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THE STUDY OF THE INTERACTION OF 1,2- AND 1,4-NAPHTHOQUINONES WITH AMINOPHOSPHONIC ESTERS

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A number of new biologically active phosphorus-containing quinones were prepared by the reaction of 2,3-dichloro-1,4-naphthoquinone and sodium 1,2-naphthoquinone-4-sulfonate with phosphonate nucleophilic reagents, aminophosphonic acid esters. The structures of the synthesized compoumds were confirmed by ESI-MS, ^{1}H NMR, IR-spectroscopies and elemental analysis. It was shown that aminophosphonic esters form products of nucleophilic substitution of a chlorine atom of 1,4-naphthoquinone or a sulfonyl group of 1,2-naphthoquinone on an aminophosphonic fragment. The products of the interaction of sodium 1,2-naphthoquinone-4-sulfonate with primary aminophosphonates existed in solution in 1,2-quinoid or 2-hydroxy-1,4-quinonimine tautomeric form depending on pH. The antimicrobial activity of the prepared compounds was investigated against *Escherichia coli* B-906, *Staphylococcus aureus* 209-P, *Mycobacterium luteum* B-917, *Candida tenuis* VKM Y-70 and *Aspergillus niger* VKM F-1119 strains by the method of diffusion in agar of their 0.1% and 0.5% solutions. 1,2-Naphthoquinone derivatives showed good activity against *S. aureus* at a concentration of 0.1%, in its turn 1,4-napthoquinone derivatives showed activity against *M. luteum* at a concentration of 0.5%.

Keywords: naphthoquinone derivatives, aminophosphonic esters, 1,4-naphthoquinones, 1,2-naphthoquinones, nucleophilic substitution, antimicrobial activity, tautomeric form, ^{1}H NMR spectra.

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Introduction

Over the last quarter of a century, in world science, special attention has been paid to research in the field of chemistry and technology of organic phosphorus compounds, including those with phosphonic acid residues. For example, an important class of phosphorus compounds is represented by nitrogen-containing phosphonic acids and their derivatives, which have a pyrophosphate fragment that ensures high affinity with calcium [1]. Some representatives of the class of aminophosphonate derivatives have been shown to be effective in the treatment of osteoporosis and disorders associated with hypercalcemia caused by malignant neoplasms [2,3]. Opportunities for expanding the use of aminophosphonates in clinical medicine for the treatment of oncological diseases are currently being explored. In particular, there is interest in the use of aminophosphonates in cancer immunotherapy, as they activate gd T-cells of the immune system to destroy tumor cells [4]. In addition, aminophosphonates are promising drug candidates for the treatment of pathogenic parasitic infections caused by *Plasmodium spp*, *Leishmania species*, *Trypanosoma cruzi* and other protozoan parasites [5]. Increasing interest in nitrogen-containing phosphonic acids and their derivatives has led to the development of various strategies for their synthesis [6].

Naphthoquinones are the most common type of quinones in nature. They are a diverse family of

derivatives that are naturally found in plants, lichens and various microorganisms. This subgroup is constantly expanding due to the discovery of new natural products and the synthesis of new compounds [7–13]. Interest in quinones and the search for new biological activities in representatives of this class have increased in recent years, as evidenced by the assessment of the potential antimicrobial activity of quinones [10–13].

Considering that the reactions of phosphonic acids and their derivatives with quinones are currently poorly studied, we decided to investigate the interaction of aminophosphonic esters with 1,2- and 1,4-naphthoquinones, which undoubtedly has significant theoretical and practical interest.

Experimental

General methods

All the chemicals were purchased from Aldrich Chemical Company (USA) and were used without further purification. Melting points were determined on a Bьchi capillary melting point apparatus and are uncorrected. The elemental analyses were performed using the Perkin-Elmer 2400 CHN analyzer. ^{1}H NMR spectra were recorded on a Bruker AC 200 spectrometer. The ^{1}H chemical shifts are reported from TMS. IR spectra were recorded on Specord M-80 IR spectrophotometer within the range of 4000–500 cm^{-1} in KBr tablets. Electrospray ionization mass spectrometry (ESI-MS) was performed on Agilent 1100 Series (LC/MSD Trap) Spectrometer applying gradient elution: A) H_2O+0.1% HCOOH; B) CH_3CN+0.1% HCOOH. TLC was performed on 5 cm×10 cm aluminum plates coated with silica gel 60 F254 (Merck) in an appropriate solvent.

