УДК 547.87:615.214.2 https://doi.org/10.33250/14.02.088

S. A. Demchenko[1], O. E. Yadlovskyi[1], O. V. Yudina[1], I. I. Tubaltseva[1], Yu. A. Fedchenkova[2], L. S. Bobkova[1], A. M. Demchenko[2]

Synthesis and anxiolytic activity of 3-aryl-1-(4[1]-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives

[1]Institute of Pharmacology and Toxicology of National Academy of Medical Sciences, Kyiv
[2]Nizhyn Mykola Gogol State University

Key words: anxiolytic activity, 3-aryl-1-(4[1]-methoxyphenyl)-1-(6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives, tranquilizing activity

Anxiety disorders, which cause chronic, disabling conditions are widespread and growing problem for healthcare across the world. According to WHO data, neurosis and psychopathy are observed in 5–15 % of world population. In developed countries, near 20 % have them on regular basis, and 30 % on periodically [1, 2]. It should be noted, that those pathologies strike various segments, from pediatric to geriatric population. Therapy of such nosologies imposes great spending for both individuals and society [3].

Anxiolytics are used for treatment of such pathologies [4]. Drugs of various pharmacological groups (barbiturates, benzodiazepins, carbamats, antihystaminea, antidepressants, opiates, sympatholytics, etc.) are classified as anxyolitics [5]. However, nowadays, there are no anxiolytics, which would optimally satisfy clinical conditions, which is related to insufficient imagination of pathology of anxiety, and insufficient effectiveness and safety of this group of drugs (negative influence on cardiovascular system, reproductive function, physical/psychological addiction, etc.). This is why search for new anxiolytic drugs is important and relevant. In the last decades, nitrogen heterocycles, which have composition, different from benzodiazepins raise significant interest. During the studies two new compounds L-838417 and TP13, selective to benzodiazepine receptior, binding with benzodiazepine fragment of GABA receptor were detected at *in vitro* conditions [6]. During the verification of data obtained in model experiments on animals (male mice C57BI/6), sedative and calming activities, which exceeded such in benzodiazepine, were detected. In other research, relationship of variety of chemical compounds to different subtypes of GABA receptor (α1, α2, α3, α5 GABAARs), influence on which has high effectiveness in relation to anxiety disorders, was studied [7]. During studies of pharmacological profile of TP003 compound at *in vitro* conditions and verification of data obtained in experiments on animals, it was shown, that TP003 implements it's action as partial, non-selective agonist of GABA-benzodiazepine receptor site *in vitro*, which shows it's anxiolytic action *in vivo* through α1, α2 i α3 GABAARs.

In the last years, significant interest in aspect of search for new anxiolytic drugs was caused by thiazoloazepine derivatives [8]. It should be noted, that anxiolytic activity of those compounds is related to influence on GABA receptors.

Therefore, we studied the tranquilizing activity of *5H*-[1,2,4]triazolo[4,3-a] azepine derivatives.

The aim of the study – to synthesize novel derivatives of of 3-aryl-1-(4[1]-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-

methyl)-urea and to study the anxiolytic activity at primary pharmacological screening stage.

Structural formulas for active ingredients of diazepam, gidazepam, mebicar and 1-(4[1]-methoxyphenyl)-3-phenyl-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea – Fig. 1.

Materials and methods. Test compounds – derivatives of 3-aryl-1-(4[1]-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea **(5 a-k)** were synthesized in department of medical chemistry, SI «Institute of Pharmacology and Toxicology NAMS of Ukraine» (Fig. 2).

[1]H-NMR spectra were recorded on the Bruker 400 MHz (Germany) with operating frequency 400.3965 MHz in DMSO-d6 using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm units with use of the δ scale.

Purity control of novel compounds was conducted by thin-layer chromatography in the system chloroform – methanol 9:1. The melting points were measured on a small-sized heating table with the observation device RNMK 05 (VEB Analytik, Dresden).

The lipophilicity (LogP) of the synthesized compounds was calculated using the program ACD LogP [9].

