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Synthesis, computational and antimicrobial studies of new 1,4-naphthoquinone aminothiazole derivatives

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The synthesis of new aminothiazole derivatives on the basis of substituted 1,4-naphthoquinones was carried out by the interaction of 2-R-3,6-dichloro-7-acylamino-1,4-naphthoquinones and potassium thiocyanate, 3,6-dichloro-2,7-diacylamino-1,4-naphthoquinone and dithiocyanate, of 2-R-3,6-dichloro-7-amino-1,4-naphthoquinones, 2-amino-7-nitro-3,6-dichloro-1,4-naphthoquinone and thiourea with the subsequent cyclization in aminothiazoles of 1,4-naphthoquinone. The physical and chemical properties of these compounds were determined, and the methods of their preparation are presented. The PASS computer program was used to predict the biological activity spectra of the new 1,4-naphthoquinone aminothiazole derivatives and to determine the most promising biological activities for experimental testing. Antibacterial and fungicidal activities were studied using the cultures of *Staphylococcus aureus*, *Escherichia coli* and *Candida tenuis* microorganisms. Some of the study compounds were found to have a moderate antibacterial and fungicidal activity, which in more than 90 % of cases coincided with the computational predictions. The analysis of the structure – antimicrobial activity relationships provides recommendations for the design of the new derivatives. Some other biological activities have been predicted by PASS for these compounds, including antiviral, antineoplastic, immunomodulating, acute neurological disease treatment, antiparkinsonian, etc., which help to identify areas for the further research. Thus, the 1,4-naphthoquinone aminothiazole derivatives have been shown to be a promising class for the preparation of novel pharmacological agents with different applications.

Key words: naphthoquinone, thiocyanate, thiourea, thiazole, heterocyclization, biological activities, computational prediction, PASS computer program, antimicrobial action

Introduction

The thiazole ring system is a useful structural motif found in numerous biologically active molecules. This structure is utilized in developing drugs for the treatment of allergies [1], hypertension [2], inflammation [3], schizophrenia [4], bacterial [5] and HIV [6] infections. Aminothiazoles are known to be ligands of estrogen receptors [7] as well as a novel class of adenosine receptor antagonists [8], whereas other analogues are used as fungicides, inhibiting *in vivo* the growth of *Xanthomonas* and as an ingredient of herbicides or as schistosomicidal and anthelmintic drugs [9].

Heterocyclic compounds based on of 1,4-naphthoquinone derivatives have many valuable properties and are used as antimicrobial, antitumor substances, dyes, catalysts, medications [10–12]. Therefore, taking into account their practical value, the synthesis of new S,N-containing heterocyclic compounds on the basis of 2,3,6,7-substituted 1,4-naphthoquinone, especially unknown in literature thiazole derivatives in positions 6 and 7, is a prospective task of the chemistry of biologically active compounds.

Materials and methods

The melting points were measured with a Nagema melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian VXR (300 MHz) spectrometer. The samples were prepared using DMSO-d_6 as a solvent. The chemical shifts are expressed as δ, ppm relative to TMS. IR spectra were recorded with a Specord M80 using KBr tablets.

Materials. 2-R-3,6-dichloro-7-acylamino-1,4-naphthoquinone, 3,6-dichloro-2,7-diacylamino-1,4-naphthoquinone, 2-R-3,6-dichloro-7-amino-1,4-naphthoquinone, 2-amino-7-nitro-3,6-dichloro-1,4-naphthoquinone [13].

Methods for the synthesis of 2-amino-7-chloro-6-R-4-ylnaphtho[2,3-d][1,3]thiazole-5,8-diones (3 a–e)

Method A. A mixture of 0.52 mmol of the corresponding 2-R-3,6-dichloro-7-acylamino-1,4-naphthoquinone **1 a**–**e** and 0.05 g (0.52 mmol) of potassium thiocyanate in 30 ml of acetone was heated at constant stirring at 30–40 °C for 4 h. The reaction mixture was cooled to room temperature, and a 20 % NaOH solution was added. The reaction mixture was heated for 4 h and filtered after cooling (the filtrate containing sodium salt of **3 c** or **3 e** was acidified with 10 % HCl and filtered). The residue was recrystallized from acetonitrile.

Method B. A mixture of 1.5 mmol of the corresponding 2-N-R-3,6-dichloro-7-amino-1,4-naphthoquinone **6 a**–**e** and 0.108 g (1.5 mmol) of thiourea in 30 ml of acetone was boiled at constant stirring for 4 h. The reaction mixture was cooled to room temperature, and a 20 % NaOH solution was added. The reaction mixture was heated for 4 h, then cooled and filtered (the filtrate containing sodium salt of **6 c** or **6 e** was acidified with 10 % HCl and filtered), and the residue was dried.

2-Amino-7-chloro-6-morpholin-4-ylnaphtho[2,3-*d*][1,3]thiazole-5,8-dione (3a). *Method B.* Yield 85 %. M_p. 173–175 °C.

