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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL NAPHTHOQUINONE DERIVATIVES CONTAINING 1,2,4-TRIAZINE AND 1,2,4-TRIAZOLE MOIETIES

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Novel naphthoquinone derivatives bearing 1,2,4-triazine- (4a–b) and 1,2,4-triazole (5a–e) pharmacophores have been synthesized; their structure was confirmed by electrospray ionization mass spectrometry, ^{1}H NMR, ^{13}C NMR, IR spectroscopies and elemental analysis. The obtained heterocyclic compounds were estimated for their anticonvulsant activity on models of chemical- and electrical-induced seizures in pentylenetetrazole (PTZ) and maximal electroshock (MES) tests, respectively. Forced swimming test was used to evaluate the antidepressant effect of the naphthoquinone derivatives under study. Compounds 4a–b and 5a–e (100 mg kg^{-1}) demonstrated anticonvulsant action comparable with valproic acid in PTZ-test and prevented the death of 100% of mice in MES model at 3 h and 24 h after oral administration. Moreover, these derivatives showed prolonged antidepressant-like properties, significantly reducing the duration of immobility time in comparison with the reference drug amitriptyline.

Keywords: naphthoquinone derivatives, 1,2,4-triazine, 1,2,4-triazole, technology, anticonvulsant, antidepressant.

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Introduction

In patients with epilepsy, depressive disorders are the most prevalent psychiatric comorbidity that significantly affects the quality of life [1]. In this context, the side effects of antiepileptic drugs are one of the major reasons representing the prominent risk factors for occurrence of depression [2]. Bearing in mind the foregoing, development and investigation of novel compounds simultaneously exhibiting the anticonvulsant and antidepressant activities is a feasible approach at this point. Recently, this concept has been implemented by our research group in design and synthesis of naphthoquinone derivatives containing pyrazole and pyrimidine fragments, as shown in Fig. 1 [3]. Pharmacological screening revealed the potency of these compounds to prevent seizures and concurrently demonstrate antidepressant action.

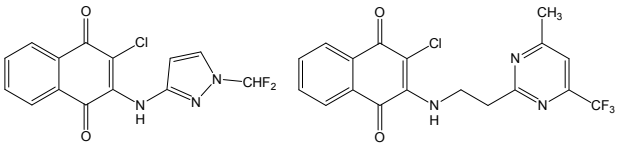


Fig. 1. Examples of previously reported compounds [3]

Despite an enormous interest in 1,4-naphthoquinone derivatives due to their wide range of biological activity (antimicrobial [4], anti-platelet [5], etc.), only a limited number of publications are aimed at studying the impact on central nervous system (CNS) (Fig. 2). The anticonvulsant effect comparable to phenytoin of 4-amino-1,2-naphthoquinone analogues along with their neurotoxicity has been examined [6]. Natural and synthetic 1,4-naphthoquinones have been explored as active inhibitors of monoamine oxidase that may be useful in treating

such disorders as depressive illness [7].

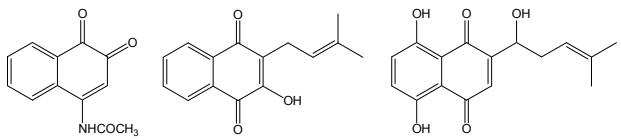


Fig. 2. Structures of naphthoquinones with anticonvulsant and antidepressant effects

In addition, significant influence on CNS was displayed by heterocyclic nitrogen systems containing 1,2,4-triazine- and 1,2,4-triazole moieties. Some examples include atypical antidepressant Nefazodone which comprises triazole nucleus and anti-epileptic drug Lamotrigine with 1,2,4-triazine in its structure [8]. Inspired by the afore-mentioned findings, here we report the synthesis of novel naphthoquinone derivatives bearing 1,2,4-triazine- and 1,2,4-triazole pharmacophores as potential anticonvulsant and antidepressant agents.

Experimental

General methods

Melting points were determined in open capillary tubes in a «Mettler Toledo MP 50» apparatus and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded using a «Varian Mercury» (Varian Inc., Palo Alto, CA) 400 MHz/100 MHz spectrometer with DMSO-d_6 as solvent and TMS as an internal standard; the coupling constants are given in Hz. The elemental analysis was performed by means of a Euro Vector EA-3000 (Eurovector SPA, Redavalle, Italy) microanalyzer. Elemental analyses were within ±0.4% inaccuracy with respect to the theoretical values. The progress of the reaction was monitored by TLC on Silufol UV-254 plates. Electrospray ionization mass spectrometry was performed on Agilent 1100 Series (LC/MSD Trap) Spectrometer using the gradient elution: A) H_2O+0.1% HCOOH; B) CH_3CN+0.1% HCOOH.