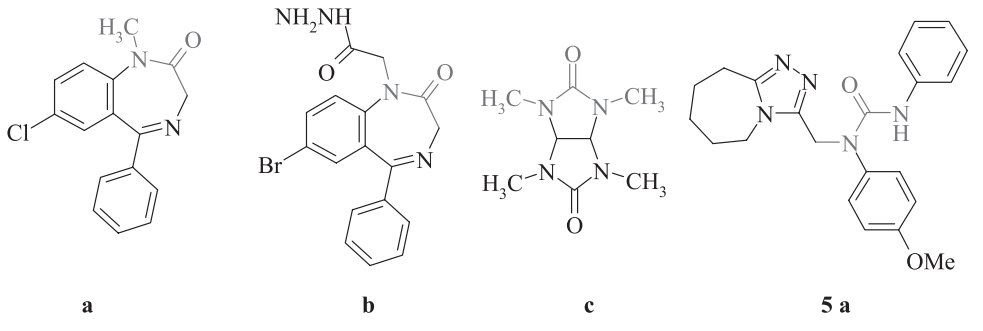
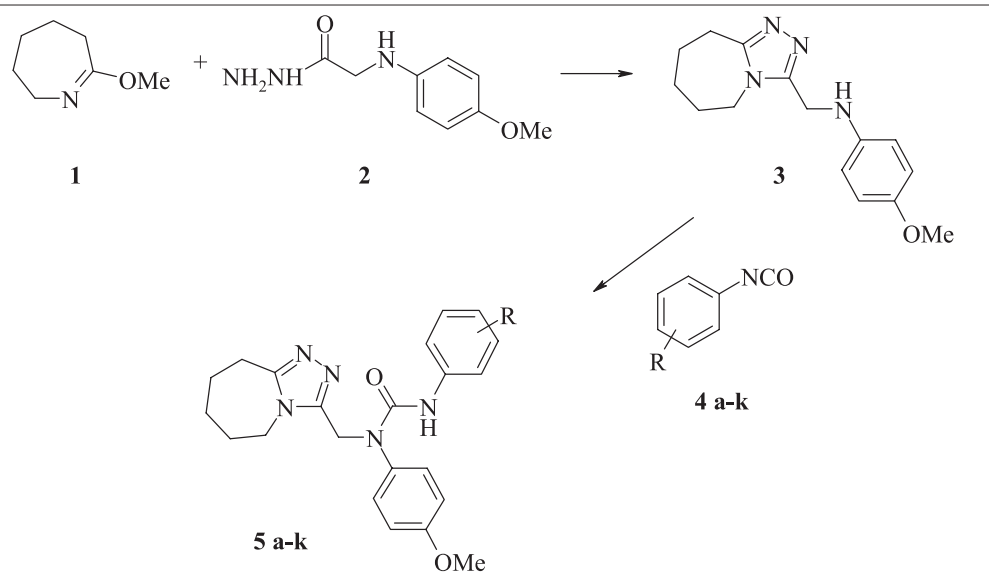


*Fig. 1. Structural formulas for active ingredients of diazepam (**a**), gidazepam (**b**), mebicar (**c**) and 1-(4[1]-methoxyphenyl)-3-phenyl-1-(6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea (**5 a**)*



Where **4,5: a)** R=H; **b)** R=2-CH_3; **c)** R=3-CH_3; **d)** R=4-CH_3; **e)** R=2-OCH_3; **f)** R=3-OCH_3; **g)** R=4-OCH_3; **h)** R= 2-Cl; **i)** R=3-Cl; **j)** 4-Cl; **k)** R=3,4Cl_2.

*Fig. 2. Scheme of synthesis of 3-aryl-1-(4[1]-methoxyphenyl)-1-(6,7,8,9-tetrahydro-5H-[1,2,4] triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives (**5 a-k**)*

2-Methoxy-3,4,5,6-tetrahydro-*7H*-azepine **1** was obtained by alkylating caprolactam with dimethyl sulfate using the method [10]. 3-(4^1-Methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-amine **3** was obtained by condensation of 2-methoxy-3,4,5,6-tetrahydro-7H-azepine **1** with (4-methoxyphenylamino)acetic acid hydrazide **2** and further cyclization of the intermediate product by the method [11]. 1-(4^1-methoxyphenyl)-3-phenyl-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea **5 a** was obtained by condensation of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-amine **3** with phenylisocyanate **4 a** in dry benzene medium according to method [12].

Synthesis of 1-(4^1-methoxyphenyl)-3-phenyl-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea 5 a. To solution of 1.36 g (0.005 M) 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-amine **2** in 25 ml dry benzene with stirring added 0.60 g (0.005 M) phenylisocyanate **4 a** and was refluxed for 1 hour. After cooling the precipitate formed is filtered off, dried. Yield 1.53 g (78%). M.p. = 190–192 °C (from ethanol). Anal. Calc. for $C_{22}H_{25}N_5O_2$, %: N 17.9. Found, %: N 17.7. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.56 (m, 2H, 7-CH_2), 1.62 (m, 2H, 8-CH_2), 1.78 (m, 2H, 6-CH_2), 2.84 (m, 2H, 9-CH_2), 3.77 (s, 3H, OCH_3), 4.07 (m, 2H, 5-CH_2), 4.94 (s, 2H, NCH_2), 6.94–7.38 (m, 9H, C_6H_5 + C_6H_4), 7.69 (s, 1H, NH). Log P = 2.22 ± 0.67.

Synthesis of 1-(4^1-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-3-(o-tolyl)-urea 5 b was obtained as urea **5 a** from 1.36 g (0.005 M) of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-amine **3** and 0.67 g (0.005 M) of ortho-tolylisocyanate 4 b. Yield 1.52 g (75 %). M.p. = 126–127 °C (from ethanol). Anal. Calc. for $C_{23}H_{27}N_5O_2$. %: N 17.3. Found, %: N 17.0. 1H NMR (400 MHz, DMSO-d6), δ (ppm): 1.56 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.79 (m, 2H, 6-CH_2), 1.97 (s, 3H, CH_3), 2.83 (m, 2H, CH_2), 3.76 (s, 3H, OCH_3), 4.09 (m, 2H, CH_2), 4.96 (s, 2H, NCH_2), 6.95–7.46 (m, 8H, C_6H_4 + C_6H_4), 7.36 (s, 1H, NH). Log P = 2.68 ± 0.67.

