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Y. N. Novodvorskyi[1], O. Y. Bahlai[2], I. V. Komarov[1], A. M. Demchenko[2,3]

Synthesis and antioxidant activity of derivatives of *8H*-[1,2,4]triazolo[4,3-в][1,2,4]triazin-7-ones

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

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Many severe diseases, such as atherosclerosis, hypertension, Alzheimer's disease, diabetes, infertility etc are connected with an oxidative stress development and formation of reactive forms of oxygen in excess amounts, which include free radicals and peroxides. Formation of such substrates result in damage of many cell components, such as lipids, DNA and proteins [1].

As a means of pathogenetic therapy of such diseases are frequently used an antioxidants. Despite rather large quantity of medicines with antioxidant properties, search of new compounds, which would meet safety and efficiency requirements is still relevant.

Previously it has been shown [2] that three derivatives of 8-(4[1]-hydroxy-3R-benzylidenamino)-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-в][1,2,4]triazine-7-ones exhibit high antioxidant properties. As the comparison compounds well-known antioxidants ionol and ascorbic acid were selected [3,4].

The aim of the study – to synthesize new derivatives of 8-(4[1]-hydroxy-3R-benzylidenamino)-6-tert-butyl-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazine-7-ones and to evaluate their antioxidant properties in screening test *in vitro* as compared with ionol and ascorbic acid.

Materials and methods. Test compounds were synthesized in Taras Shevchenko National University of Kyiv (Figure). Antioxidant activity was studied in department of medical chemistry of SI «Institute of Pharmacology and Toxicology NAMS of Ukraine».

4-Amino-6-tert-butyl-3-methylthio-4H-[1,2,4]triazinine-5-one **1** was obtained by alkylating 4-Amino-6-tert-butyl-3-thio-4H-[1,2,4]triazinine-5-one with iodomethane in alkaline conditions using the method [5]. 4-Amino-6-tert-butyl-3-hydrazino-4H-[1,2,4]triazinine-5-one **2** was obtained by boiling of 3-methylthio-derivative **1** with excess of hydrazinhydrate in propanol-2 by method [6]. 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-в][1,2,4]triazine-7-он **3** was obtained by boiling of compound **2** with orthoformic ether in ethanol. Substituted 8-benzylidenamino-derivatives **5–10** were synthesized by standard Schiff method of alkali obtainment [7].

^{1}H-NMR spectra were recorded on the Bruker VXR-360 (Germany), the working frequency of 360.191 MHz, in DMSO-d6 using tetramethyl silane (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.

Synthesis of 8-amino-6-tert-butyl-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **4**. To solution of 7.93 g (0.04 M) 4-amino-6-tert-butyl-3-hydrazino-*4H*-[1,2,4]triazin-5-one **3** in 100 ml ethanole 5.93 g (0.04 M) diethoxymethoxyethane and 2 drops of acetic acid were added. The reaction mixture was refluxed for 8 hour. After cooling the solid **4** was filtered, washed with ethanole, then dried on air. Yield 5.24 g (63 %). M.p. = 190–191 °C. Anal. Calc. for $C_8H_{12}N_6O$. %: N 40.3. Found, %: N 40.0. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): Found, %: N 40.0.