3-(2-Aminophenyl)-6-aryl-1,2,4-triazin-5(2H)-ones (2a–b) and (3-R-1H-1,2,4-triazol-5-yl)anilines (3a–e) were synthesized by known method [9]. Other starting materials and solvents were obtained from commercially available sources and used without additional purification.

General procedure for the synthesis of 1,2,4-triazine- and 1,2,4-triazole-containing derivatives of 2,3-dichloro-1,4-naphthoquinone (4a–b, 5a–e)

To the solution (1 mmol) of the corresponding 1,2,4-triazine- or 1,2,4-triazole-containing derivative (2a–b, 3a–e) in 10 ml of DMSO, 0.17 g (1.5 mmol) of 2,3-dichloro-1,4-naphthoquinone (1) was added with constant stirring and kept at 70^{0}C with stirring for 12 h in the presence of 0.27 g (2 mmol) of K_2CO_3. The completion of 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 DCM. Combined organic layers were extracted with 3×20 ml 5% NaOH, water phase was acidified with 20% HCl to pH 3–4, the formed precipitate was filtered, washed with water, dried in vacuum over $CaCl_2$, recrystallized from EtOAc and dried in vacuum. Products are yellow-orange crystalline substances, soluble in organic solvents and poorly soluble in water, which easily form water-soluble salts in an alkaline environment.

2-((2-(3-(4-Fluorophenyl)-5-oxo-2,5-dihydro-1,2,4-triazin-6-yl)phenyl)amino)-3-hydroxynaphthalene-1,4-dione (4a)

Yield 62%, light orange crystals, m.p.=225–227^{0}C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.59 (d, J=7.9 Hz, 1H), 8.39 (d, J=7.6 Hz,1H), 8.27 (d, J=7.6 Hz, 1H), 8.04–8.12 (m, 3H), 7.83 (d, J=8.3 Hz, 1H), 7.72 (t, J=7.7 Hz, 1H), 7.56 (t, J=7.8 Hz, 1H), 7.48 (t, J=7.8 Hz, 1H), 7.25 (br.t, J=8,8 Hz, 2H). IR, (KBr, cm^{-1}): 3460–3280 (–NH), 3260, 2930, 2580 (–OH), 1730, 1675 (C=O), 1640 (–NH), 1584, 1550 (C=N–), 1461 (C=C), 950 (–OH). Calcd. (%): C 66.08; H 3.33; F 4.18; N 12.33. Found: C 66.14; H 3.38; F 4.13; N 12.37. LC–MS m/z (% relative intensity): $[M+H]^+$ 455 (100), 456 (29), 457 (5). t_r=1.105 min.

2-Hydroxy-3-((2-(3-(4-isopropylphenyl)-5-oxo-2,5-dihydro-1,2,4-triazin-6-yl)phenyl)amino)-naphthalene-1,4-dione (4b)

Yield 68%, light orange crystals, m.p.=230–232^{0}C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.51 (d, J=7.8 Hz, 1H), 8.34 (d, J=7.7 Hz, 1H), 8.29 (d, J=7.7 Hz, 1H), 8.22 (d, J=8.3 Hz, 2H), 8.09 (t, J=7.7 Hz, 1H), 7.83–7.55 (m, 6H), 2.94 (sept, J=6.9 Hz, 1H), 1.23 (d, J=6.9 Hz, 6H). IR, (KBr, cm^{-1}): 3450–3380 (–NH), 3280, 2925, 2582 (–OH), 1738, 1680 (C=O), 1630 (–NH), 1585, 1548, 1525 (C=N–), 1470 (C=C), 990 (–OH). Calcd. (%): C 70.28; H 4.63; N 11.71. Found: C 70.33; H 4.68; Cl 7.35; N 11.67. LC–MS m/z (% relative intensity): $[M+H]^+$ 479 (100), 480 (33), 481 (6). t_r=1.035 min.