General procedures for the synthesis

General method of synthesis of aminophosphonate derivatives of 1,4-naphthoquinone 1.2 (a–d)

To a solution of 2.27 g (0.01 mol) of 2,3-dichloro-1,4-naphthoquinone 1 in 50 ml of acetonitrile with magnetic stirring at room temperature an equimolar amount of the corresponding aminoalkylphosphonate 1.1 (a–d) (0.01 mol), 2.76 g (0.02 mol) of potassium carbonate and 0.36 g (5 mol.%) of dibenzo-18-crown-6 were added. The reaction mixture was heated and kept under reflux for 2 hours. The completion of the reaction was monitored by TLC (EtOAc/acetone 1:1). The reaction mass was cooled and filtered, the filtrate was evaporated *in vacuo*. The resulting crystals were dissolved in 30% ethyl alcohol, neutralized with 10% hydrochloric acid to pH 5 and poured onto ice. The resulting precipitate was filtered and recrystallized from 50% ethyl alcohol.

Diethyl (((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)methyl)phosphonate 1.2a. Yield 81%, m.p. 86–87^{0}C. IR (KBr): 3302 (NH); 1676 (C–O); 1600, 1594 (C–C); 1294 (P=O); 1084 (P–O–C). ^{1}H NMR (200 MHz, $CDCl_3$), δ, ppm: 9.22 (br.s, 1H, NH), 8.09 (d, *J*=7.8 Hz, 1H quinone), 7.96 (d, *J*=7.8 Hz, 1H, quinone), 7.61–7.44 (m, 2H, quinone), 4.38–3.94 (m, 4H, $2CH_2$), 3.85 (d, *J*=12.4 Hz, 2H, CH_2), 1.31 (t, *J*=7.1 Hz, 3H, CH_3), 1.09 (t, *J*=7.1 Hz, 3H, CH_3). Calculated ($C_{15}H_{17}ClNO_5P$), %: C 50.36, H 4.79, Cl 9.91, N 3.92, P 8.66. Found, %: C 50.24, H 4.68, Cl 9.84, N 3.86, P 8.76. LC-MS m/z (% relative intensity): $[M+H]^+$ 358 (100), 359 (17), 360 (35), 361 (6).

Diethyl (((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)(phenyl)methyl) phosphonate 1.2b. Yield 76%. m.p. 78–79^{0}C. IR (KBr): 3312 (NH); 1686 (C–O); 1606, 1596 (C–C); 1296 (P=O); 1026 (P–O–C). ^{1}H NMR (200 MHz, $CDCl_3$), δ, ppm: 9.32 (br.s, 1H, NH), 8.10 (d, *J*=7.8 Hz, 1H quinone), 7.95 (d, *J*=7.8 Hz, 1H, quinone), 7.75–7.49 (m, 4H), 7.33 (t, *J*=7.4 Hz, 2H Ar), 7.27 (t, *J*=7.4 Hz, 1H Ar), 5.62 (d, *J*=18.6 Hz, 1H, CH), 4.29–3.80 (m, 4H, $2CH_2$), 1.25 (t, *J*=7.0 Hz, 3H, CH_3) , 1.07 (t, *J*=7.0 Hz, 3H, CH_3). Calculated ($C_{21}H_{21}ClNO_5P$), %: C 58.14, H 4.88, Cl 8.17, N 3.23, P 7.14. Found, %: C 58.08, H 4.80, Cl 8.22, N 3.20, P 7.26. LC-MS m/z (% relative intensity): $[M+H]^+$ 434 (100), 435 (24), 436 (36), 437 (8).

Diethyl (2-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)propan-2-yl) phosphonate 1.2c. Yield 72%, m.p. 160–162^{0}C. IR (KBr): 3344 (NH); 1672 (C–O); 1612, 1600 (C–C); 1286 (P=O); 1036 (P–O–C). ^{1}H NMR (200 MHz, $CDCl_3$), δ, ppm: 9.25 (br.s, 1H, NH), 7.99 (d, *J*=7.8 Hz, 1H quinone), 7.93 (d, *J*=7.8 Hz, 1H, quinone), 7.71–7.48 (m, 2H, quinone), 4.29–3.91 (m, 4H, $2CH_2$), 1.59 (d, *J*=16.6 Hz, 6H, $2CH_3$), 1.35–1.22 (m, 6H, $2CH_3$). Calculated ($C_{17}H_{21}ClNO_5P$), %: C 52.93, H 5.49, Cl 9.19, N 3.63, P 8.03. Found, %: C 52.85, H 5.48, Cl 9.25, N 3.54, P 8.11. LC-MS m/z (% relative intensity): $[M+H]^+$ 386 (100), 387 (19), 388 (35), 389 (6).