IR ν (cm^{-1}): 3530, 3460 (NH_2); 1680, 1640 (C=O).

^{1}H NMR (DMSO-d_6) δ: 3.06–3.18 (4H, m, CH_2); 3.62–3.70 (4H, m, CH_2); 7.35 (2H, broad s, NH_2); 8.50 (1H, s, CH_{arom}); 8.76 (1H, s, CH_{arom}).

Calculated, %: C 51.51; H 3.46; Cl 10.14; N 12.01; S 9.17. $C_{15}H_{12}ClN_3O_3S$.

Found, %: C 51.55; H 3.52; Cl 10.12; N 12.07; S 9.21.

2-Amino-7-chloro-6-piperidin-1-ylnaphtho[2,3-*d*][1,3]thiazole-5,8-dione (3b). *Method B.* Yield 88 %. M_p. 197–198 °C.

IR ν (cm^{-1}): 3524, 3462 (NH_2), 1678, 1649 (C=O).

^{1}H NMR (DMSO-d_6) δ: 1.47–1.60 (4H, m, CH_2); 1.52–1.58 (2H, m, CH_2); 3.32–3.40 (4H, m, CH_2); 7.35 (2H, broad s, NH_2); 8.48 (1H, s, CH_{arom}); 8.76 (1H, s, CH_{arom}).

Calculated, %: C 55.25; H 4.06; Cl 10.19; N 12.08; S 9.22. $C_{16}H_{14}ClN_3O_2S$

Found, %: C 55.26; H 4.08; Cl 10.23; N 12.05; S 9.29.

1-(2-Amino-7-chloro-5,8-dioxo-5,8-dihydro-naphtho[2,3-*d*][1,3]thiazol-6-yl)piperidine-4-carboxylic acid (3c). *Method B.* Yield 86 %. M_p. 207–208 °C.

IR ν (cm^{-1}): 3534, 3431 (NH_2), 3000–2500 (COOH), 1760, 1678, 1660 (C=O).

^{1}H NMR (DMSO-d_6) δ: 1.73–1.92 (4H, m, CH_2); 2.49–2.57 (1H, m, CH); 4.24–4.32 (4H, m, CH_2); 8.29 (2H, s, NH_2); 8.48 (1H, s, CH_{arom}); 8.77 (1H, s, CH_{arom}); 12.36 (1H, broad, OH).

Calculated, %: C 52.11; H 5.10; Cl 3.60; N 10.72; S 8.18. $C_{17}H_{14}ClN_3O_4S$.

Found, %: C 52.10; H 5.14; Cl 3.61; N 10.71; S 8.20.

2-Amino-7-chloro-6-(dibutylamino)naphtho[2,3-*d*][1,3]thiazole-5,8-dione (3d). *Method A.* Yield 82 %. M_p. 192–194 °C.

IR ν (cm^{-1}): 3534, 3431 (NH_2), 1678, 1660 (C=O).

^{1}H NMR (DMSO-d_6) δ: 0.94 (6H, t, J = 3.5 Hz, CH_3); 1.35–1.44 (4H, m, CH_2); 1.86–1.93 (4H, m, CH_2); 3.63 (4H, t, J = 15.1 Hz, CH_2); 7.35 (2H, broad s, NH_2); 8.53 (1H, s, CH_{arom}); 8.76 (1H, s, CH_{arom}).

Calculated, %: C 58.23; H 5.66; Cl 9.05; N 10.72; S 8.18. $C_{19}H_{22}ClN_3O_2S$.

Found, %: C 58.16; H 5.72; Cl 9.04; N 10.68; S 8.15.

4-[(2-Amino-7-chloro-5,8-dioxo-5,8-dihydro-naphtho[2,3-*d*][1,3]thiazol-6-yl)amino]butanoic acid (3e). *Method A.* Yield 87 %. M_p. 185–186 °C.

IR ν (cm^{-1}): 3520, 3472 (NH_2), 3000–2500 (COOH), 1670, 1659 (C=O).

^{1}H NMR (DMSO-d_6) δ: 2.07 (2H, m, CH_2); 2.27 (2H, t, J = 16.1 Hz, CH_2); 3.40 (2H, t, J = 13.2 Hz, CH_2); 8.13 (3H, broad s, NH_2, NH); 8.53 (1H, s, CH_{arom}); 8.81 (1H, s, CH_{arom}); 12.36 (1H, broad s, OH).

Calculated, %: C 49.25; H 3.31; Cl 9.69; N 11.49; S 8.77. $C_{15}H_{12}ClN_3O_4S$.

Found, %: C 49.21; H 3.33; Cl 9.72; N 11.47; S 8.81.