2-Hydroxy-3-((2-(5-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazol-3-yl)phenyl)amino)naphthalene-1,4-dione (5a)

Yield 78%, light orange crystals, m.p.>250^{0}C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.65 (d, J=7.9 Hz, 1H), 8.48 (d, J=8.1 Hz, 2H), 8.25 (d, J=7.6 Hz, 1H), 8.11 (d, J=7.6 Hz, 1H), 7.81–7.92 (m, 3H), 7.59–7.73 (m, 3H), 7.45 (d, J=7.9 Hz,

1H). IR, (KBr, cm^{-1}): 3480–3370 (–NH), 3290, 2932, 2580 (–OH), 1736, 1682 (C=O), 1620 (–NH), 1584, 1546 (C=N–), 1472 (C=C), 996 (–OH). Calcd. (%): C 63.03; H 3.17; F 11.96; N 11.76. Found: C 63.07; H 3.19; F 11.92; N 11.80. LC–MS m/z (% relative intensity): $[M+H]^+$ 477 (100), 478 (30), 479 (5). t_r=0.983 min.

2-((2-(5-(3-Fluorophenyl)-1H-1,2,4-triazol-3-yl)phenyl)amino)-3-hydroxynaphthalene-1,4-dione (5b)

Yield 80%, light orange crystals, m.p.=208–210°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.63 (d, J=7.9 Hz, 1H), 8.36 (d, J=7.9 Hz, 1H), 8.22 (d, J=7.6 Hz, 1H), 8.01–8.13 (m, 2H), 7.82 (t, J=7.8 Hz, 1H), 7.64 (t, J=7.7 Hz, 1H), 7.47–7.62 (m, 3H), 7.35 (d, J=7.9 Hz, 1H), 7.28 (t, J=8.5 Hz, 1H). IR, (KBr, cm^{-1}): 3480–3390 (–NH), 3300, 2930, 2578 (–OH), 1732, 1682 (C=O), 1620 (–NH), 1587, 1550 (C=N–), 1470 (C=C), 947 (–OH). Calcd. (%): C 67.60; H 3.55; F 4.46; N 13.14. Found: C 67.64; H 3.57; F 4.39; N 13.17. LC–MS m/z (% relative intensity): $[M+H]^+$ 427 (100), 428 (29), 429 (4). t_r=0.785 min.

2-((2-(5-(2-Chlorophenyl)-1H-1,2,4-triazol-3-yl)phenyl)amino)-3-hydroxynaphthalene-1,4-dione (5c)

Yield 83%, orange crystals, m.p.=223–225°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.64 (d, J=7.9 Hz, 1H), 8.33 (d, J=7.6 Hz, 1H), 8.24 (d, J=7.7 Hz, 1H), 8.13 (d, J=7.6 Hz, 1H), 7.88–7.97 (m, 2H), 7.63–7.80 (m, 5H), 7.35 (d, J=7.9 Hz, 1H). IR, (KBr, cm^{-1}): 3480–3370 (NH), 3320, 2928, 2583 (–OH), 1738, 1680 (C=O), 1630 (–NH), 1585, 1548 (C=N–), 1471 (C=C), 996 (–OH), 740 (C–Cl). Calcd. (%): C 65.09; H 3.41; Cl 8.00; N 12.65. Found: C 65.12; H 3.38; Cl 7.96; N 12.69. LC–MS m/z (% relative intensity): $[M+H]^+$ 443 (100), 444 (27), 445 (38), 446 (10), 447 (2). t_r=0.803 min.

2-((2-(5-(2-Bromophenyl)-1H-1,2,4-triazol-3-yl)phenyl)amino)-3-hydroxynaphthalene-1,4-dione (5d)