Diethyl (1-(butyl(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)ethyl) phosphonate 1.2d. Yield 64%, m.p. 180–181^{0}C. IR (KBr): 1644 (C–O); 1608, 1588 (C–C); 1254 (P=O); 1024 (P–O–C). ^{1}H NMR (200 MHz, $CDCl_3$), δ, ppm: 8.02 (d, *J*=7.8 Hz, 1H quinone), 7.94 (d, *J*=7.8 Hz, 1H, quinone), 7.74–7.44 (m, 2H, quinone), 4.23–3.71 (m, 5H, $2CH_2$, CH), 3.59–3.21 (m, 2H, CH_2), 1.80 (quint., *J*=7.4 Hz, 2H, CH_2), 1.48–1.10 (m, 11H), 0.94 (t, *J*=7.2 Hz, 3H, CH_3). Calculated

($C_{20}H_{27}ClNO_5P$), %: C 56.14, H 6.36, Cl 8.29, N 8.29, P 7.24. Found, %: C 56.06, H 6.30, Cl 8.34, N 3.20, P 7.32. LC-MS m/z (% relative intensity): $[M+H]^+$ 428 (100), 429 (23), 430 (36), 431 (8).

General method of synthesis of aminophosphonate derivatives of 1,2-naphthoquinone 1.4 (a–c)

To a solution of the corresponding aminophosphonic ester 1.1 (a–c) (0.01 mmol) in 10 ml of DMSO 2.60 g (0.01 mol) of sodium 1,2-naphthoquinone-4-sulfonate 1.3 was added and kept at 60°C with stirring for 12 hours. The completion of the reaction was monitored by TLC (EtOAc/acetone 1:1). The reaction mixture was precipitated with water (50 ml), acidified with 5% HCl (aq.) to pH 4–5 and extracted with 3×20 ml of DCM. The combined organic layers were extracted with 3×20 ml of 5% NaOH, the aqueous phase was acidified with 20% HCl to pH 3–4, the formed precipitate was filtered, washed with water, dried in a vacuum over $CaCl_2$, recrystallized from EtOAc and dried in a vacuum.

Diethyl (((3,4-dioxo-3,4-dihydronaphthalen-1-yl)amino)methyl)phosphonate 1.4a. Yield 86%, m.p. 134–136°C. IR (KBr): 3308(NH); 1686(C=O); 1280(P=O); 1026(P–O–C). ^{1}H NMR (200 MHz, $CDCl_3$), δ, ppm: 9.50 (br.s, 1H, NH), 8.00 (d, *J*=7.6 Hz, 1H quinone), 7.93–7.68 (m, 2H, quinone), 7.35 (t, *J*=7.7 Hz, 1H, quinone), 5.54 (s, 1H, quinone), 4.39–3.92 (m, 4H, $2CH_2$), 3.49 (d, *J*=12.4 Hz, 2H, CH_2), 1.34 (t, *J*=7.1 Hz, 3H, CH_3), 1.11 (t, *J*=7.1 Hz, 3H, CH_3). Calculated ($C_{15}H_{18}NO_5P$), %: C 55.73, H 5.61, N 4.33, P 9.58. Found, %: C 55.67, H 5.57, N 4.36, P 10.03. LC-MS m/z (% relative intensity): $[M+H]^+$ 324 (100), 325 (17), 326 (2).

Diethyl (((3,4-dioxo-3,4-dihydronaphthalen-1-yl)amino)(phenyl)methyl)phosphonate 1.4b. Yield 83%. m.p. 162–164°C. IR (KBr): 3342(NH); 1680(C=O); 1284(P=O); 1048(P–O–C). ^{1}H NMR (200 MHz, $CDCl_3$), δ, ppm: 9.44 (br.s, 1H, NH), 8.07 (d, *J*=7.8 Hz, 1H quinone), 7.87 (d, *J*=7.8 Hz, 1H, quinone), 7.70 (t, *J*=7.6 Hz, 1H quinone), 7.63–7.46 (m, 2H, Ar), 7.43–7.19 (m, 3H, Ar), 5.49 (s, 1H quinone), 7.27 (t, *J*=7.4 Hz, 1H Ar), 5.23 (d, *J*=18.6 Hz, 1H, CH), 4.29–3.80 (m, 4H, $2CH_2$), 1.25–1.02 (m, 6H, $2CH_3$). Calculated ($C_{21}H_{22}NO_5P$), %: C 63.16, H 5.55, N 3.51, P 7.76. Found, %: C 63.08, H 5.54, N 3.60, P 7.81. LC-MS m/z (% relative intensity): $[M+H]^+$ 400 (100), 401 (24), 402 (4). t_r=1.035 min.