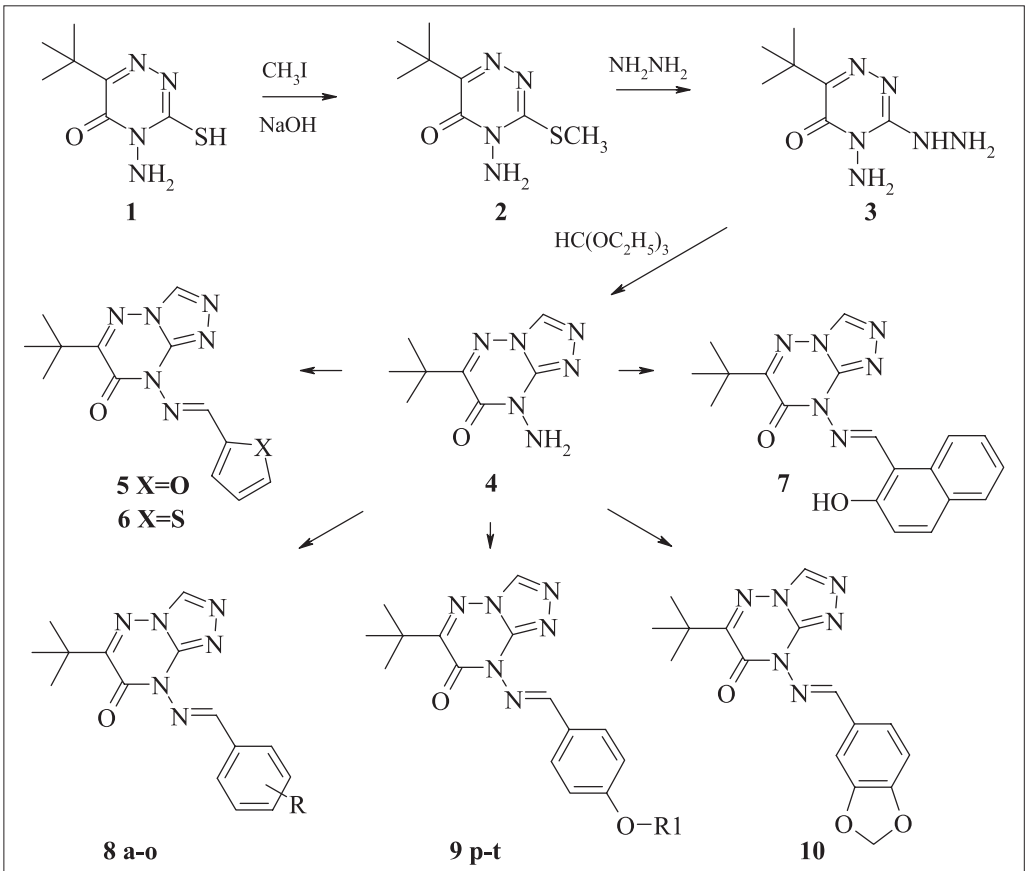

*Figure. Scheme of synthesis of 8-(4[1]-hydroxy-3R-benzylidenamino)-6-tert-butyl-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-ones (**5–10**)*

*Where 8: **a**) R=H; **b**) R=4-CH_3; **c**) R= 4-F; **d**) R= 4-Cl; e) R= 4-Br; f) R= 4-NMe_2; **g**) R= 4-COOMe; **h**) R= 2-Cl; i) R=2-OH; **j**) R= 2-OCH_3; **k**) R= 3-OH; **l**) R= 2-OH, 3-OCH_3; **m**) R= 4-OH, 3-OCH3; n) 3-OH, 4-OCH3; o) 4-OH, 3-OC_2H_5.*
*9: **p**) R=H; **q**) R=CH3; r) CH_2Ph; s) $CH_2C_6H_4Cl$(para); t) $CH_2CONPh(CH(CH_3)_2)$.*

$C_8H_{12}N_6O$. Calc., %: N 40.3. NMR ^1H (δ, ppm, DMSO-d6): 1.42 (s, 3H, $C(CH_3)$), 6.56 (s, 2H, NH_2), 8.82 (s, 1H, 3-CH).

Synthesis of 6-tert-butyl-8-[(furan-2-ylmethylene)-amino]-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **5**. To solution of 2.08 g (0.01 M) 8-amino-6-tert-butyl-***8H***-[1,2,4]triazolo[4,3-b] [1,2,4]triazin-7-one 4 in 50 ml ethanol 0.96 g (0.01 M) furan-2 carbaldehyde was added. The reaction mixture was refluxed for 3 hour and evaporated to volume 15 ml. After cooling the solid **5** was filtered, recrystallized from propanole-2. Yield 1.46 g (51 %). M.p. = 216–217 °C. Anal. Calc. for $C_{13}H_{14}N_6O_2$. %: N 29.3. Found, %: N 29.5. ^1H NMR (360 MHz, DMSO-d6), δ (ppm): 1.41 (s, 9H, $C(CH_3)_3$), 6.77–8.03 (m, 3H, C_4H_3S), 9.05 (s, 1H, CH), 9.27 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-[(thiophen-2-ylmethylene)-amino]-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **6** was obtained as compound **5** from 2.08 r (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one 4 and 1.12 g (0.01 M) of thiophene-2 carbaldehyde. Yield 1.72 g (57 %). M.p. = 217–218 °C. Anal. Calc. for $C_{13}H_{14}N_6O_2$. %: N 29.3. Found, %: N 29.5. ^1H NMR (500 MHz, DMSO-d6), δ (ppm): 1.40 (s, 9H, $C(CH_3)_3$), 7.29 (t, 1H, C_4H_3S), 7.88 (d, 3H, C_4H_3S), 7.99 (d, 3H, C_4H_3S), 9.18 (s, 1H, CH), 9.54 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-[(2-hydroxynaphthalen-1-ylmethylene)-amino]-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **7** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]