Yield 69%, light orange crystals, m.p.=198–200°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.05 (d, J=7.8 Hz, 1H), 8.00–7.93 (m, 2H), 7.82 (t, J=7.4 Hz, 1H), 7.76 (d, J=7.5 Hz, 1H), 7.72 (d, J=7.7 Hz, 1H), 7.45 (d, J=7.8 Hz, 1H), 7.43–7.33 (m, 2H), 7.24 (t, J=7.6 Hz, 1H), 6.98 (d, J=8.0 Hz, 1H), 6.85 (t, J=7.5 Hz, 1H). ^{13}C NMR (DMSO, 100 MHz): d (ppm) 182.1 (C=O), 177.3 (C=O), 160.5 (C), 160.4 (C), 154.6 (C), 143.1 (C), 134.8 (CH), 133.1 (CH), 132.9 (CH), 131.9 (C), 131.8 (CH), 131.1 (CH), 130.5 (C), 130.0 (CH), 129.2 (C), 128.1 (CH), 127.4 (CH), 126.2 (CH), 126.0 (CH), 122.3 (C), 118.4 (C), 118.2 (CH), 117.7 (CH), 114.4 (C). IR, (KBr, cm^{-1}): 3480–3370 (–NH), 3310, 2932, 2580 (–OH), 1736, 1682 (C=O), 1630 (–NH), 1584, 1546 (C=N–), 1472 (C=C), 960 (–OH). Calcd. (%): C 59.15; H 3.10; Br 16.40; N 11.50. Found: C 59.17; H 3.12; Br 16.35; N 11.53. LC–MS m/z (% relative intensity): $[M+H]^+$ 487 (99), 488 (26), 489 (100), 490 (26), 491 (5). t_r=0.795 min.

2-((2-(5-(3-Bromophenyl)-1H-1,2,4-triazol-3-yl)phenyl)amino)-3-hydroxynaphthalene-1,4-dione (5e)

Yield 72%, light orange crystals, m.p.>250°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.65 (d, J=7.9 Hz, 1H), 8.38 (br.s, 1H), 8.22–8.29 (m, 2H), 8.11 (d, J=7.6 Hz, 1H), 7.85–7.95 (m, 2H), 7.76 (t, J=7.6 Hz, 1H), 7.55–7.64 (m, 2H), 7.20–7.37 (m, 2H). IR, (KBr, cm^{-1}): 3490–3380 (–NH), 3360, 2928, 2580 (–OH), 1740, 1682 (C=O), 1630 (–NH), 1585, 1548 (C=N–), 1471 (C=C), 992 (–OH). Calcd. (%): C 59.15; H 3.10; Br 16.40; N 11.50. Found: C 59.18; H 3.13; Br 16.36; N 11.46. LC–MS m/z (% relative intensity): $[M+H]^+$ 487 (98), 488 (25), 489 (100), 490 (26), 491 (5). t_r=0.799 min.

Drug administration

Anticonvulsant and antidepressant effects of compounds 4a–b, 5a–e were evaluated at 3 h and 24 h after administration. 1,4-Naphtoquinone derivatives were administered orally to mice in Tween 80/water emulsion at a dose of 100 mg kg^{-1} and Tween 80/water emulsion was used as a vehicle control. Valproic acid (VPA, 400 mg kg^{-1}, p.o.) and amitriptyline (20 mg kg^{-1}, p.o.) served as reference drugs (positive control), respectively.

Anticonvulsant activity

The anticonvulsant action of compounds 4a–b, 5a–e was estimated by pentylenetetrazole model (PTZ) as described elsewhere [10]. Doses of PTZ for inducing clonic-tonic convulsions (DCTC) and tonic extension (DTE) were calculated relative to control. The anticonvulsant activity of 1,4-naphtoquinone derivatives was evaluated at 3 h and 24 h after their administration from the increase of pentylenetetrazole MED compared with a control group. MED in percent was calculated using the following formula:

$$MED=V/m\cdot10^4$$

where MED is the minimum effective dose of PTZ inducing DCTC or DTE; V is the volume of PTZ solution (ml); and m is the animal weight (g).

Antidepressant effect

Forced swim test (FST) was used to determine antidepressant action of compounds 4a–b, 5a–e according to the procedure described elsewhere [11]. Briefly, mice were placed individually into glass cylinder filled with water (24±3^{0}C) and total duration of their immobility (in seconds) during 5 minutes has been measured.

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).

