Identification of sensitivity to drugs was carried out by the method of serial dilutions in 96-well plates according to the recommendations of the European Committee for the identification of sensitivity to antimicrobial drugs [19].

Cultures grown on LB agar medium were inoculated into Mueller-Hinton broth (MHB, OXOID, UK) and cultured overnight. Stock solutions of compounds with a concentration of 10 mg/ml were prepared using sterile Milli-Q water (Merk, Germany). The final cell concentration of the overight broth culture of the strains was adjusted to ~5×105 KOE/ml in MHBT. The final concentrations of drugs in the medium were 100, 50, 25, 12,5, 6,3 etc mkg/ml. The preparation was incubated together with cultures in 96-well plates at 37°C and at 580 rpm/min in a volume of 200 mkl during 24h. Culture growth was assessed after 24 hours by measuring OD595 in each well (Uniplan, PIKON, Russia).

As a result, the minimum inhibitory concentration (MIC) was established for the preparations by measuring the OD595 and the minimum bactericidal concentration (MBC) by plating the suspension on an agar LB medium after 24 hours. All

experiments were performed in triplicate. The minimum inhibitory concentration was taken to be the minimum concentration at which there was no visible growth of the culture. The minimum bactericidal concentration was defined as the lowest concentration at which the growth of the test culture was completely absent.

The kinetics of the drug 4d activity

The kinetics of the action of preparation 4d and ciprofloxacin hydrochloride against *S. aureus* ATCC 25923 was assessed. The culture grown on agar LB medium was inoculated into MCB medium and cultured overnight. The cell suspension was mixed with the drugs and incubated at 37 °C in MCB. The final cell concentration was 1×105 CFU/ml. Ciprofloxacin hydrochloride at a concentration of 25 mkg/ml was used as a negative control. Bacterial samples (100 mkg) were taken at different time intervals (0, 30, 60, 120, 240, 360 min and 24h) and plated on agar LB medium to count grown colonies (20).

Results

The general scheme for the synthesis of polycationic compounds based on DABCO conjugated with ciprofloxacin is shown in Figure 1. Ciprofloxacin has two reactive groups that can be used for conjugation with a cationic amphiphile – the carboxyl group and the amino group of the piperazine ring. A fragment of 3-oxo-4-carboxylic acid is an active site of DNA gyrase binding. Thus, replacement or modification of the carboxyl group leads to the disappearance or significant decrease in antibacterial activity [20, 21].

On the contrary, modification of the pipyrazine ring, including acylation of the amino group does not lead to a decrease in antibacterial activity [23]. Based on these data, we synthesized compounds 2a-c which were later used for the synthesis of target conjugates. In order to establish whether the antibiotic residue can act as a hydrophobic part of the cationic amphiphile we synthesized compounds with an alkyl chain length of 4, 5 and 10 methylene fragments. To assess the effect of the linker group (L), compounds with flexible (4d) and rigid linker groups (4a, b, c) were synthesized (Figure I).

For the synthesis of target compounds 4a-d, two synthesis options were tested (Fig. I (A) and Fig. I (B). Treatment of 1, 4 – diazabicyclo[2.2.2]octane with bromo derivatives of ciprofloxacin 2a-c leads to the formation of mono-quaternary salts 3a-c with a yield of 67 to 97 %. Subsequent treatment with p-dichloroxylene or 1,5 – dibromopentane leads to the formation of the target dimers 4a-d in 23-84% exit.

Figure 1. here

On the contrary, the reaction of p-dichloroxylene or 1,5 – dibromopentane with 1,4 – diazabicyclo[2.2.2]octane leads to the formation of dimeric products 5a-d in almost quantitative exit. However, the reaction of bromo derivatives of ciprofloxacin with compounds 5a-d failed. The structures of all the obtained compounds were confirmed by NMR (1H, 13C) and mass spectroscopy. The resulting compounds were tested against a number of gram-positive and gram-

negative microorganisms. Unmodified ciprofloxacin (Cip) was used as a control. The results obtained are represented in Table 1.
Table 1 here

As can be seen from the presented data, the antibacterial activity was comparable to the antibacterial activity of the control antibiotic. In this case, the activity decreased with an increase in the length of the hydrophobic fragment connecting the polycationic part with ciprofloxacin.

Kinetics of drug action in vitro

For compound **4d** which showed the highest efficacy the antibacterial activity was investigated depending on the time of exposure using the example of the suppression of *S. aureus* ATCC 25923 similar to the work of Wang et.al [22].

The kinetics of the substance activity in vitro obtained as a result of experimental investigation is represented in Figure II.

Figure II here

The study of the kinetics of the action of drugs in vitro showed that compound 4d begins to show activity almost immediately after administration. After 4 hours, 100 % death of microorganisms is observed. While in the case of ciprofloxacin, approximately 10% of bacteria die in 4 hours.

Discussion

The antimicrobial activity of cationic amphiphiles strongly depends on its structure of the hydrophobic fragment. It usually consists of one or more linear saturated hydrocarbon chains but may also contain branches, points of unsaturation or rings. A number of researchers have demonstrated the relationship between the length of the hydrophobic chain of amphiphile and its antibacterial activity and the optimal efficacy is usually found at some intermediate chain length above and below which the activity decreases.