Diethyl (2-((3,4-dioxo-3,4-dihydronaphthalen-1-yl)amino)propan-2-yl)phosphonate 1.4c. Yield 75%, m.p. 154–156°C. IR (KBr): 3336(NH); 1684(C=O); 1290(P=O); 1032(P–O–C). ^{1}H NMR (200 MHz, $CDCl_3$), δ, ppm: 9.48 (br.s, 1H, NH), 8.00 (d, *J*=7.8 Hz, 1H quinone), 7.81–7.60 (m, 2H, quinone), 7.30 (t, *J*=7.7 Hz, 1H, quinone), 5.47 (s, 1H quinone), 4.29–3.89 (m, 4H, $2CH_2$), 1.53 (d, *J*=16.6 Hz, 6H, $2CH_3$), 1.29 (t, *J*=7.1 Hz, 3H, CH_3), 1.09 (t, *J*=7.1 Hz, 3H, CH_3). Calculated ($C_{21}H_{22}NO_5P$), %: C 58.18, H 6.31, N 3.99, P 8.82. Found, %: C 58.04, H 6.29, N 4.04, P 8.90. LC-MS m/z (% relative intensity): $[M+H]^+$ 352 (100), 353 (19), 354 (3).

Method of synthesis of diethyl (1-(butyl(3,4-dioxo-3,4-dihydronaphthalen-1-yl)amino)ethyl)phosphonate 1.4d

To a solution of 2.37 g (0.01 mmol) of diethyl (1-(butylamino)ethyl)phosphonate 1.1d in 10 ml of DMSO 2.60 g (0.01 mol) of sodium 1,2-naphthoquinone-4-sulfonate 1.3 was added and kept at 60°C with stirring for 12 hours. The completion of the reaction was monitored by TLC (EtOAc/acetone 1:1). The reaction mixture was precipitated with water (50 ml), acidified with 5% HCl (aq.) to pH 4–5 and extracted with 3×20 ml of DCM. The combined organic layers were extracted with 3×20 ml of water, organic phase was evaporated in vacuum and the residue was recrystallized from EtOAc and dried in a vacuum. Yield 72%, m.p. 140–142°C. IR (KBr): 3340(NH); 1680(C=O); 1296(P=O); 1064(P–O–C). ^{1}H NMR (200 MHz, $CDCl_3$), δ, ppm: 8.09 (d, *J*=7.6 Hz, 1H quinone), 7.83 (t, *J*=7.6 Hz, 1H, quinone), 7.39–7.19 (m, 2H, quinone), 5.70 (s, 1H quinone), 4.23–3.71 (m, 5H, $2CH_2$, CH), 3.40–3.12 (m, 2H, CH_2), 1.85 (quint., *J*=7.4 Hz, 2H, CH_2), 1.65–1.17 (m, 11H), 0.87 (t, *J*=7.2 Hz, 3H, CH_3). Calculated ($C_{20}H_{28}NO_5P$), %: C 61.06, H 7.17, N 3.56, P 7.87. Found, %: C 61.01, H 7.16, N 3.60, P 7.79. LC-MS m/z (% relative intensity): $[M+H]^+$ 394 (100), 395 (23), 396 (3).

Antimicrobial and antifungal activity

Antimicrobial and antifungal activity has been studied by diffusion in agar on solid nutrient medium (beefextract agar for bacteria, wort agar for fungi). Petri plates containing 20 ml of nutrient medium were used for all the microorganisms that were tested. The inoculums (the microbial loading 10^9 cells (spores)/1 ml) was spread on the surface of the solidified media and Whatman no.1 filter paper discs (6 mm in diameter) impregnated with the test compound (0.1 and 0.5%) were placed on the plates. The duration of bacteria incubation was 24 h at 35°C and of fungi incubation 48–72 h at 28–30°C [14]. The antimicrobial effect and degree of activity of the tested compounds were evaluated by measuring the zone diameters. Control disk contained vancomicine (for bacteria) or nistatine (for fungi) as a standard. Every experiment

was repeated three times.