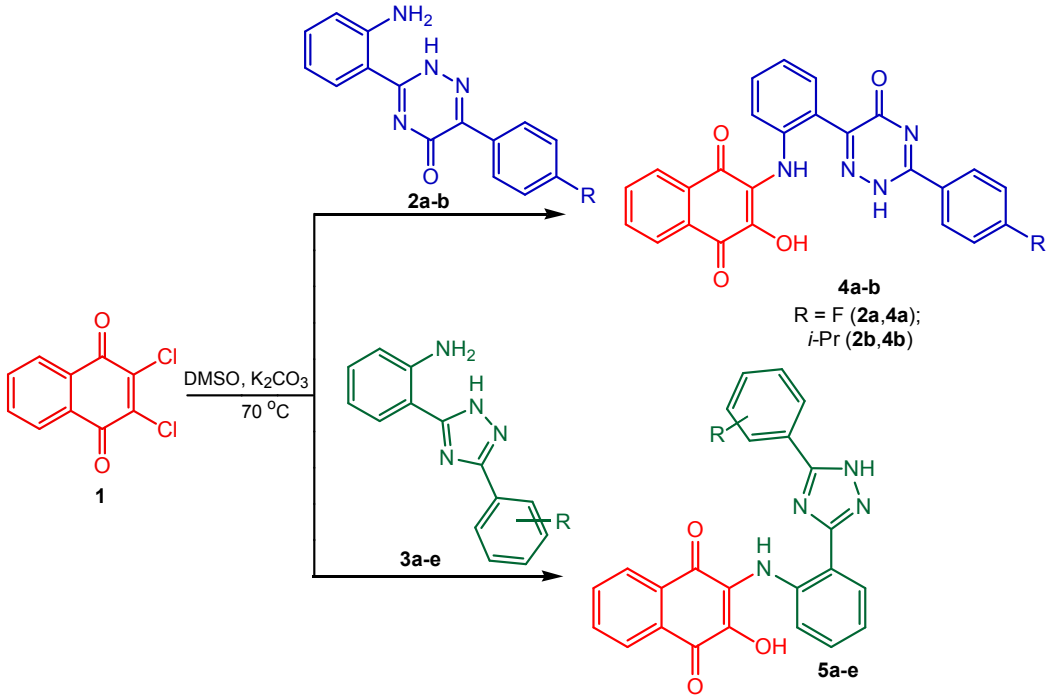
Results and discussion

Synthesis

A variety of methods are available to synthesize nitrogen-containing heterocyclic compounds (quinoxalines, phenazines, phenothiazines, and phenoxazines), these methods being based on the interaction between 1,4-quinones and aliphatic/aromatic diamines (or their derivatives). However, the interaction of naphthoquinones with some bifunctional amines has not been sufficiently studied. The direction of the reaction between 1,4-naphthoquinone derivatives and 1,2,4-triazines/triazoles might be determined both by tautomeric equilibrium in intermediate azomethine (amide-imide tautomerism) and by the nature of electrophilic reagent.

As shown earlier [12], the reaction of 2,3-dichloro-1,4-naphthoquinone (1) with 1,5-N,N-binucleophiles can proceed with the formation of the diazepine cycle. Studying the reaction of 2,3-diloro-1,4-naphthoquinone with heterocyclic 1,5-N,N-binucleophiles (2a–b, 3a–e), we expected to obtain similar condensed heterocyclic systems. However, the formation of such diazepine systems was not detected in any of the studied conditions. When carrying out the reaction in acetonitrile at boiling temperature (potassium carbonate as a base), the reaction practically does not occur. Only under harsh conditions (120^{0}C, microwave irradiation), an ion corresponding to the substitution of one chlorine atom of 2,3-dichloro-1,4-naphthoquinone (1) by the amino group of heterocycles (2a–b, 3a–e) was registered on the mass spectrum. In parallel, many side effects were observed, in particular the decomposition of the original heterocycles, so it was not possible to obtain a substitution product with a preparative yield. Instead, in strongly polar aprotic solvents such as DMF and DMSO, the reaction takes place under milder conditions (70^{0}C, 12 h) in the presence of potassium carbonate and leads to the formation of products with molecular weights corresponding to the expected cyclocondensation products. However, detailed analysis of the isotopic signature of the mass spectra signals of the products and especially the results of ^{13}C NMR spectroscopy and elemental analysis showed that the reaction proceeds with parallel hydrolysis of the second chlorine atom to form products (4a–b, 5a–e). The substitution of one chlorine atom products as well as the cyclocondensation products was not detected at all. The erroneous assumption about the formation of diazepine systems was due to the fact that the obtained products (4a–b, 5a–e) and their corresponding cyclocondensation products have the same molecular weight. The highest yields were obtained with a 1.5-fold excess of naphthoquinone, a 2-fold excess of potassium carbonate and the use of DMSO as a solvent (Scheme). When using DMF, the formation of a by-product of the substitution of the chlorine atom of 2,3-dichloro-1,4-naphthoquinone (1) by dimethylamine was observed. The presence of salt-forming groups OH and NH in the molecules of products (4a–b, 5a–e) allows isolating them from the reaction mass by acid-base extraction.