For gram-positive bacteria the optimal alkyl chain length is 12-24 carbon atoms while for gram-negative bacteria the maximum activity is achieved for compounds with a chain length of 14-16 carbon atoms. Compounds with an n-alkyl chain length of less than 4 or more than 18 carbon atoms are practically inactive [24, 25]. It should be noted that this effect is observed for compounds with different topologies, regardless of the number of cationic groups and alkyl residues [9, 26, 27].

The maximum activity with the same structure of the linker group (compopunds 4a-c) was observed with the smallest number of methylene units between ciprofloxacin and the polycationic part of the conjugate. Thus, it can be assumed that the total size of the hydrophobic part (alkyl fragment and antibiotic) is greater than or equal to the optimal size for gram-negative microorganisms.

Compounds 4a-c showed lower activity in the case of gram-positive microorganisms which is consistent with the literature data. The last one notes the need for a smaller size of the hydrophobic fragment for effective action on these microorganisms. Thus, when designing hybrid antibacterial drugs, it is necessary to take into account the hydrophobic properties of the antibiotic molecule.

DABCO-based polycationic amphiphiles containing rigid L linker groups have the same antibacterial activity against both *Staphylococcus aureus* and *Enterococcus faecalis*. We obtained similar results for ciprofloxacin [8]. However, in the case of conjugates of cationic amphiphiles with an antibiotic containing a similar rigid linker group there is a significantly lower activity against both gram-positive and all tested gram-negative microorganisms.

Conclusion

Thus, the results obtained generally confirm the possibility of obtaining new compounds with antibacterial activity by combining antibiotics and cationic amphiphiles into one structure. A significant acceleration of the death of *S. aureus* under the influence of compound **4d** can probably be due to the more rapid penetration of the conjugate into microorganisms due to the disruption of the cell membranes of microorganisms. However, to unambiguously establish the mechanism of action of the conjugates, additional studies are required.

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Conflicts of interest

There are no conflicts of interest.

Table 1: Antibacterial activity of compounds 4 a-d.

Tested compound		**S. aureus 2,0×10^5 KOE/ml**	***E. faecalis* 7,0×10^5 KOE/ml**	**S. enterica 2,0×10^5 KOE/ml**	***P. aeruginosa* 1,7×10^5 KOE/ml**	***C. freundii* 7,0×10^5 KOE/ml**	**E. coli 1,0×10^5 KOE/ml**	***P. mirabilis* 2,0×10^5 KOE/ml**
4a	МРК	25	100	6.3	25	3.2	1.6	12.5
	МБК	12.5	>100	6.3	25	3.2	3.2	25
4b	МРК	12.5	100	6.3	25	6.3	3.2	12.5
	МБК	12.5	>100	3.2	50	6.3	3.2	12.5
4c	МРК	25	100	12.5	25	12.5	6.3	25
	МБК	25	>100	12.5	50	12.5	12.5	25
4d	МРК	6.3	25	1.6	6.3	0.8	0.4	0.8
	МБК	12.5	50	1.6	12.5	3.2	0.4	1.6
Cip	МРК	3.2	3.2	3.2	3.2	3.2	0.1	3.2
	МБК	3.2	3.2	3.2	3.2	3.2	0.1	3.2

CFU – quantity of colony embodied units in 1 mL.

МБК – minimal bactericidal concentration.

МПК – minimal pressing concentration

Fig 1.