Statistical analysis

All results are expressed as mean±standard error mean (SEM). One-way analysis of variance (ANOVA) was performed to determine the statistical significance of the results followed by Tukey's *post hoc* comparison. ** $p<0.01$ and * $p<0.05$ was considered as significant. All statistical analyses were conducted using GraphPad Prism 8.4.2 (GraphPad Software Inc., San Diego, CA).

Results and discussion

Study of the interaction of 1,2- and 1,4-naphthoquinones with esters of aminophosphonic acids

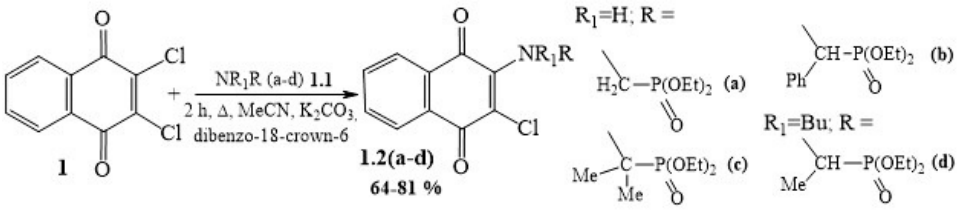
Derivatives of naphthoquinones are used in organic synthesis as convenient reagents for obtaining various products with useful properties. Some ways used in the preparation of naphthoquinone derivatives are nucleophilic substitution of halogenated quinones [10], cycloaddition and cyclocondensation of 1,4-naphthoquinone derivatives [7,8,11], Michael addition to 1,4- and 1,2-naphthoquinone derivatives [9]. Reactions of aminophosphonic acids and their derivatives with quinones are currently poorly studied, so we decided to investigate the interaction of aminophosphonic esters with derivatives of 1,4- (Scheme 1) and 1,2- (Scheme 2) naphthoquinones and to investigate the antimicrobial activity of the obtained compounds.

It was established that the optimal conditions for the interaction of 2,3-dichloro-1,4-naphthoquinone 1 with esters of aminophosphonic acids 1.1 (a–d) are to conduct the interaction in the presence of anhydrous K_2CO_3 in MeCN with the addition of 5% dibenzo-18-crown-6 or in DMF at 70–80^{0}C for 2 hours. Products 1.2 (a–d) were obtained with the yields of 75–82%.

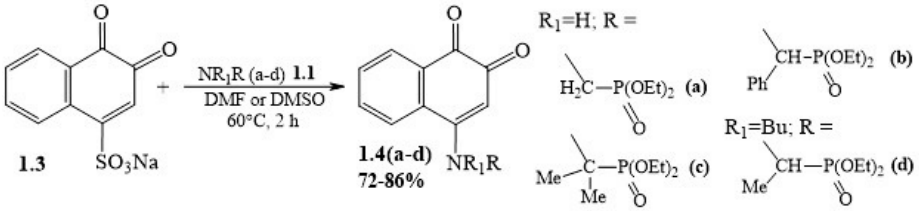
The structure of aminophosphonic derivatives of 1,4-naphthoquinone 1.2 (a–d) was confirmed by the results of elemental analysis and spectral data (^{1}H NMR and IR). For example, in ^{1}H NMR spectrum of diethyl (((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)(phenyl)methyl)-phosphonate 1.2b in $CDCl_3$ solution characteristic shifts of protons of two methyl groups are observed as two triplets of six protons at 1.25 ppm and 1.07 ppm, a multiplet of four protons of methylene groups is located at 4.29–3.80 ppm, a doublet of a methine proton is observed at 5.62 ppm, the signal of an amino group proton is observed as a broadened singlet at 9.32 ppm, the signals of aromatic and quinoid of protons are observed as a characteristic system of signals at 8.10 (d, J=7.8 Hz, 1H quinone), 7.95 (d, J=7.8 Hz, 1H, quinone), 7.75–7.49 (m, 4H), 7.33 (t, J=7.4 Hz, 2H Ar), 7.27 (t, J=7.4 Hz, 1H Ar), accordingly. In addition, the structure of obtained compounds was confirmed by IR spectroscopy. In the IR spectra of compounds 1.2 (a–d), characteristic absorption bands of quinoid carbonyl groups are observed at 1686–1640 cm^{-1}, a characteristic band of valence vibrations of the NH group at 3360–3310 cm^{-1} and intense absorption of the P=O bond at 1296–1256 cm^{-1} and P–O–C_{Et} group at 1056–1024 cm^{-1}.