2,7-Diamino-1,8-dithio-3,6-diazodicyclopentane-naphthalene-4,10-dione (5). A mixture of 1 g (3 mmol) of 3,6-dichloro-2,7-diacylamino-1,4-naphthoquinone (**4)** and 0.348 g (3 mmol) of dithiocyanate in 25 ml of acetone was boiled for 4 h. The reaction mixture was cooled to room temperature, and 0.12 g (3 mmol) of NaOH was added. The reaction mixture was heated for 4 h, cooled and filtered, and the residue was recrystallized from acetonitrile.

Yield 57 %. M_p. 164–165 °C.

IR ν (cm^{-1}): 3534, 3431 (NH_2), 1670, 1659 (C=O).

^{1}H NMR (DMSO-d_6) δ: 7.60 (4H, s, NH_2); 8.50 (1H, s, CH_{arom}); 8.59 (1H, s, CH_{arom}).

Calculated, %: C 47.67; H 2.00; N 18.53; S 21.21. $C_{12}H_6N_4O_2S_2$.

Found, %: C 47.65; H 2.01; N 18.55; S 21.22.

***Method for the synthesis of 2-amino-4-imino-8-mercapto-7-nitronaphtho[1,2-d][1,3]thiazol-5(4H)-one* (8) *and 2-amino-7-mercapto-6-nitronaphtho[2,3-d][1,3]-thiazole-4,9-dione* (9).** A mixture of 1 g (7 mmol) of 2-amino-7-nitro-3,6-dichloro-1,4-naphthoquinone (**7)** and 1.14 g (14 mmol) of thiourea in 30 ml of acetone was heated at constant stirring for 3 h, and a 20 % KOH solution was added. The mixture was heated for 5 h, then cooled to room temperature, filtered, the residue was recrystallized from benzene and divided on a chromatographic column (eluent – benzene : chloroform, 2 : 1).

2-Amino-4-imino-8-mercapto-7-nitronaphtho[1,2-d][1,3]thiazol-5(4H)-one (8). Yield 35 %. M_p. 152–153 °C.

IR ν (cm^{-1}): 3467, 3441 (NH_2), 3367 (NH), 1690 (C=O).

^{1}H NMR (DMSO-d_6) δ: 3.37 (1H, s, SH); 7.43 (1H, s, CH_{arom}); 8.82 (1H, s, CH_{arom}); 9.30 (3H, s, NH_2, NH).

Calculated, %: C 43.13; H 1.97; N 18.29; S 20.94. $C_{11}H_6N_4O_3S_2$.

Found, %: C 43.09; H 2.02; N 18.21; S 20.83.

2-Amino-7-mercapto-6-nitronaphtho[2,3-d][1,3]-thiazole-4,9-dione (9). Yield 12 %. M_p 225–226 °C.

IR ν (cm^{-1}): 3465, 3442 (NH_2), 1672, 1652 (C=O).

^{1}H NMR (DMSO-d_6) δ: 3.35 (1H, s, SH); 7.85 (2H, s, NH_2); 7.93 (1H, s, CH_{arom}); 9.14 (1H, s, CH_{arom}).

Calculated, %: C 42.99; H 1.64; N 13.67; S 20.87. $C_{11}H_5N_3O_4S_2$.

Found, %: C 43.03; H 1.61; N 13.63; S 20.83.

Results and discussion

Computer-aided prediction of biological activity spectra using PASS software

The prediction of the biological activity spectra of the synthesized compounds was performed employing the PASS computer program (Prediction of Activity Spectra for Substances) [14, 15]. The latest PASS version (2012 06 22) predicts 6400 kinds of biological activity based on the analysis of the training set including information about ~330000 drugs, drug-candidates, lead compounds, etc. The average prediction accuracy estimated in a leave-one-out cross-validation procedure for the whole training set is about 95 %. The PASS online version is freely available for scientific community on the website [16]. Based on PASS predictions, new pharmaceutical agents from diverse chemical classes with various kinds of biological activity have been discovered (for a review, see: [16, 17]).

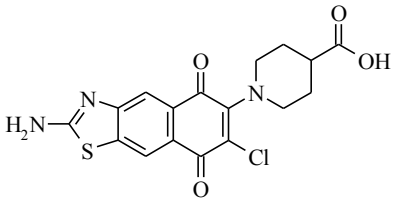
PASS input information is presented by MOL or SD files with structural formulae of compounds under study; PASS output is presented by a list of probable activities with two estimated probabilities, *Pa* – the probability to be "active", and *Pi* – the probability to be "inactive".

The list of predicted activities for a certain chemical compound is called its biological activity spectrum. It includes the main and side pharmacological effects, (e. g., antihypertensive, hepatoprotective, sedative, etc.), the biochemial mechanisms of action, (5-hydroxytryptamine agonist, acetylcholinesterase inhibitor, adenosine uptake inhibitor, etc.), specific toxicities (carcinogenic, hallucinogenic, hepatotoxic, etc.), antitargets (ATPase inhibitor, CYP3A4 inhibitor, HERG channel blocker, etc.), terms associated with drug metabolism (CYP1A substrate, CYP1A1 human substrate, CYP3A4 substrate, etc.), terms associated with the transport of drugs (P-glycoprotein substrate, P-glycoprotein inhibitor, P-glycoprotein inductor, etc.) and terms associated with gene expression (APOA1 expression enhancer, ErbB-2 expression inhibitor, etc.).