Synthesis of 1-(4^1-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-3-(m-tolyl)-urea 5 c was obtained as urea **5 a** from 1.36 g (0.005 M) of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-amine **3** and 0.67 g (0.005 M) of metha-tolylisocyanate 4 c. Yield 1.56 g (77 %). M.p. = 144–145 °C (from ethanol). Anal. Calc. for $C_{23}H_{27}N_5O_2$. %: N 17.3. Found, %: N 17.4. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.57 (m, 2H, 7-CH_2), 1.63 (m, 2H, 8-CH_2), 1.79 (m, 2H, 6-CH_2), 2.21 (s, 3H, CH_3), 2.83 (m, 2H, 9-CH_2), 3.78 (s, 3H, OCH_3), 4.07 (m, 2H, 5-CH_2), 4.94 (s, 2H, NCH_2), 6.75–7.23 (m, 8H, C_6H_4 + C_6H_4), 7.60 (s, 1H, NH). Log P = 2.68 ± 0.67.

Synthesis of 1-(4^1-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-3-(p-tolyl)-urea 5 d was obtained as urea **5 a** from 1.36 g (0.005 M) of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-amine **3** and 0.67 g (0.005 M) of para-tolylisocyanate **4 d**. Yield 1.62 g (80 %). M.p. = 205–206 °C (from propanole-2). Anal. Calc. for $C_{23}H_{27}N_5O_2$. %: N 17.3. Found, %: N 17.6. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.56 (m, 2H, 7-CH_2), 1.62 (m, 2H, 8-CH_2), 2.21 (s, 3H, CH_3), 1.80 (m, 2H, 6-CH_2), 2.83 (m, 2H, 9-CH_2), 3.76 (s, 3H, OCH_3), 4.07 (m, 2H, 5-CH_2), 4.93 (s, 2H, NCH_2), 6.94 and 7.14 (d-d, 4H, C_6H_4, J=9.1 Hz), 7.01 and 7.24 (d-d, 4H, C_6H_4, J=8.7 Hz),7.58 (s, 1H, NH). Log P = 2.68 ± 0.67.

Synthesis of 1-(4^1-methoxyphenyl)-3-(2^2-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-urea 5 e was obtained as urea **5 a** from 1.36 g (0.005 M) of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-amine **3** and 0.75 g (0.005 M) of 2-methoxyphenylisocyanate **4 e**. Yield 1.45 g (69 %). M.p. = 176–177 °C (from propanole-2). Anal. Calc. for $C_{23}H_{27}N_5O_3$. %: N 16.6. Found, %: N 16.8. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.57 (m,

2H, 7-CH_2), 1.63 (m, 2H, 8-CH_2), 1.81 (m, 2H, 6-CH_2), 2.83 (m, 2H, 9-CH_2), 3.56 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.05 (m, 2H, 5-CH_2), 4.96 (s, 2H, NCH_2), 6.86–8.07 (m, 4H, C_6H_4), 7.02 and 7.23 (d-d, 4H, C_6H_4, J=8.7 Hz), 6.97 (s, 1H, NH). Log P = 2.12 ± 0.68.

Synthesis of 1-(4^1-methoxyphenyl)-3-(3^2-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-urea 5 f was obtained as urea **5 a** from 1.36 g (0.005 M) of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-amine **3** and 0.75 g (0.005 M) of 3-methoxyphenylisocyanate **4 f**. Yield 1.54 g (73 %). M.p. = 168–169 °C (from propanole-2). Anal. Calc. for $C_{23}H_{27}N_5O_3$. %: N 16.6. Found, %: N 16.5. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.56 (m, 2H, 7-CH_2), 1.62 (m, 2H, 8-CH_2), 1.79 (m, 2H, 6-CH_2), 2.80 (m, 2H, 9-CH_2), 3.67 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 4.06 (m, 2H, 5-CH_2), 4.93 (s, 2H, NCH_2), 6.52–7.18 (m, 8H, C_6H_4 + C_6H_4), 7.68 (s, 1H, NH). Log P = 2.48 ± 0.68.

Synthesis of 1,3-bis-(4^1-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-urea 5 g was obtained as urea **5 a** from 1.36 g (0.005 M) of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-amine **3** and 0.75 g (0.005 M) of 4-methoxyphenylisocyanate **4 g**. Yield 1.60 g (76 %). M.p. = 185–186 °C (from propanole-2). Anal. Calc. for $C_{23}H_{27}N_5O_3$. %: N 16.6. Found, %: N 16.4. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.56 (m, 2H, 7-CH_2), 1.62 (m, 2H, 8-CH_2), 1.78 (m, 2H, 6-CH_2), 2.83 (m, 2H, 9-CH_2), 3.69 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 4.08 (m, 5-2H, CH_2), 4.93 (s, 2H, NCH_2), 6.79 and 7.14 (d-d, 4H, C_6H_4, J=9.1 Hz), 6.94 and 7.25 (d-d, 4H, C_6H_4, J=9.0 Hz), 7.55 (s, 1H, NH). Log P = 2.17 ± 0.68.