triazin-7-one 4 and 1.72 g (0.01 M) of 2-hydroxynaphtalene-1 carbaldehyde. Yield 2.35 g (65%). M.p. = 240–241 °C. Anal. Calc. for $C_{19}H_{18}N_6O_2$. %: N 23.2. Found, %: N 23.4. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.50 (s, 9H, $C(CH_3)_3$), 7.25 (d, 1H, $C_{10}H_5$), 7.40 (t, 1H, $C_{10}H_5$), 7.63 (t, 1H, $C_{10}H_5$), 7.83 (d, 1H, $C_{10}H_5$), 7.96 (d,1H, $C_{10}H_5$), 8.41 (d, 1H, $C_{10}H_5$), 8.89 (s, 1H, CH), 10.7 (s, 1H, CH), 12.1 (s, 1H, OH).

Synthesis of 6-tert-butyl-8-(benzylideneamino)-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8** a was obtained as compound **5** from 2.08 r (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one 4 and 1.06 g (0.01 M) of benzaldehyde. Yield 1.81 g (61 %). M.p. = 217–218 °C. Anal. Calc. for $C_{15}H_{16}N_6O$. %: N 28.4. Found, %: N 28.2. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.47 (s, 9H, $C(CH_3)_3$), 7.52–8.00 (m, 5H, C_6H_5), 8.80 (s, 1H, CH), 9.49 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-[(4^1-methylbenzylidene)-amino]-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **8 b** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one 4 and 1.20 g (0.01 M) of 4-methylbenzaldehyde. Yield 1.95 g (63 %). M.p. = 256–257 °C. Anal. Calc. for $C_{16}H_{18}N_6O$. %: N 27.1. Found, %: N 26.8. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.47 (s, 9H, $C(CH_3)_3$), 2.47 (S, 3H, CH_3), 7.31 and 7.89 (d-d, 4H, C_6H_4, J=8.0 Hz), 8.62 (s, 1H, CH), 9.46 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-[(4^1-fluorobenzylidene)-amino]-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 c** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **4** and 1.24 g (0.01 M) of 4-fluorobenzaldehyde. Yield 2.04 g (65 %). M.p. = 205–207 °C. Anal. Calc. for $C_{15}H_{15}FN_6O$. %: N 26.7. Found, %: N 26.4. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.47 (s, 9H, $C(CH_3)_3$), 7.21–8.09 (m, 4H, C_6H_4), 8.64 (s, 1H, CH), 9.56 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-[(4^1-chlorobenzylidene)-amino]-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 d** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one 4 and 1.41 g (0.01 M) of 4-chlorobenzaldehyde. Yield 2.22 g (67 %). M.p. = 255–256 °C. Anal. Calc. for $C_{15}H_{15}ClN_6O$. %: N 25.4. Found, %: N 25.7. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.47 (s, 9H, $C(CH_3)_3$), 7.51 and 8.04 (d-d, 4H, C_6H_4, J=8.8 Hz), 8.63 (s, 1H, CH), 9.64 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-[(4^1-bromobenzylidene)-amino]-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 e** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **4** and 1.85 g (0.01 M) of 4-bromobenzaldehyde. Yield 2.81 g (75 %). M.p. = > 260 °C. Anal. Calc. for $C_{15}H_{15}BrN_6O$. %: N 22.4. Found, %: N 22.2. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.40 (s, 9H, $C(CH_3)_3$), 7.81 and 9.92 (d-d, 4H, C_6H_4, J=8.5 Hz), 9.19 (s, 1H, CH), 9.46 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-[(4^1-N,N-dimethylaminobenzylidene)-amino]-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 f** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **4** and 1.49 g (0.01 M) of 4-N,N-dimethylaminobenzaldehyde. Yield 2.48 g (73 %). M.p. = 231–232 °C. Anal. Calc. for $C_{17}H_{21}N_7O$. %: N 28.9. Found, %: N 28.6. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.46 (s, 9H, $C(CH_3)_3$), 3.12 (s, 9H, $N(CH_3)_2$), 6.75 and 7.79 (d-d, 4H, C_6H_4, J=8.9 Hz), 8.61 (s, 1H, CH), 9.03 (s, 1H, CH).