The ^{1}H NMR spectra of (4a–b, 5a–e) show the signals from two ABCD systems corresponding to the quinoid and ortho-substituted aniline moieties as well as proton signals from the corresponding aromatic substituent, but no signals corresponding to the OH and NH protons. This can be explained by the mobility of their hydrogen atoms and their ability to form hydrogen bonds, which is also indicated by the strong displacement of the water signal. The latter is also confirmed by the results of IR spectroscopy, where there is a significant broadening of the absorption bands of OH and NH valence vibrations. In the IR spectra of compounds 4a–b, 5a–e, there are no intense fluctuation bands of the NH_2 group at 3550–3474 cm^{-1}, which are characteristic of amino derivatives, and there are oscillation bands («amide-II») at 1626–1574 cm^{-1}, which are characteristic mixed valence-strain oscillations of CN, NH bonds. This confirms the confirmatory course of nucleophilic substitution on the aniline amino group of heterocycles (2a–b, 3a–e). The oscillations of νCO groups are observed at 1738–1630 cm^{-1}. In addition, low-intensity bands of νC=C– bond oscillations at 1510–1466 cm^{-1} and intense bands of valence-deformation of C=N bonds oscillations of heterocyclic fragments at 1610–1585 cm^{-1} are present. The absorption bands of the



Scheme. Synthesis of 1,2,4-triazine- (4a–b) and 1,2,4-triazole-containing derivatives of 2,3-dichloro-1,4-naphthoquinone (5a–e)

associated νOH valence vibrations at 3510–3430 cm^{-1} confirm the presence of the hydroxyl group in (4a–b, 5a–e). The band of valence oscillations of one carbonyl group is shifted to the region of 1740–1720 cm^{-1}.

The final confirmation of the absence of diazepine cycle products is the ^{13}C NMR spectrum of compound 5d, in which two signals at 182 and 177 ppm are observed that indicates the preservation of two carbonyl groups of the quinoid system and the absence of an anil group.

Pharmacology

The influence of the synthesized compounds on CNS has been proven by investigation of their anticonvulsant and antidepressant potency. In order to estimate the anticonvulsant effect of 1,4-naphthoquinone (4a–b, 5a–e), chemically (PTZ) and electrically (MES) induced models of epileptic seizures have been applied. These two commonly used nonclinical approaches reflect different types of seizures, since MES test is associated with generalized tonic-clonic convulsions, while PTZ model simulates absence seizures [13]. In our study, the synthesized compounds were tested using technique of intravenous PTZ infusion (i.v.PTZ) whereby chemoconvulsant is injected into the tail vein in mice with constant flow rate (0.01 ml s^{-1}). This procedure provides several advantages over the more widely used traditional subcutaneous PTZ test (s.c.PTZ): lower number of animals, insight into the seizure susceptibility, low interanimal variability in the clonus onset, and possibility to determine not only anticonvulsant but also proconvulsant action [13,14]. Heterocyclic compounds (4a–b, 5a–e) were orally administered into mice (100 mg kg^{-1}) followed by the detecting of PTZ dose that provoke clonic-tonic convulsions (DCTC) and tonic extension (DTE). As follows from Fig. 3, all triazine- and triazole-containing naphthoquinone derivatives along with valproic acid (VPA, positive control) were found to possess antiseizure effect at 3 h after their

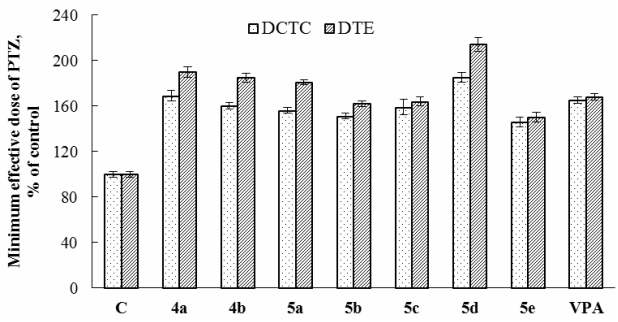


Fig. 3. Anticonvulsant activity of compounds (4a–b, 5a–e) at 3 h after oral administration. Values are given as mean±SEM, n=5 mice; for all groups $p<0.01$ as compared with control (C)

administration as confirmed by increasing of DCTC and DTE values ($p<0.01$ vs. control). All obtained compounds at this time point demonstrated average action (161% DCTC and 176% DTE) comparable to VPA activity (165% DCTC and 168% DTE).