Synthesis of 3-(2^2-chlorophenyl)-1-(4^1-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-urea 5 h was obtained as urea **5 a** from 1.36 g (0.005 M) of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-amine **3** and 0.77 g (0.005 M) of 2-chlorophenylisocyanate **4 h**. Yield 1.53 g (72 %). M.p. = 123–124 °C (from propanole-2). Anal. Calc. for $C_{22}H_{24}ClN_5O_2$. %: N 16.4. Found, %: N 16.3. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.57 (m, 2H, 7-CH_2), 1.64 (m, 2H, 8-CH_2), 1.80 (m, 2H, 6-CH_2), 2.83 (m, 2H, 9-CH_2), 3.77 (s, 3H, OCH_3), 4.06 (m, 2H, 5-CH_2), 4.99 (s, 2H, NCH_2), 7.17–7.92 (m, 8H, C_6H_4+C_6H_4), 7.19 (s, 1H, NH). Log P = 2.73 ± 0.68.

Synthesis of 3-(3^2-chlorophenyl)-1-(4^1-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-urea 5 i was obtained as urea **5 a** from 1.36 g (0.005 M) of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-amine **3** and 0.77 g (0.005 M) of 3-chlorophenylisocyanate **4 i**. Yield 1.62 g (76 %). M.p. = 183–184 °C (from propanole-2). Anal. Calc. for $C_{22}H_{24}ClN_5O_2$. %: N 16.4. Found, %: N 16.6. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.59 (m, 2H, 7-CH_2), 1.65 (m, 2H, 8-CH_2), 1.82 (m, 2H, 6-CH_2), 2.84 (m, 2H, 9-CH_2), 3.76 (s, 3H, OCH_3), 4.07 (m, 2H, 5-CH_2), 4.91 (s, 2H, NCH_2), 6.91–7.84 (m, 8H, C_6H_4+C_6H_4), 7.05 (s, 1H, NH). Log P = 3.25 ± 0.68.

Synthesis of 3-(4^2-chlorophenyl)-1-(4^1-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-urea 5 j was obtained as urea **5 a** from 1.36 g (0.005 M) of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-amine **3** and 0.77 g (0.005 M) of 4-chlorophenylisocyanate **4 j**. Yield 1.70 g (80 %). M.p. = 223–224 °C (from ethanol). Anal. Calc. for $C_{22}H_{24}ClN_5O_2$. %: N 16.4. Found, %: N 16.7. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.56 (m, 2H, 7-CH_2), 1.64 (m, 2H, 8-CH_2), 1.78 (m, 2H, 6-CH_2), 2.84 (m, 2H, 9-CH_2), 3.77 (s, 3H, OCH_3), 4.06 (m, 2H, 5-CH_2), 4.94 (s, 2H, NCH_2), 6.94 and 7.16 (d-d, 4H, C_6H_4, J=8.6 Hz), 7.25 and 7.44 (d-d, 4H, C_6H_4, J=8.8 Hz), 7.92 (s, 1H, NH). Log P = 3.21 ± 0.68.

Synthesis of 3-(3^2,4^2-dichlorophenyl)-1-(4^1-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-urea 5 k was obtained as urea **5 a** from 1.36 g (0.005 M) of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-amine **3** and 0.94 g (0.005 M) of 3,4-dichlorophenylisocyanate **4 k**. Yield 1.96 g (85%).

M.p. = 211-212 °C (from ethanol). Anal. Calc. for $C_{22}H_{23}Cl_2N_5O_2$. %: N 15.2. Found, %: N 15.5. ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.58 (m, 2H, 7-CH_2), 1.65 (m, 2H, 8-CH_2), 1.83 (m, 2H, 6-CH_2), 2.85 (m, 2H, 9-CH_2), 3.78 (s, 3H, OCH_3), 4.06 (m, 2H, 5-CH_2), 4.91 (s, 2H, NCH_2), 6.94 and 7.14 (d-d, 4H, C_6H_4, J=8.6 Hz), 7.36–8.01 (m, 4H, C_6H_4), 7.75 (s, 1H, NH). Log P = 4.12 ± 0.68.

Researches of anxiolytic activity were conducted on white non-linear female mice with mass of 20 ± 2g, bred in SI IFT of NAMS of Ukraine. Animals were held on standard food ration, received food and water *ad libitum*.

Since anxiolytic drugs are characterized by sedative, anticonvulsant and muscle relaxation action, evaluation of GABA-ergic mechanism of action of new derivatives of triazol-azepines was conducted on models of «open field», korazol induced seizures and wire test in comparison with diazepam and gidazepam [13–15].

In all tests following pharmacological agents were used: diazepam (substance) pharmacopoeia standard, 2 mg/kg ED_{50}, intragastrically; gidazepam (substance), pharmacopoeia standard 2 mg/kg, intragastrically; (6,7,8,9-tetrahydro-*5H*-[1,2,4] triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives **5 a-k** were administrated intragastrically in equimolar doses to ED_{50} of diazepam in form of water-alcohol emulsion.

Activity studies of (6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives were conducted on model of «open field» in comparative aspect with diazepam and gidazepam. Large, rectangle cell (100 × 100 cm) with 40 cm high wooden walls and with small holes on the crossings between squares. It had wooden floor with a black grid, which divides field into 64 (8 × 8) equal squares. The mouse was placed inside the cell and observed for 3 minutes. Feces droppings, grooming, looking in the holes, standing on back paws and number of passed squares were registered. Square, on which animal stepped with both front paws was counted as a passed square. The animals were split into groups of five. Studied animals intragastrically received drugs or other compounds in doses equimolar to diazepam. Animals of control group, intragastrically received solvent in volume of 0,2 ml. After 30 min, animals were placed in «Open field» and then the measurement was conducted. Compound activity was judged by it's registered indicators changes compared with control.