The higher the *Pa* value, the lower is the predicted probability of obtaining false positives in biological testing. For example, if one selects for testing only compounds for which a particular activity is predicted with *Pa* > 0.9, the expected probability to find inactive compounds in the selected set is very low; however, about 90 % of active compounds are missed. If one lowers the *Pa* threshold to 0.8, the probability to find inactive compounds is still low, but (only) about 80 % of active compounds are missed, etc. PASS uses the criteria *Pa* = *Pi* as the default threshold, i. e. all compounds with *Pa* > *Pi* are declared as being active.

Another aspect of the predictions is the compounds' novelty. If one limits oneself to high *Pa* values, one may find close analogues of known biologically active substances among the test compounds. For example, for *Pa* > 0.7, the chance to find the activity in experiment is high, but some of the activities may be a close analogue of the known pharmaceutical agents. If one chooses 0.5 < *Pa* < 0.7, the chance of obtaining activity in the experiment is lower, but the compounds may be less similar to the known pharmaceutical agents. For *Pa* < 0.5, the chance of obtaining activity in the experiment are even lower, but if the activity is found, the compound might happen to be a new chemical entity.

An example of the predicted pharmacological effects for compound **3c** is given below (only data with *Pa* > 0.3 are shown):



Pa	*Pi*	Pharmacological effects
0.610	0.036	Acute neurologic disorders treatment
0.512	0.049	Urolithiasis treatment
0.429	0.037	Antineoplastic (astrocytoma)
0.414	0.027	Antibacterial
0.412	0.025	Immunomodulatory
0.396	0.171	Antiviral (Arbovirus)
0.307	0.083	Cell adhesion molecule inhibitor
0.301	0.080	Antifungal
0.409	0.197	Neuroprotector
0.356	0.150	Antiviral (Rhinovirus)
0.380	0.175	Potassium sparing diuretic
0.366	0.190	Antineoplastic (myeloid leukemia)
0.371	0.211	Antiischemic, cerebral
0.342	0.192	Antinephrotoxic
0.354	0.237	Antineoplastic (genitourinary cancer)
0.340	0.262	Myasthenia Gravis treatment
0.327	0.280	Antineoplastic (oral cancer)

The most probable predicted activity for compound **3c** is *Acute neurologic disorders treatment* (*Pa* = 0.610, *Pi* = 0.036), followed by *Urolithiasis treatment* (*Pa* = 0.512, *Pi* = 0.049), *Antineoplastic (astrocytoma)* (*Pa* = 0.429, *Pi* = 0.037), etc. In most cases, the *Pa* values are not close to the *Pi* values; therefore, only *Pa* values can be used to characterize the likelihood of activity.

If PASS predictions are analyzed for a set of compounds from a certain chemical series, the predicted activities can be defined as "typical", i. e. are predicted for the majority of derivatives from this series, and "minor", i. e. predicted only for some compounds [18].

Some predicted typical pharmacological effects for the seven compounds studied are shown in Table 1. These activities include antibacterial, antifungal, antiviral (arbovirus), antineoplastic, immunomodulatory, acute neurological disease treatment, and antiparkinsonian.

Two of the predicted activities (antibacterial and antifungal) were tested in biological experiments (see below).

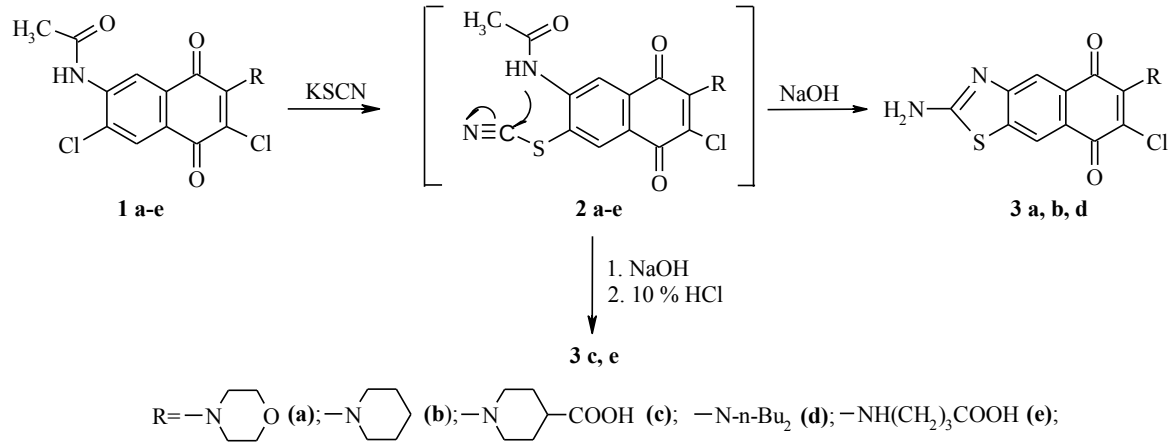
Table 1. Typical activities predicted for new 1,4-naphthoquinone aminothiazole derivatives