Synthesis of 4-[(6-tert-butyl-7-oxo-7H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-8-ylimino)-methyl]-benzoic acid methyl ester **8 g** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b] [1,2,4] triazin-7-one **4** and 1.64 g (0.01 M) of 4-formylbenzoic acid methyl ester. Yield 2.12 g (60 %). M.p. = > 260 °C. Anal. Calc. for $C_{17}H_{18}N_6O_3$. %: N 23.7. Found, %: N 23.6. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.42 (s, 9H, $C(CH_3)_3$), 3.91 (S, 3H, CH_3), 8.11 and 8.15 (d-d, 4H, C_6H_4), 9.22 (s, 1H, CH), 9.64 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-[(21-chlorobenzylidene)-amino]-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 h**

was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **4** and 1.41 g (0.01 M) of 2-chlorobenzaldehyde. Yield 2.12 g (64 %). M.p. = 201–202 °C. Anal. Calc. for $C_{15}H_{15}ClN_6O$. %: N 25.4. Found, %: N 25.1. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.44 (s, 9H, $C(CH_3)_3$), 7.52–8.22 (m, 4H, C_6H_4), 9.09 (s,1H, CH), 10.0 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-[(2^1-hydroxybenzylidene)-amino]-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **8 i** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **4** and 1.22 g (0.01 M) of 2-hydroxybenzaldehyde. Yield 1.72 g (55 %). M.p. = 228–229 °C. Anal. Calc. for $C_{15}H_{16}N_6O_2$. %: N 26.9. Found, %: N 26.8. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.48 (s, 9H, $C(CH_3)_3$), 6.96 (t, 1H, C_6H_4), 7.00 (d, 1H, C_6H_4), 7.39 (t, 1H, C_6H_4), 7.70 (d, 1H, C_6H_4), 8.66 (s, 1H, CH), 9.87 (s, 1H, CH), 10.8 (s, 1H, OH).

Synthesis of 6-tert-butyl-8-[(2^1-methoxybenzylidene)-amino]-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **8 j** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **4** and 1.36 g (0.01 M) of 2-methoxybenzaldehyde. Yield 1.92 g (59 %). M.p. = 181-182 °C. Anal. Calc. for $C_{16}H_{18}N_6O_2$. %: N 25.8. Found, %: N 25.6. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.41 (s, 9H, $C(CH_3)_3$), 3.92 (s, 3H, OCH_3), 7.14 (t, 1H, C_6H_4), 7.24 (d, 1H, C_6H_4), 7.64 (t, 1H, C_6H_4), 8.07 (d, 1H, C_6H_4), 9.18 (s, 1H, CH), 9.63 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-[(3^1-hydroxybenzylidene)-amino]-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **8 k** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **4** and 1.22 g (0.01 M) of 3-hydroxybenzaldehyde. Yield 1.78 g (57 %). M.p. = 241–242 °C. Anal. Calc. for $C_{15}H_{16}N_6O_2$. %: N 26.9. Found, %: N 27.1. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.40 (s, 9H, $C(CH_3)_3$), 7.03 (d, 1H, C_6H_4), 7.34 (d, 1H, C_6H_4), 7.38 (t, 1H, C_6H_4), 7.40 (s, 1H, C_6H_4), 9.18 (s, 1H, CH), 9.28 (s, 1H, CH), 9.89 (s, 1H, OH).