Muscle relaxation action of researched compounds was evaluated on wire test. The animals were divided in groups of five. Initial muscle activity was studied before administration of researched compounds. For that animals were hanged on wire and observed for 60 seconds. Duration of hanging was registered by stopwatch. 30 minutes after administration of compound, muscle relaxation activity was measured again. Levels of muscle relaxation activity was judged by changes in latent period of hanging on wire, compared to initial values. Activity of compounds was judged by reliable changes of values, registered in relation to control.

Evaluation of anticonvulsant activity of researched compounds was conducted on model of corazol seizures. For modeling seizures mice received pentylenetetrazol (corazol) in dose of 100 mg/kg subcutaneously. Lab animals were divided in groups of 5. Studied animals received diazepam in ED_{50} = 2,0 mg/kg, or gidazepam or researched compounds in doses equimolar to diazepam in form of water-alcohol emulsion intragastrically. Animals from control group received solvent in volume of 0,2 ml. 30 minutes after injection of researched compounds, animals received corazol subcutaneously. Evaluation of anticonvulsant effect was conducted by duration of latent period of seizures development, duration of tonic-clonic seizures, intensity of seizures, and lethality during 15 minutes after injection. Intensity of seizure development was evaluated by M. C. Gerald 6 point scale [15].

- 0 – absence of convulsant activity;
- 1 – hyperkinesia;
- 2 – trembling, twitching, stereotypical movements;
- 3 – clonic convulsions of front paws with standing on back paws;

- 4 – pronounced tonic-clonic convulsions, collapsing of the animal, presence of tonic extension phase;
- 5 – repeated tonic-clonic convulsions, loss of position;
- 6 – full tonic extension, death of the animal.

Compound activity was judged by reliable changes in indicators, which are registered in relations to control.

Statistical treatment of data obtained was conducted with Student t-criteria method. Changes on $P < 0.05$ were considered reliable [16].

Results and discussion. (6,7,8,9-Tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives **5 a-k** demonstrated sedative activity on open field model. Horizontal activity inhibition by studied compounds is significantly lower (7.9–36.1 %) in comparison to diazepam (-62.3 %), and all compounds except **5 g** and **5 d** demonstrated unreliable effect, close to that of day tranquilizer gidazepam (-2.0 %). Simultaneously studied animals moved through the open field and diametrical movement wasn't dominant, which indicates absence of fear in animals.

Established, that (6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives **5 a-k** showed diverse effect on exploration activity of animals. Compounds **5 e**, **5 i**, **5 a**, **5 j** inhibit exploration activity in animals by 52.38 %, 66.66 %, 80.85 % and 80.95 % correspondingly, demonstrating effects, close to those of diazepam and gidazepam.

Studied (6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives **5 a-k** moderately inhibit vertical activity (24.1–44.8 %), being inferior in effectiveness to diazepam (90.2 %) and not being inferior to gidazepam (36.6 %).

Inhibition in grooming is one of the most important characteristics of anxiety. Compounds **5 d**, **5 e**, **5 i**, **5 k** and **5 a** reliably inhibit grooming in animals by 41.9–100 %. Grooming inhibition is related to modulation of substitutes in para-position of benzol ring of N`-group of urea. Besides, introduction of chlorine atoms into meta- and para- positions shows maximum effect. Another characteristic of anxiety is quantity of feces droppings in animals. Indicated, that both comparative compounds and studied (6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives **5 a-k** decrease quantity of feces droppings by 50–100 %, compared to control, however unreliability of this data allows to observe this effect as tendency.

Evaluation of muscle relaxation action showed, that absence of such in studied (6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives **5 a-k**, unlike in comparative drugs (58.2 % and 21.3 % for diazepam and gidazepam), correspondingly.

During evaluation of anticonvulsant activity, it was indicated, that researched compounds influenced development of hyperkinesias, and compounds **5 c**, **5 i**, **5 k** та **5 g** decreased it by 45.5–89.1 %, thus exceeding or not being inferior to diazepam (0 %) and gidazepam (50 %).

Indicated, that (6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives **5 a-k** moderately influence on the development of tremor and stereotypical movements, which appeared in several minutes after injection of corazol. Reliable effect (36.4–54.5 %), is demonstrated by compounds **5 e**, **5 d** та **5 c**. It exceeds that of gidazepam (0 %), but is inferior to to that of diazepam (-60 %).

All researched compounds inhibit clonic seizures in approximately same degree (50.0–68.8 %), exceeding gidazepam, but being inferior to diazepam, which points out presence of such activity in compounds.

Reliable inhibition of clonic-tonic seizures characterize compounds **5 a** and **5 j**, which exceed comparative drugs diazepam and gidazepam by activity.

Thus, researched line of (6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives **5 a-k** is characterized by anticonvulsant and axiolytic activities. Absense of muscle relaxation activity in wire test and absence/moderate inhibition of horizontal activity suggests absence of GABA-ergic component of specific activity of

studied compounds. Anticonvulsant activity of compounds and influence on «open field» behavior depend on modification of substitutes in para- and ortho- positions of benzene ring of N-group of urea. By anticonvulsant activity, which is due to methyl group in meta- or para- positions of benzene ring of N`-group of urea, most significant results were obtained for compounds **5 c** (meta-) and **5 d** (para-), which in variety of cases are not inferior to diazepam by activity. By it's characteristics in «open field» model, compound **5 d** exceeds tranquilizer gidazepam.