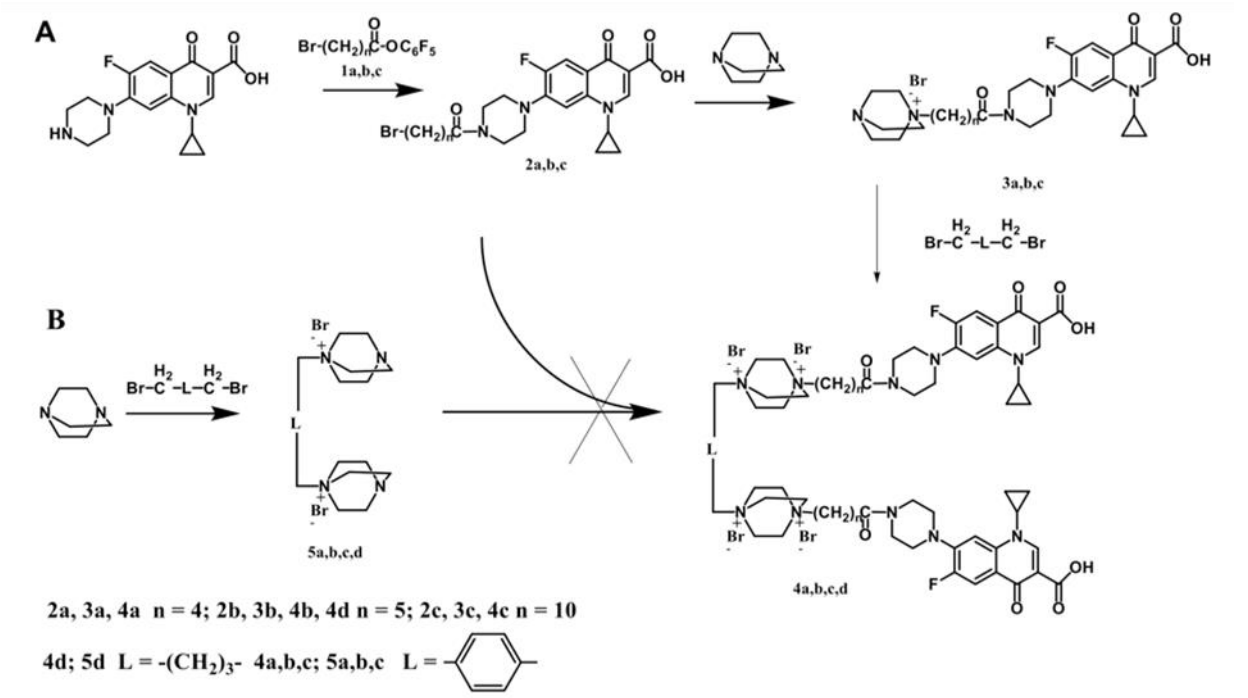
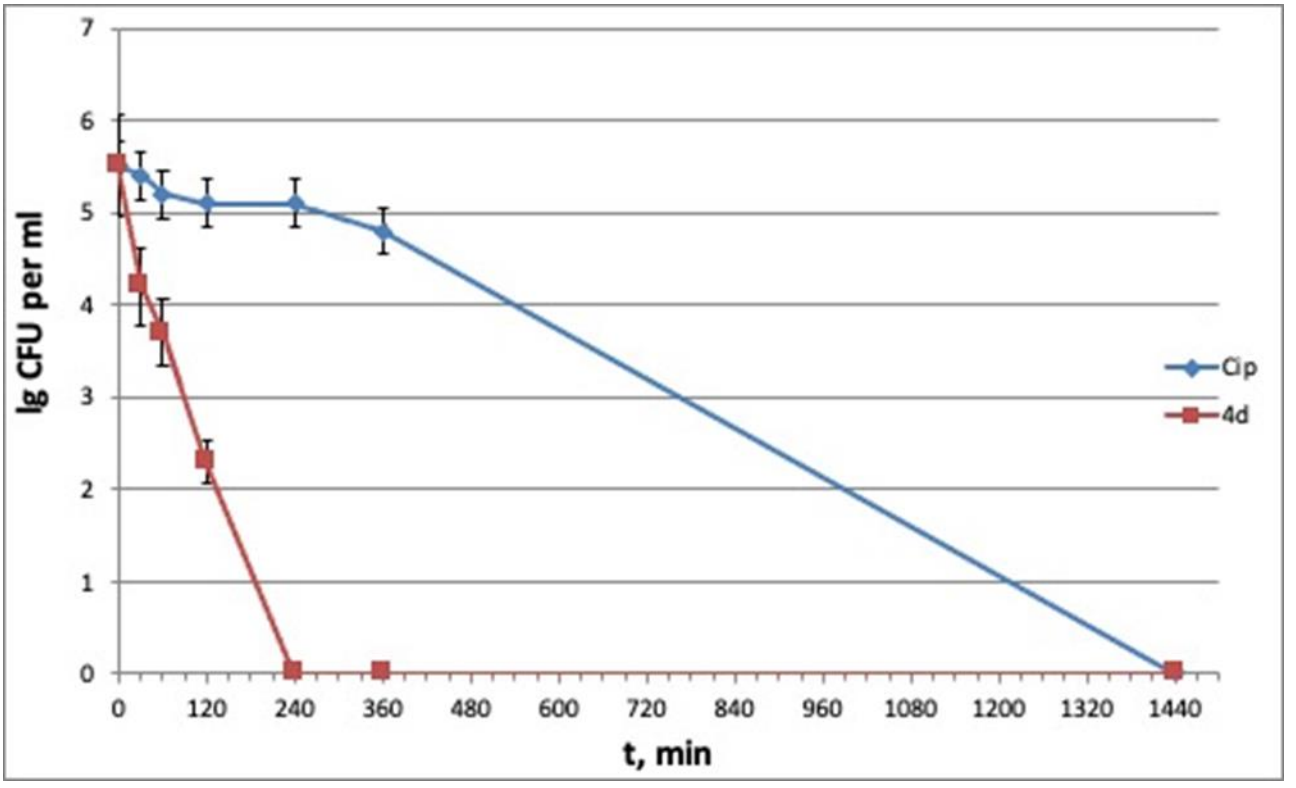

A
B
1a,b,c
2a,b,c
3a,b,c
5a,b,c,d
4a,b,c,d
2a, 3a, 4a n = 4; 2b, 3b, 4b, 4d n = 5; 2c, 3c, 4c n = 10
4d; 5d L = -(CH2)3- 4a,b,c; 5a,b,c L =

Fig 2.


lg CFU per ml
t, min
Cip
4d
0
1
2
3
4
5
6
7
0
120
240
360
480
600
720
840
960
1080
1200
1320
1440