Bearing in mind the high lipophilicity of naphthoquinone derivatives (4a–b, 5a–e) (logP range is from 5.01 to 6.25; ACD/Labs software), they were additionally examined for their prolonged anticonvulsant efficiency at 24 h after oral administration (Fig. 4). According to our data, all compounds retained their activity with an average DCTC and DTE values 142% and 152%, respectively, which is not statistically different from the reference drug VPA (164% DCTC and 166% DTE).

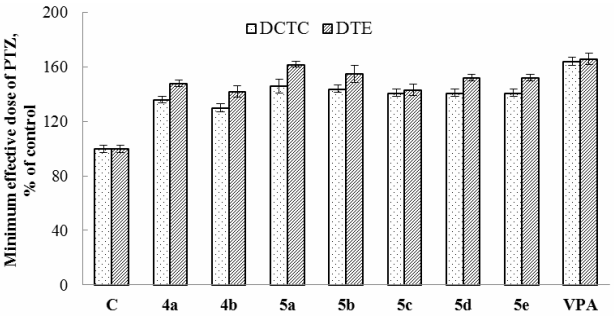


Fig. 4. Anticonvulsant activity of compounds (4a–b, 5a–e) at 24 h after oral administration. Values are given as mean±SEM, n=5 mice; for all groups $p<0.01$ as compared with control (C)

1,4-naphthoquinone derivatives (4a–b, 5a–e) were also analyzed for anticonvulsant action with the use of MES method and were revealed as promising anti-seizure agents. In this test, clonic-tonic seizures are induced by transcorneal electrical stimulus (50 mA) followed by the determining of mortality protection. Mice treated with the synthesized compounds have been completely protected against mortality over 24 h after single oral administration (100%, Table 1). In contrast, electrical stimuli in MES test were found to induce the rigid extension of the hind limbs in all animals from control group. As regards the positive control (VPA), it was able to prevent seizures in experimental animals with 80% and 60% at 3 h and 24 h after oral treatment, respectively.

Given that activity in PTZ test is typically widespread for drugs which enhance GABAergic neurotransmission or may act through T-type Ca^{2+} channels whereas blocking voltage dependent sodium channels is an action mechanism for active compounds in MES model [13], we may propose that naphthoquinone derivatives (4a–b, 5a–e) are involved in interaction with all aforementioned pharmacological targets.

For patients with epilepsy suffering from seizures, depression is a serious and frequent comorbidity. Taking this into account, synthesized triazine- and triazole-containing naphthoquinones were screened for their antidepressant activity in forced swim test (FST). As demonstrated in Table 2, the duration of immobility has been reduced in animals treated both with compounds (4a–b, 5a–e) and amitriptyline (reference drug) compared to control at 3 h after oral administration. At this time point, however, only derivative 4a exhibited remarkable activity that exceeded positive control (immobility period was 10.7 s for 4a vs. 25.7 s for amitriptyline).

With respect to investigation at long time period (24 h), the inefficiency of amitriptyline should be emphasized (immobility time: 93.7 s vs. 95.0 s of control). Meanwhile, all synthesized compounds were found to demonstrate significant antidepressant-like effect ($p<0.01$ vs. amitriptyline) affirming a prolonged activity of naphthoquinone derivatives (4a–b, 5a–e).

It is notorious that forced swim test (FST) aims to measure the reduction of behavioral immobility while investigating the compounds as potential antidepressant agents. However, two active behaviors (swimming and climbing) were discovered in the FST reflecting the possible action mechanism of antidepressants. Increased climbing with no effect on swimming was detected for antidepressants that selectively inhibit the reuptake of norepinephrine. In turn, inhibitors of selective serotonergic reuptake along with the immobility reduction were found to increase swimming activity [15]. In FST study of naphthoquinone derivatives (4a–b, 5a–e), the

Table 1

Anticonvulsant effect of compounds (4a–b, 5a–e) against maximal electroshock (MES)-induced seizures in mice

Compound	4a	4b	5a	5b	5c	5d	5e	VPA	Control
3 h after single oral administration									
% Mortality protection	100	100	100	100	100	100	100	80	0
24 h after single oral administration									
% Mortality protection	100	100	100	100	100	100	100	60	0