Conclusions

1. New derivatives of 3-aryl-1-(4^1-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea were synthesized by the condensation of 3-(4^1-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-amine with appropriate arylisocyanates in dry benzene medium at 69–85 % yield.
2. Studies of muscle relaxation and anticonvulsant action of 3-aryl-1-(4^1-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-ure derivatives, conducted on models of «open field», korazol induced convulsions and hanging wire test showed, that this array of compounds demonstrates anxiolytic and anticonvulsant activity, which is not inferior or exceeds that of diazepam and gidazepam.
3. There was established, that compound 1-(4^1-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepin-3-yl-methyl)-3-(p-tolyl)-urea **5 d** exceeds daytime tranquilizer gidazepam by results of primary screening.

1. *Griebel G., Holmes A.* 50 years of hurdles and hope in anxiolytic drug discovery. *Nat Rev Drug Discov.* 2013. V. 12 (9). P. 667–87.
2. *Kessler R. C., Merikangas K. R*. The National Comorbidity Survey Replication (NCS-R): background and aims. *Int J Methods Psychiatr Res*. 2004. V. 13 (2). P. 60–68.
3. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. R. C. Kessler, W. T. Chiu, O. Demler et al. *Arch Gen Psychiatry*. 2005. V. 62 (6). P. 617–627.
4. Section Review Central & Peripheral Nervous Systems: Novel anxiolytic agents – 1994 to present. A. D. Jordan, C. P. Kordik, A. B. Reitz, P. J. Sanfilippo. *Expert Opinion on Therapeutic Patents*. 1996. V. 6 (10). P. 1047–1060.
5. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 13th Edition by Laurence Brunton, Bjorn Knollman, Randa Hilal-Dandan. 1440 p.
6. Subtype-selective GABAergic drugs facilitate extinction of mouse operant behavior. C. McCabe, D. Shaw, J. R. Atack et al. *Neuropharmacology*. 2004. V. 46 (2). P. 171–178.
7. TP003 is a non-selective benzodiazepine site agonist that induces anxiolysis via α2 GABAA receptors. E. Neumann, W. T. Ralvenius, M. A. Acuña et al. *Neuropharmacology*. 2018. V. 18 (143). V. 71–78.
8. *Atack R. J.* GABAa Receptor Subtype-Selective Modulators. I. α2 / α3-Selective Agonists as Non-Sedating Anxiolytics. *Current Topics in Medicinal Chemistry.* 2011. V. 11 (9). P. 1176–1202.
9. ACD/Structure Elucidator, version 2018.1, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2019.
10. Ацетали лактамов. VII. Исследование алкилирования N-метиллактамов и лактимных эфиров диметилсульфатом. В. Г. Граник, А. М. Жидкова, Н. С. Курятов и др. *ХГС*. 1973. № 11. С. 1532–1535.
11. Synthesis and Anticorrosion and Biocidal Activity of Triazoloazepine Derivatives. A. M. Demchenko, K. G. Nazarenko, A. P. Makei et al. *Russian Journal of Applied Chemistry.* 2004. V. 77 (5). P. 790–793.
12. Synthesis of derivatives of 5,6,7,8-tetrahydro-2,2a,8a-triazacyclophenta[cd]azulenes and their effect on platelet aggregation. S. A. Demchenko, L. S. Bobkova, O. E. Yadlovskyi, T. A. Bukhtiarova, A. M. Demchenko. *Pharmacology and drug toxicology*. 2019. V. 13, № 1. P. 28–34.
13. *Calcaterra N. E., Barrow J. C.* Classics in chemical neuroscience: diazepam (valium). *ACS Chem Neurosci*. 2014. V. 16, 5 (4). P. 253–60.
14. *Vogel Gerhard H.* Drug Discovery and Evaluation. Pharmacological Assays Second Completely Revised, Updated, and Enlarged Edition. Springer Verlag. Berlin Heidelberg, 2002. 1026 p.
15. *Gerald M. C., Riffee W. H*. Acute and chronic effects of d- and l-amphetamine on seizure susceptibility in mice. *Eur. J. Pharmacol*. 1973. V. 21, № 3. P. 323–330.
16. *Лапач С. Н., Чубенко А. В., Бабич П. Н*. Статистика в науке и бизнесе. Киев : Морион, 2002. 640 с.

S. A. Demchenko, O. E. Yadlovskyi, O. V. Yudina, I. I. Tubaltseva, Yu. A. Fedchenkova , L. S. Bobkova, A. M. Demchenko

Synthesis and anxiolytic activity of 3-aryl-1-(4[1]-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives

Anxiety disorders are a widespread and growing problem of healthcare across the world. According to WHO data, neurosis and psychopaties are observed in 5–15 % of world population. In developed countries, about 20 % of population take anxiolytics permanently, and 30 % periodically. According to the WHO data it should be noted, that those pathologies affect different segments of population, from pediatric to geriatric population. Therapy of such nosologies imposes great loses on both individuals and society.