The interaction of sodium 1,2-naphthoquinone-4-sulfonate 1.3 with aminophosphonic acids esters 1.1 (a–d) occurs in milder conditions than the similar reaction of 1.1 (a–d) with 2,3-dichloro-1,4-quinone 1. Reaction of sodium 1,2-naphthoquinone-4-sulfonate 1.3 with aminophosphonic acids 1.1 (a–d) was carried out in DMF or DMSO at 60^{0}C for 2 hours. Products 1.4 (a–d) were obtained with the preparative yields of 72–86%.

The structure of 1,2-naphthoquinone aminophosphonic derivatives 1.4 (a–d) was confirmed by the results of elemental analysis and spectral data (^{1}H NMR and IR). For example, in ^{1}H NMR spectrum of diethyl (((3,4-dioxo-3,4-dihydronaphthalen-1-yl)amino)methyl)phosphonate 1.4a in $CDCl_3$ solution the signals of protons of two methyl groups are observed as two triplets of six protons at 1.25 ppm and 1.07 ppm (J=7.0 Hz), two methylene groups are observed as a multiplet of four protons at 4.29–3.80 ppm, the doublet of two protons of the methylene group is observed at 3.49 ppm (J=12.4 Hz). A wide singlet at 9.50 ppm corresponding to the signal of the amino group proton and a characteristic system of quinoid proton signals is present at 8.00 (d, J=7.6 Hz, 1H), 7.93–7.68 (m, 2H), 7.35 (t, J=7.7 Hz, 1H), 5.54 (s, 1H) indicate



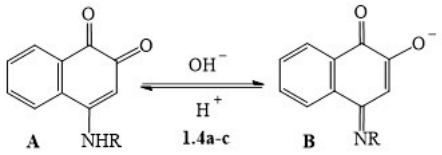
Scheme 1. Synthesis of aminophosphonic derivatives of 1,4-naphthoquinone 1.2 (a–d)



Scheme 2. Synthesis of aminophosphonic derivatives of 1,2-naphthoquinone 1.4 (a–d)

1,2-quinoid tautomeric form. In addition, the structure of obtained compounds was confirmed by IR spectroscopy. In the IR spectra of compounds 1.4 (a–d), characteristic absorption bands of quinoid carbonyl groups are observed at 1686–1640 cm^{-1}, a characteristic band of valence vibrations of the NH group is observed at 3342–3308 cm^{-1}, intense absorption of the P=O bond at 1288–1264 cm^{-1} and P–O–C_{Et} group vibrations are observed at 1096–1080 cm^{-1}.

When studying the structure of the interaction products of sodium 1,2-naphthoquinone-4-sulfonate 1.3 with aminophosphonic acids 1.1 (a–d), it was established that the products 1.4 (a–c) undergo tautomeric transformations (Scheme 3), which is confirmed by the results of ultraviolet spectroscopy and is consistent with literature data on similar structures [15].



Scheme 3. Tautomeric equilibrium of products 1.4 (a–c)

Thus, in acidic and neutral conditions, compounds 1.4 (a–c) exist in the 4-(R)-amino-1,2-naphthoquinone tautomeric form A ($\nu_{C=O}$ 1700 and 1684 cm^{-1} and λ_{max} 529.5 nm). In an alkaline medium, the color changes from red to violet (λ_{max} 584 nm): the tautomeric form of 2-hydroxy-1,4-naphthoquinone-4-(R)-imines form B of the structures of products 1.4 (a–c) is formed. This makes it possible to use acid-base extraction for their isolation, since in molecular form the products are well soluble in the organic phase and in anionic form in the aqueous phase.

Study of antimicrobial activity of 1,2- and 1,4-naphthoquinone containing aminophosphonic acids derivatives.

The synthesized compounds were evaluated for antibacterial and antifungal activity against *Escherichia coli* B-906, *Staphylococcus aureus* 209-P, *Mycobacterium luteum* B-917, *Candida tenuis* VKM Y-70 and *Aspergillus niger* VKM F-1119 strains by the agar diffusion method [14]. Their activity was compared to that of the known antibacterial agent, vancomycin and the antifungal agent nystatin.