Compound ID	AB	AF	AV	AN	IM	ANDT	AP
3a	0.33	–	–	0.53	0.50	–	0.40
3b	0.32	0.25	0.48	0.46	0.43	0.43	0.36
3c	0.41	0.30	0.40	0.43	0.41	0.61	0.24
3d	0.22	0.28	0.69	0.37	0.34	0.33	0.27
3e	0.32	0.26	0.69	0.51	0.37	0.38	0.21
5	0.32	0.20	0.65	0.76	0.36	0.36	0.71
9	–	0.18	0.55	0.34	0.21	0.53	0.32

Note: AB – antibacterial; AF – antifungal; AV – antiviral (Arbovirus); AN – antineoplastic; IM – immunomodulator; ANDT – acute neurological disease treatment; AP – antiparkinsonian activity.

Interaction of 2-N-R-3,6-dichloro-7-acylamino-1,4-naphthoquinone with potassium thiocyanate and 3,6-dichloro-2,7-diacylamino-1,4-naphthoquinone with dithiocyanate

The synthesis of aminothiazoles **3 a–e** was carried out similarly to the method described in literature [19, 20]. The corresponding thiocyanate derivative **2 a–e** appears in the interaction of potassium thiocyanate with 2-R-3,6-dichloro-7-acylamino-1,4-naphthoquinone **1 a–e** in acetone for 30–40 °C (Method A). 2-Amino-7-chloro-6-R-4-yl-naphtho[2,3-d][1,3]-thiazole-5,8-diones **3 a–e** were obtained at heating with a 20 % NaOH solution for 4 h (Scheme 1). To obtain **3 c, e**, the filtrate containing sodium salt **3 c** or **3 e** was acidified with 10 % HCl and filtered.

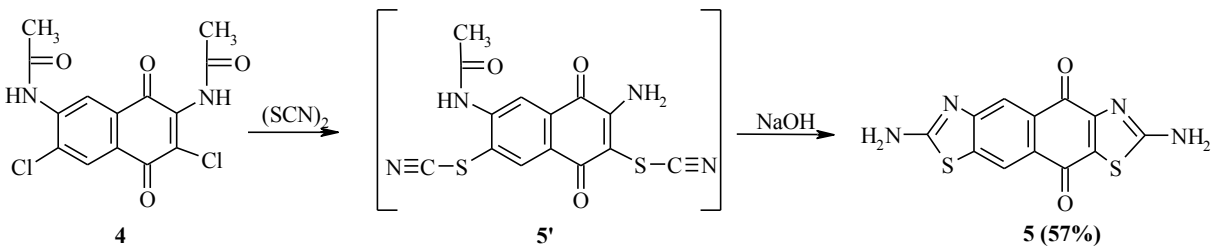


Scheme 1. Synthesis of 2-amino-7-chloro-6-R-4-yl-naphtho[2,3-d][1,3]-thiazole-5,8-diones **3 a–e**

An atom of carbon in position 6 of a molecule of 2-R-3,6-dichloro-7-acylamino-1,4-naphthoquinone **1** was activated by amino group acylation in position 7 [13]; this is why the nucleophilic anion of the thiocyanate group attacks this atom with the formation of **2**, which converts into aminothiazole **3** in the presence of NaOH (Scheme 1). Compounds **3 a–e** were obtained by Method A in 72–85 % yields.

The IR spectra of the obtained compounds **3 a–e** have absorption bands in the interval 3431–3534 cm^{-1}, which are characteristic of the vibration of NH_2 groups. The 1680–1640 cm^{-1} scope is characteristic of *p*-quinonic C=O groups. In the spectra of 6-thiocyanate-7-N-acylaminonaphthoquinones **1 a–e** [13], there are signals of NH group absorption in the area of 3310 cm^{-1} and in the area of 2348 cm^{-1} for the SCN group. Also, in the ^1H NMR of compounds **1 a–e** [13], there are signals of acyl group protons in the area of 2.12–2.06 ppm, which are absent in the spectra of aminothiazole derivatives **3 a–e**.

For the synthesis of diaminothiazole, we used dithiocyanate **5'** obtained by the method described in [19]. In this case, the substitution of two chlorine atoms was realized; as a result, 2,7-diamino-1,8-dithio-3,6-diazodicyclopentanaphthalene-4,10-dione (**5)** appeared in the presence of NaOH (Scheme 2).