3-(4[1]-Methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-amine was obtained by condensation of 2-methoxy-3,4,5,6-tetrahydro-*7H*-azepine with (4-methoxyphenylamino) acetic acid hydrazide and further cyclization of the intermediate product. 3-Aryl-1-(4[1]-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives were obtained by condensation of 3-(4[1]-methoxyphenyl)-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-amine with appropriate arylisocyanates in dry benzene medium.

The aim of the study – to synthesize and study an anxiolytic activity of 3-aryl-1-(4[1]-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives in comparison with known drugs diazepam and gidazepam on the stage of primary pharmacological screening.

Tranquilizing activity of compounds was evaluated on models of «open field», «corazol convulsions» and «wire test» on mice, after administration at doses, equimolar to ED_{50} of diazepam. Diazepam (2 mg/kg) and gidazepam (2 mg/kg) were used as referent compounds.

There were established, that 3-aryl-1-(4[1]-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives are characterized by anticonvulsant and anxiolytic activity. The absence of muscle relaxant effect and absence or moderate inhibition of horizontal activity, suggest the absence of GABAergic component in specific activity of studied compounds. Anticonvulsant and behavioral activity («open field») of studied compounds depend on modification of substitutes on para- and ortho- positions of benzene ring of N`-group of urea. By specific activity, researched compounds are not inferior (or exceed) daytime tranquilizer gidazepam.

On open field model compound 1-(4[1]-methoxyphenyl)-1-(6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a] azepin-3-yl-methyl)-3-(p-tolyl)-urea **5 d** is characterized by lesser influence on horizontal, vertical and exploration activity, while grooming and quantity of feces droppings are significantly inhibited. This indicates less sedation in comparison to such of diazepam. However, on open field model compound **5 d** exceeds daytime tranquilizer gidazepam by results of primary screening.

Key words: anxiolytic activity, 3-aryl-1-(4[1]-methoxyphenyl)-1-(6,7,8,9-tetrahydro-5H-[1,2,4] triazolo[4,3-a]azepine-3-yl-methyl)-urea derivatives, tranquilizing activity

С. А. Демченко, О. Є. Ядловський, О. В. Юдіна, І. І. Тубальцева, Ю. А. Федченкова, Л. С. Бобкова, А. М. Демченко

Синтез та анксіолітична активність похідних 3-арил-1-(4[1]-метоксифеніл)-1-(6,7,8,9-тетрагідро-*5H*-[1,2,4]триазол[4,3-а]азепін-3-іл-метил)-сечовини

Тривожні розлади є поширеною проблемою охорони здоров'я в усьому світі. За даними Всесвітньої організації охорони здоров'я, неврози та психопатії спостерігаються в 5–15 % населення земної кулі. У розвинених країнах близько 20 % населення приймає анксіолітичні препарати постійно і 30 % – періодично. Слід зазначити, що дані патологічні стани вражають різні верстви населення, від педіатричної до геріатричної популяції. Терапія даних нозологій накладає величезні витрати як на окремих осіб, так і на суспільство.

Мета дослідження – синтезувати та вивчити анксіолітичну активність похідних 3-арил-1-(4[1]-метоксифеніл)-1-(6,7,8,9-тетрагідро-*5H*-[1,2,4]триазоло [4,3-а]азепін-3-іл-метил)-сечовини порівняно з відомими препаратами діазепамом і гідазепамом на етапі первинного фармакологічного скринінгу.

3-(4[1]-Метоксифеніл)-(6,7,8,9-тетрагідро-*5H*-[1,2,4]триазоло[4,3-а]азепін-3-іл-метил)-амін був одержаний конденсацією 2-метокси-3,4,5,6-тетрагідро-*7H*-азепіну з гідразидом (4-метоксифеніламіно)оцтової кислоти та подальшою циклізацією проміжного продукту. Похідні 3-арил-1-(4[1]-метоксифеніл)-1-(6,7,8,9-тетрагідро-*5H*-[1,2,4]триазоло[4,3-а]азепін-3-іл-метил)-сечовини були синтезовані взаємодією 3-(4[1]-метоксифеніл)-(6,7,8,9-тетрагідро-*5H*-[1,2,4] триазоло[4,3-а]азепін-3-іл-метил)-аміну з відповідними арилізотіоціанатами в середовищі сухого бензину.

Транквілізуючу активність оцінювали на моделях «відкритого поля», «коразолових судом», «дротовому тесті» на мишах за введення в дозах, еквімолярних $ЕД_{50}$ діазепаму. Референтними препаратами були субстанції діазепаму (2 мг/кг) та гідазепаму (2 мг/кг).

Встановлено, що для досліджуваного ряду 3-арил-1-(4[1]-метоксифеніл)-1-(6,7,8,9-тетрагідро-*5H*-[1,2,4]триазоло[4,3-а]азепін-3-іл-метил)-сечовини є характерною протисудомна та анксіолітична активність. Відсутність м'язово релаксуючої дії та відсутність або помірне пригнічення горизонтальної активності передбачає відсутність ГАМК-ергічного компонента специфічної активності вивчених сполук. Протисудомна та поведінкова активність («відкрите поле») досліджених сполук

залежить від модифікації замісників у пара- або орто-положенні бензольного кільця N`-групи сечовини. За специфічною активністю досліджені сполуки не поступаються (або переважають) денний транквілізатор гідазепам.