Antibacterial and antifungal activity of derivatives of quinones has been studied in our laboratory previously [10,11]. Depending on the substituent, the results of antimicrobial activity of synthesized compounds differed in the degree of influence on bacteria and fungi. Analyzing the data of investigations, it can be summarized that products of monosubstitution of 2,3-dichloro-1,4-naphthoquinone possess higher activity than derivatives of 1,2-naphthoquinone ones.

The synthesized compounds 1.2–1.4 (a–d) were evaluated for antibacterial and antifungal activity. Results of estimate diameter of microorganism growth inhibition zones according to the parameters are listed in Tables 1 and 2.

Antimicrobial activity data analysis of heterocyclic quinoid derivative series showed that studied microorganisms were predominantly insensitive to the synthesized derivatives. The compounds 1.2–1.4 (a–d) had good activity against *S. aureus* at a concentration of 0.1% and 0.5% (Table 1). The strain *M. luteum* was most sensitive to compounds 1.2 (b–d); 1.4 (b, d) at a concentration of 0.5% (Table 1). The compounds 1.2–1.4 (a–d) had low antifungal activity against *C. tenuis* and *A. niger* (Table 2) and had no antibacterial activity against *E. coli* at 0.1 and 0.5% concentration evaluated by the diffusion method (Table 1).

Conclusions

Methods of the synthesis of new biologically active phosphorus-containing quinones by the interaction of 1,2- and 1,4-naphthoquinones with aminophosphonic nucleophilic reagents were developed. It has been proven that aminophosphonic acids form products of nucleophilic substitution of the chlorine atom of 1,4-naphthoquinone or the sulfonyl group of 1,2-naphthoquinone on an aminophosphorus-containing fragment. It was established that the products of the interaction of 1,2-naphthoquinone with primary aminophosphonates

Table 1

Antibacterial activity of investigated compounds by agar diffusion method*

Compound	Diameter of the zones of microbial growth inhibition, mm; Mean±SD					
	E. coli		*S. aureus*		*M. luteum*	
	0.10%	0.50%	0.10%	0.10%	0.50%	0.10%
1.2a	0	0	11.5±0.27	15.1±0.39	9.7±0.43	11.8±0.41
1.2b	0	0	12.4±0.21	16.4±0.43	10.7±0.35	16.9±0.49
1.2c	0	0	13.2±0.34	16.2±0.43	7.7±0.35	19.1±0.43
1.2d	0	0	11.7±0.30	16.5±0.47	14.6±0.39	19.2±0.45
1.4a	0	0	10.5±0.27	17.1±0.39	10.6±0.43	11.8±0.41
1.4b	0	0	12.4±0.21	19.4±0.43	11.9±0.35	16.9±0.49
1.4c	0	0	12.2±0.34	18.2±0.43	9.7±0.35	11.1±0.43
1.4d	0	0	14.7±0.30	18.5±0.47	13.6±0.39	17.2±0.45
C**	0	14±0.31	11.4±0.31	18.6±0.43	17.8±0.41	19.7±0.47

Notes: * – all analyses were carried out in triplicate, and results are reported as the mean±standard deviation (SD).
** – vancomycin was used as a control in the tests of antibacterial activity of the synthesized compounds.

Table 2

Antifungal activity of investigated compounds by agar diffusion method*

Comp.	Diameter of the zones of microbial growth inhibition, mm; Mean±SD			
	C. tenuis		*A. niger*	
	0.10%	0.50%	0.10%	0.50%
1.2a	10.7±0.35	10.8±0.25	6.8±0.50	13.1±0.53
1.2b	7.7±0.35	9.9±0.35	6.2±0.35	15.5±0.58
1.2c	10.7±0.35	10.5±0.55	6.8±0.30	14.2±0.57
1.2d	8.5±0.57	12.1±0.45	6.6±0.35	15.9±0.51
1.4a	0	10.8±0.25	6.3±0.40	12.1±0.53
1.4b	0	9.9±0.35	0	10.5±0.58
1.4c	0	10.5±0.55	0	11.2±0.57
1.4d	8.5±0.57	12.1±0.45	6.6±0.35	11.9±0.51
C**	15.3±0.51	21.0±0.51	8.7±0.50	22.1±0.58

Notes: * – all analyses were carried out in triplicate, and results are reported as the mean±standard deviation (SD).
** – nystatin was used as a control in the tests of antifungal activity of the synthesized compounds.