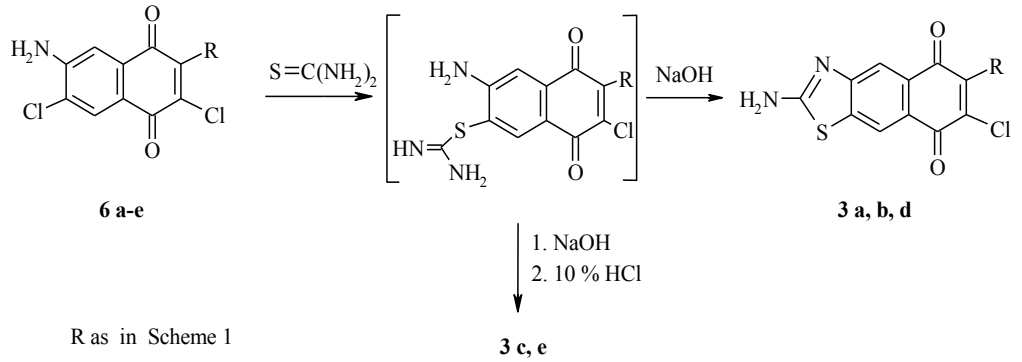


Scheme 2. Synthesis of 2,7-diamino-1,8-dithio-3,6-diazodicyclopentanaphthalene-4,10-dione **5**

A comparison of ^{1}H NMR spectra of compounds 3,6-dichloro-2,7-diacylamino-1,4-naphthoquinone **4** [13] and **5** shows the absence of signals of CH_3 group protons of the acylic fragment in the area of 2.20–2.10 ppm; at the same time, signals of benzene cycle protons are almost identical (difference is 0.01 ppm) as compared with the spectrum of compound **4** where the signals of protons are present as two singlets at 8.76 and 8.31 ppm, respectively; this confirms formation of the aminothiazole cycle. In the IR spectrum of compounds **4** [13], a characteristic absorption band at 3200 cm^{-1} of the amide group is present, and in the IR spectrum of compounds **5** absorption bands, which are characteristic of the vibration of the NH_2 group, were observed.

Interaction of 2-R-3,6-dichloro-7-amino-1,4-naphthoquinone and 2-amino-3,6-dichloro-7-nitro-1,4-naphthoquinone with thiourea

The interaction of 2-R-3,6-dichloro-7-amino-1,4-naphthoquinones **6 a–e** with thiourea was carried out similarly to the method presented in [21]. We obtained aminothiazoles of 1,4-naphthoquinone **3 a–c** (Method B)**,** which did not require recrystallization (Scheme 3). To obtain **6 c, e**, the filtrate containing a sodium salt of **6 c** or **6 e** was acidified with 10 % HCl and filtered.

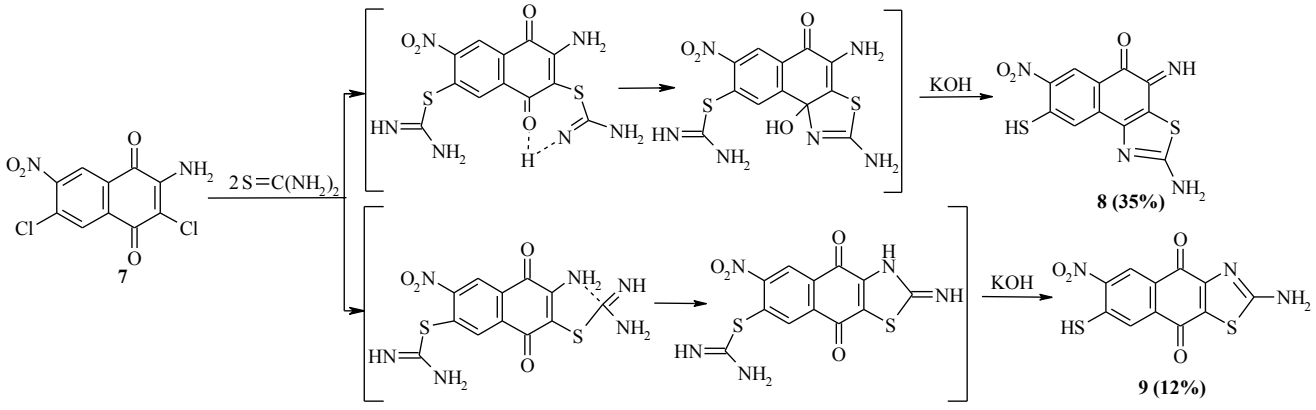


Scheme 3. Synthesis of 2-amino-7-chloro-6-R-4-yl-naphtho[2,3-d][1,3]-thiazole-5,8-diones **3 a–c**

In this case, cyclization takes place by the Hantzch method [22]. In the process of the reaction, there is a nucleophilic attack by the sulphur atom on the halogen atom of carbon; as a result, we obtained isothiourea intermediates, which in the presence of NaOH lead to the formation of aminothiazoles **3 a–c** (Scheme 3).

Compounds **3 a–e** were obtained by Method B with yields of 85–88 %. A comparison of methods for the synthesis of aminothazoles **3 a–e** has shown that Method A is suitable for the preparation of **3 d, e** (82 %, 87 %, respectively), and Method B is convenient for obtaining **3 a–c** (85–88 %).