Встановлено, що на моделі відкритого поля сполуці 1-(4^1-метоксифеніл)-1-(6,7,8,9-тетрагідро-*5H*-[1,2,4]триазоло[4,3-а]азепін-3-іл-метил)-(3-пара-толіл)-сечовини **5 d** притаманний менший вплив на горизонтальну, вертикальну та дослідницьку активність зі значним пригніченням грумінгу та кількості фекальних болюсів. Це вказує на меншу седативну дію порівняно з такою діазепаму. Але на моделі відкритого поля сполука **5 d** за результатами первинного скринінгу переважає денний транквілізатор гідазепам.

Ключові слова: анксіолітична активність, похідні 3-арил-1-(4^1-метоксифеніл)-1-(6,7,8,9-тетрагідро-5H-[1,2,4]триазоло[4,3-а]азепін-3-іл-метил)-сечовини, транквілізуюча активність

С. А. Демченко, О. Е. Ядловский, О. В. Юдина, И. И. Тубальцева, Ю. А. Федченкова, Л. С. Бобкова, А. М. Демченко

Синтез и анксиолитическая активность производных 3-арил-1-(4^1-метоксифенил)-1-(6,7,8,9-тетрагидро-*5H*-[1,2,4]триазоло[4,3-а]азепин-3-ил-метил)-мочевины

Тревожные расстройства являются распространенной и постоянно растущей проблемой здравоохранения мира. По данным Всемирной организации здравоохранения, неврозы и психопатии наблюдаются у 5–15 % населения земного шара. В промышленно развитых странах около 20 % населения принимают анксиолитические препараты постоянно и 30 % – периодически. Следует отметить, что данные патологические состояния поражают различные группы населения – от педиатрической до гериатрической популяции. Терапия данных нозологий требует значительных затрат, как индивидуальных, так и государственных.

Цель исследования – синтезировать и изучить анксиолитическую активность производных 3-арил-1-(4^1-метоксифенил)-1-(6,7,8,9-тетрагидро-*5H*-[1,2,4]триазоло[4,3-а]азепин-3-ил-метил)-мочевины по сравнению с известными препаратами диазепамом и гидазепамом на этапе первичного фармакологического скрининга.

3-(4^1-Метоксифенил)-(6,7,8,9-тетрагидро-*5H*-[1,2,4]триазоло[4,3-а]азепин-3-ил-метил)амин был получен конденсацией 2-метокси-3,4,5,6-тетрагидро-*7H*-азепина с гидразидом (4-метоксифениламино)уксусной кислоты и последующей циклизацией образовавшегося промежуточного продукта. Производные 3-арил-1-(4^1-метоксифенил)-1-(6,7,8,9-тетрагидро-*5H*-[1,2,4]триазоло[4,3-а] азепин-3-ил-метил)-мочевины были синтезированы взаимодействием 3-(4^1-метоксифенил)-(6,7,8,9-тетрагидро-*5H*-[1,2,4]триазоло[4,3-а] азепин-3-ил-метил)-амина с соответствующими арилизотиоцианатами в среде сухого бензола.

Транквилизирующую активность оценивали на мышах с использованием моделей «открытого поля», «коразоловых судорог» и «проволочного теста» при внутрижелудочном введении в дозах, эквимолярных $ЕД_{50}$ диазепама. Референтными препаратами служили субстанции диазепама (2 мг/кг) и гидазепама (2 мг/кг).

Установлено, что для исследуемого ряда 3-арил-1-(4^1-метоксифенил)-1-(6,7,8,9-тетрагидро-*5H*-[1,2,4]триазоло[4,3-а] азепин-3-ил-метил)-мочевины характерна противосудорожная и анксиолитическая активность. Отсутствие мышечно-релаксирующего действия и отсутствие или умеренное угнетение горизонтальной активности предполагает отсутствие ГАМК-эргического компонента специфической активности изученных соединений. Противосудорожная и поведенческая активность («открытое поле») исследованных соединений зависит от модификации заместителей в пара- или орто-положениях бензольного кольца N`-фрагмента мочевины. По выраженности специфической активности соединения не уступают (или превосходят) дневной транквилизатор гидазепам.

Показано, что на модели открытого поля для соединения 1-(4^1-метоксифенил)-1-(6,7,8,9-тетрагидро-*5H*-[1,2,4]триазоло[4,3-а]азепин-3-ил-метил)-(3-пара-толил)-мочевины **5 d** характерно меньшее влияние на горизонтальную, вертикальную и исследовательскую активность при значительном снижении груминга и количества фекальных болюсов, что указывает на меньшее седативное действие по сравнению с таковым диазепама. В тесте открытого поля соединение **5 d** по результатам первичного скрининга превосходит дневной транквилизатор гидазепам.

Ключевые слова: анксиолитическая активность, производные 3-арил-1-(4^1-метоксифенил)-1-(6,7,8,9-тетрагидро-5H-[1,2,4]триазоло[4,3-а]азепин-3-ил-метил)-мочевины, транквилизирующая активность

Надійшла: 3 березня 2020 р.

Прийнята до друку: 22 квітня 2020 р.

Контактна особа: Демченко С. А., ДУ «Інститут фармакології та токсикології НАМНУ», буд. 14, вул. Антона Цедіка, м. Київ, 03057. Тел.: + 38 0 44 456 42 56.