1,2-naphthoquinone with primary aminophosphonates exist in the acidic and neutral medium in the 1,2-quinone tautomeric form, and in the alkaline medium in the 2-hydroxy-1,4-quinonimine tautomeric form. The synthesized compounds were evaluated for antibacterial and antifungal activity against *Escherichia coli* B-906, *Staphylococcus aureus* 209-P, *Mycobacterium luteum* B-917, *Candida tenuis* VKM Y-70 and *Aspergillus niger* VKM F-1119 strains by the agar diffusion method. The products 1.4 (b–d) show good activity against *S. aureus* at a concentration of 0.1% and 0.5%. The strain *M. luteum* is sensitive to compounds 1.2 (c,d) at a concentration of 0.5%. Analyzing the data of investigations, it can be summarized that products of monosubstitution of 2,3-dichloro-1,4-naphthoquinone possess higher activity than derivatives of 1,2-naphthoquinones.

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ВИВЧЕННЯ ВЗАЄМОДІЇ 1,2- ТА 1,4-НАФТОХІНОНІВ З АМІНОФОСФОНОВИМИ ЕСТЕРАМИ

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Реакцією 2,3-дихлор-1,4-нафтохінону та 1,2-нафтохінон-4-сульфонату натрію з фосфонатними нуклеофільними реагентами – ефірами амінофосфонової кислоти одержано низку нових біологічно активних фосфоровмісних хінонів, будова яких підтверджено ESI-MS, ^{1}H-ЯМР, ІЧ-спектроскопією та елементним аналізом. Показано, що естери амінофосфонової кислоти утворюють продукти нуклеофільного заміщення атома хлору 1,4-нафтохінону або сульфонільної групи 1,2-нафтохінону на амінофосфоновий фрагмент. Продукти взаємодії 1,2-нафтохінон-4-сульфонату натрію з первинними амінофосфонатами знаходяться в розчині в 1,2-хіноїдній або 2-гідрокси-1,4-хініміновій таутомерній формі залежно від pH. Антимікробну активність одержаних сполук досліджено щодо штамів *Escherichia coli* B-906, *Staphylococcus aureus* 209-P, *Mycobacterium luteum* B-917, *Candida tenuis* VKM Y-70 and *Aspergillus niger* VKM F-1119 методом дифузії в агар їх 0,1% та 0,5% розчинів. Похідні 1,2-нафтохінону показали хорошу активність проти *S. aureus* у концентрації 0,1%, у свою чергу похідні 1,4-нафтохінону виявляли активність проти *M. luteum* у концентрації 0,5%.

Ключові слова: похідні нафтохінону, естери амінофосфонових кислот, 1,4-нафтохінони, 1,2-нафтохінони, нуклеофільне заміщення, антимікробна активність, таутомерна форма, спектри ^{1}H NMR.

THE STUDY OF THE INTERACTION OF 1,2- AND 1,4-NAPHTHOQUINONES WITH AMINOPHOSPHONIC ESTERS

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A number of new biologically active phosphorus-containing quinones were prepared by the reaction of 2,3-dichloro-1,4-naphthoquinone and sodium 1,2-naphthoquinone-4-sulfonate with phosphonate nucleophilic reagents, aminophosphonic acid esters. The structures of the synthesized compoumds were confirmed by ESI-MS, ^{1}H NMR, IR-spectroscopies and elemental analysis. It was shown that aminophosphonic esters form products of nucleophilic substitution of a chlorine atom of 1,4-naphthoquinone or a sulfonyl group of 1,2-naphthoquinone on an aminophosphonic fragment. The products of the interaction of sodium 1,2-naphthoquinone-4-sulfonate with primary aminophosphonates existed in solution in 1,2-quinoid or 2-hydroxy-1,4-quinonimine tautomeric form depending on pH. The antimicrobial activity of the prepared compounds was investigated against *Escherichia coli* B-906, *Staphylococcus aureus* 209-P, *Mycobacterium luteum* B-917, *Candida tenuis* VKM Y-70 and *Aspergillus niger* VKM F-1119 strains by the method of diffusion in agar of their 0.1% and 0.5% solutions. 1,2-Naphthoquinone derivatives showed good activity against *S. aureus* at a concentration of 0.1%, in its turn 1,4-napthoquinone derivatives showed activity against *M. luteum* at a concentration of 0.5%.

Keywords: naphthoquinone derivatives; aminophosphonic esters; 1,4-naphthoquinones; 1,2-naphthoquinones; nucleophilic substitution; antimicrobial activity; tautomeric form; ^{1}H NMR spectra.

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