The synthesis of aminothiazole derivatives of 2-amino-3,6-dichloro-7-nitro-1,4-naphthoquinone **7** [13] was carried out by the previously mentioned method. We obtained and identified the compounds as 2-amino-4-imino-8-mercapto-7-nitronaphtho[1,2-*d*][1,3]thiazol-5(4*H*)-one **8** and 2-amino-7-mercapto-6-nitronaphtho[2,3-d][1,3]-thiazole-4,9-dione **9** (Scheme 4) in low yields (35 % and 12 %, respectively) and a mixture of by-products which were difficult to divide and identify.



Scheme 4. Interaction of 2-amino-3,6-dichloro-7-nitro-1,4-naphthoquinones with thiourea

During the reaction, isothiourea intermediates appear in positions **3** and **6** (Scheme 4). The mercaptonitroaminothiazole derivatives 2-amino-4-imino-8-mercapto-7-nitronaphtho[1,2-*d*][1,3]thiazol-5(4*H*)-one (**8)** and 2-amino-7-mercapto-6-nitronaphtho[2,3-d][1,3]-thiazole-4,9-dione (**9)** were obtained as a result of the action of KOH at heating. Depending on the isothiourea group position **3** or **6**, it can be hydrolysis to mercapto group (pos. **6**) or cyclization to aminothiazole (pos. **3**).

In the IR spectrum of compound **8**, the absorption band attributed to the NH group was observed at 3367 cm^{-1} and an intensive absorption band was identified in the area

of 1690 cm^{-1}, characteristic of the C=O group vibration. In the ^1H NMR spectra of 2-amino-4-imino-8-mercapto-7-nitronaphtho[1,2-*d*][1,3]thiazol-5(4*H*)-one **8** 7.43–8.82 ppm regions, resonances of two benzene ring protons are present; furthermore, spectral lines characteristic of NH_2, NH and SH groups are also present. In the IR spectrum of 2-amino-7-mercapto-6-nitronaphtho[2,3-d][1,3]-thiazole-4,9-dione **9**, two absorption bands characteristic of *p*-quinonic C=O groups were observed at 1652, 1672 cm^{-1}, and an absorption band of the NH_2 group was present in the region of 3442, 3465 cm^{-1}. In ^1H NMR spectrum of compound **9**, a signal of the SH group proton at 3.35 ppm was noted.

The structure of the newly obtained mercaptoaminothiazoles **8** and **9** of naphthoquinone was also confirmed by qualitative reactions of the free SH group.

The reaction mixture of **8**, **9** after evaporation of the solvent in vacuum was divided on a chromatographic column (eluent – benzene : chloroform, 2 : 1). Compounds **8** and **9** were selected in a 3 : 1 correlation.

Thus, new aminothiazole derivatives notdescribed in the literature were synthesized by the reaction of 2,3,6,7-substituted 1,4-naphthoquinones with thiocyanate and thiourea. The obtained compounds open possibilities for obtaining of new derivatives with a wide spectrum of biological activity.

The structure of all obtained aminothiazole naphthoquinone derivatives was confirmed by element analysis, ^1H NMR and IR spectral data.

Antibacterial and antifungal activity of the synthesized compounds

The antimicrobial activity was studied by the disk method, using the followings cultures of microorganisms: *Staphylococcus aureus*, *Escherichia coli*, and C*andida tenuis*.

All cultures were grown at 37 °C in the medium with peptone, yeast extract. Disks (5 mm in diameter) were soaked in 0.02 mg ml^{-1} of thiols as solutions in DMF. A disk was put on an exponentially growing plated culture with the appropriate dilution to 10^6 cells per ml. The plates were then incubated for 24 h at 37 °C. The results were recorded by measuring the zones surrounding the disk. Control disks contained DCNQ (2,3-dichloro-1,4-naphthoquinone) and oxacillin [23–26].

The antibacterial and antifungal activities were estimated by the size of the diameter of inhibition zones of microorganisms' growth.

Most of the compounds were found to show a moderate antibacterial and antifungal activity (Table 2), and some substances superseded the reference drugs (oxacillin and DCNQ).

Table 2. Antibacterial and antifungal activity of the synthesized compounds

Compound / Microorganism	Diameter of inhibition zone, mm								
	3a	**3b**	**3c**	**3d**	**3e**	**5**	**9**	DCNQ	Oxacillin
E. coli	0	0	0	0	0	17	27	15	0
S. aureus	18	17	20	22	22	24	26	18	24
C. tenuis	16	17	19	19	28	17	27	23	–

Data presented in Table 2 show that *S. aureus* has a high sensitivity to compounds **5** and **9** in comparison with oxacillin and DCNQ, which exert a selective action on gram-positive bacteria. The majority of the compounds showed a higher antimicrobial action on gram-positive bacteria than did DCNQ and oxacillin. The absence of inhibition zones of *E. coli* bacteria has shown that the study compounds at these concentrations have no antibacterial action. *E. coli* appeared to be sensible to compounds **5** and **9**, at the same time oxacillin had not antimicrobial activity as regards this culture. Compound **9** had a higher inhibition zone than DCNQ for the action on gram-negative bacteria (Fig.1).