Table 2

Antidepressant activity of compounds (4a–b, 5a–e) in forced swim test (FST)*

Compound	Immobility time, s	
	3 h after administration	24 h after administration
Control	95.0±8.7	95.0±8.7
4a	10.7±0.7**	11.3±2.8**
4b	12.3±1.2**	13.7±4.4**
5a	23.0±4.4	10.3±2.3**
5b	67.7±1.2	12.0±4.0**
5c	50.0±4.2	23.3±2.3**
5d	33.3±3.3	12.3±1.5**
5e	68.3±9.4	12.7±1.3**
Amitriptyline	25.7±3.5	93.7±4.4

Note: * – all values are expressed as mean±SEM; n=5 mice; for all experimental groups $p<0.01$ as compared with control; ** – $p<0.01$ as compared with amitriptyline.

increased swimming behavior of experimental animals was registered that enable to suggest the direction of further detailed research.

Conclusions

In summary, a series of novel naphthoquinones containing 1,2,4-triazine- and 1,2,4-triazole moieties have been synthesized with a good yield and characterized by analytical and spectroscopic methods (ESI-MS, ^{1}H NMR, ^{13}C NMR, IR spectroscopy and elemental analysis). The preliminary results of pharmacological screening demonstrated that the target compounds 4a-b, 5a-e possess antiseizure action both in PTZ and MES tests along with antidepressant properties in FST model. Consequently, heterocycle-fused naphthoquinones synthesized in the present study exhibit combined effect on CNS and might be further explored as potential compounds for reducing depression symptoms in patients with convulsions.

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СИНТЕЗ ТА ФАРМАКОЛОГІЧНЕ ОЦІНЮВАННЯ НОВИХ ПОХІДНИХ НАФТОХІНОНУ, ЩО МІСТЯТЬ ФРАГМЕНТИ 1,2,4-ТРИАЗИНУ ТА 1,2,4 ТРИАЗОЛУ

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Синтезовано нові похідні нафтохінону, що містять фармакофорні фрагменти 1,2,4-триазину (4a–b) та 1,2,4-триазолу (5a–e) з подальшим підтвердженням їх структури за допомогою ESI-MS, ^{1}H-ЯМР, ^{13}C-ЯМР, ІЧ та елементного аналізу. Одержані гетероциклічні сполуки оцінювали за їх антиконвульсантною активністю на моделях хімічних та електричних судом у тесті пентилентетразолу (PTZ) та максимального електрошоку (MES). Тест на примусове плавання (FST) був використаний для оцінки антидепресивного ефекту досліджуваних похідних нафтохінону. Сполуки 4a–b та 5a–e (100 мг/кг) продемонстрували протисудомну дію, порівнянну з вальпроєвою кислотою в PTZ-тесті, і запобігли загибелі 100% мишей на моделі MES через 3 год та 24 год після перорального прийому. Більше того, ці похідні показали тривалу дію, подібну антидепресантам, значно зменшували тривалість часу нерухомості порівняно з референтним препаратом амітриптиліном.

Ключові слова: похідні нафтохінону, 1,2,4-триазин, 1,2,4-триазол, технологія, протисудомний засіб, антидепресант.

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL NAPHTHOQUINONE DERIVATIVES CONTAINING 1,2,4-TRIAZINE AND 1,2,4-TRIAZOLE MOIETIES

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Novel naphthoquinone derivatives bearing 1,2,4-triazine-(4a–b) and 1,2,4-triazole (5a–e) pharmacophores have been synthesized; their structure was confirmed by electrospray ionization mass spectrometry, ^{1}H NMR, ^{13}C NMR, IR spectroscopies and elemental analysis. The obtained heterocyclic compounds were estimated for their anticonvulsant activity on models of chemical- and electrical-induced seizures in pentylenetetrazole (PTZ) and maximal electroshock (MES) tests, respectively. Forced swimming test was used to evaluate the antidepressant effect of the naphthoquinone derivatives under study. Compounds 4a–b and 5a–e (100 mg kg^{-1}) demonstrated anticonvulsant action comparable with valproic acid in PTZ-test and prevented the death of 100% of mice in MES model at 3 h and 24 h after oral administration. Moreover, these derivatives showed prolonged antidepressant-like properties, significantly reducing the duration of immobility time in comparison with the reference drug amitriptyline.

Keywords: naphthoquinone derivatives; 1,2,4-triazine; 1,2,4-triazole; technology; anticonvulsant; antidepressant.

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