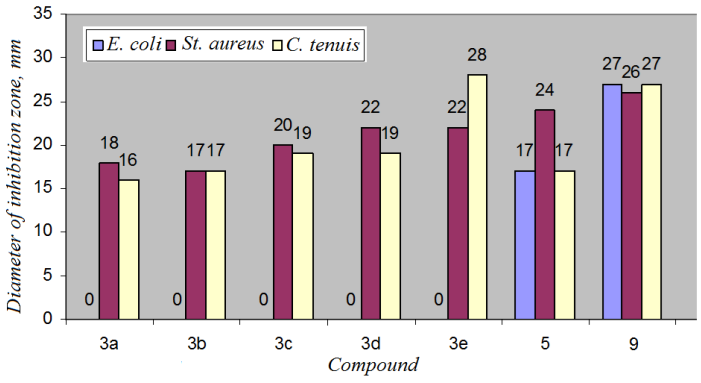


Fig. 1. Antimicrobial action of the synthesized compounds

It was established that the synthesized compounds **3e** and **9** showed an antifungal activity higher than that of DCNQ.

On the basis of data presented in Table 2, the following conclusions as to the structure – antimicrobial activity relationships can be done:

for bacteria: 9 > 5 > 3d ~ 3e > 3c > 3a ~ 3b;

for fungi: 9 > 3e > 3c ~ 3d > 3a ~ 3b ~ 5.

Therefore, as a result of our experimental studies, we have confirmed the computational prediction of antibacterial and antifungal activities for the study compounds on *S. aureus*, *E. coli* and *C. tenuis* cultures. Since the *Pa* values estimate the probability of belonging to the class of "actives", there is no correlation between the *Pa* values and the diameters of the inhibition zone, which characterize the potency of the compounds.

Conclusions

For the first time, new aminothiazole derivatives of 2,3,6,7-substituted 1,4-naphthoquinones have been synthesized. The physical and chemical properties of these compounds have been determined, and the methods of their preparation are presented.

Using the PASS computer program the biological activity spectra of the synthesized compounds have been estimated. Among the effects that were predicted as typical of this chemical series there are antibacterial, antifungal, antiviral (arbovirus), antineoplastic, immunomodulator, acute neurological disease treatment, and antiparkinsonian activities.

The experimental testing of the antimicrobial action of the study compounds on the *S. aureus*, *E. coli* and *C. tenuis* cultures have confirmed these two activities to be typical of this chemical series. The activity of some 1,4-naphthoquinone aminothiazole derivatives supersedes that of the reference drugs oxacillin and DCNQ.

Since the results of computational predictions regarding antibacterial and antifungal activities were confirmed in more than 90 % of cases, the other biological activities predicted for 1,4-naphthoquinone aminothiazole derivatives deserve further studies.

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NAUJŲ 1,4-NAFTOCHINONO AMINOTIAZOLO DARINIŲ SINTEZĖ, BIOLOGINIO AKTYVUMO PROGNOZAVIMAS IR ANTIMIKROBINIŲ SAVYBIŲ TYRIMAS

S a n t r a u k a

2-R-3,6-Dichlor-7-acilamino-1,4-naftochinonų ir kalio tiocianato, 3,6-dichlor-2,7-diacilamino-1,4-naftochinono ir ditiocianato, 2-R-3,6-dichlor-7-amino-1,4-naftochinonų, 2-amino-7-nitro-3,6-dichlor-1,4-naftochinono ir tiokarbamido tarpusavio reakcijų metu gauti nauji 1,4-naftochinono aminotiazolo dariniai, nustatytos jų fizikinės ir cheminės savybės. Naudojant kompiuterinę programą PASS įvertintas prognozuojamas susintetintų junginių biologinis aktyvumas, atlikti jų biologiniai tyrimai. Ištirtas antibakterinis ir priešgrybelinis poveikis *Staphylococcus aureus*, *Escherichia coli* ir *Candida tenuis* mikroorganizmams ir nustatyta, kad dalis tiriamųjų junginių pasižymi vidutiniu aktyvumu, daugiau nei 90 proc. sutampančiu su kompiuteriniais duomenimis. Junginio struktūros ir antimikrobinio aktyvumo ryšio analizė suteikia galimybę sintetinti naujus darinius. Taikant PASS metodą, taip pat įvertintos galimos susintetintų junginių antivirusinės, antineoplastinės, imunomodulatorių savybės, jų tinkamumas neurologinėms, Parkinsono ligoms gydyti. Gauti duomenys leidžia daryti prielaidą, kad 1,4-naftochinono aminotiazolų dariniai yra perspektyvūs vaistiniai preparatai.