Synthesis of 6-tert-butyl-8-[(2^1-hydroxy-3^1-methoxybenzylidene)-amino]-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 l** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4] triazin-7-one **4** and 1.52 g (0.01 M) of 2-hydroxy-3-methoxybenzaldehyde. Yield 1.78 g (52 %). M.p. = > 260 °C. Anal. Calc. for $C_{16}H_{18}N_6O_3$. %: N 24.6. Found, %: N 24.9. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.41 (s, 9H, $C(CH_3)_3$), 3.88 (s, 3H, OCH_3), 6.95 (t, 1H, C_6H_3), 7.20 (d, 1H, C_6H_3), 7.49 (t, 1H, C_6H_3), 9.17 (s, 1H, CH), 9.67 (s, 1H, CH), 10.2 (s, 1H, OH).

Synthesis of 6-tert-butyl-8-[(4^1-hydroxy-3^1-methoxybenzylidene)-amino]-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 m** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4] triazin-7-one **4** and 1.52 g (0.01 M) of 4-hydroxy-3-methoxybenzaldehyde. Yield 2.36 g (69 %). M.p. = 223–224 °C. Anal. Calc. for $C_{16}H_{18}N_6O_3$. %: N 24.6. Found, %: N 24.4. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.46 (s, 9H, $C(CH_3)_3$), 3.94 (s, 3H, OCH_3), 6.89 (d, 1H, C_6H_3), 7.32 (d, 1H, C_6H_3), 7.55 (s, 1H, C_6H_3), 8.64 (s, 1H, CH), 9.13 (s, 1H, CH), 9.30 (s, 1H, OH).

Synthesis of 6-tert-butyl-8-[(3^1-hydroxy-4^1-methoxybenzylidene)-amino]-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 n** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4] triazin-7-one 4 and 1.52 g (0.01 M) of 4-hydroxy-3-methoxybenzaldehyde. Yield 2.26 g (66 %). M.p. = 252–253 °C. Anal. Calc. for $C_{16}H_{18}N_6O_3$. %: N 24.6. Found, %: N 24.7. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.40 (s, 9H, $C(CH_3)_3$), 3.87 (s, 3H, OCH_3), 7.09 (d, 1H, C_6H_3), 7.32 (d, 1H, C_6H_3), 7.46 (s, 1H, C_6H_3), 9.07 (s, 1H, CH), 9.17 (s, 1H, CH), 9.58 (s, 1H, OH).

Synthesis of 6-tert-butyl-8-[(4^1-hydroxy-3^1-ethoxybenzylidene)-amino]-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 o** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4] triazin-7-one *4* and 1.66 g (0.01 M) of

4-hydroxy-3-ethoxybenzaldehyde. Yield 2.36 g (69 %). M.p.= 218–219 °C. Anal. Calc. for $C_{17}H_{20}N_6O_3$. %: N 23.6. Found, %: N 23.3. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.45 (s, 9H, $C(CH_3)_3$), 1.48 (t, 3H, CH_2CH_3), 4.17 (q., 2H, CH_2CH_3), 6.89 (d, 1H, C_6H_3), 7.32 (d, 1H, C_6H_3), 7.53 (s, 1H,C_6H_3), 8.63 (s, 1H, CH), 9.12 (s, 1H, CH), 9.18 (s, 1H, OH).

Synthesis of 6-tert-butyl-8-[(4^1-hydroxybenzylidene)-amino]-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **9 p** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **4** and 1.22 g (0.01 M) of 4-hydroxybenzaldehyde. Yield 2.25 g (72 %). M.p. = > 250 °C. Anal. Calc. for $C_{15}H_{16}N_6O_2$. %: N 26.9. Found, %: N 26.6. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.45 (s, 9H, $C(CH_3)_3$), 6.90 and 7.79 (d-d, 4H, C_6H_4), 8.62 (s, 1H, CH), 9.12 (s, 1H, CH), 9.95 (s, 1H, OH).

Synthesis of 6-tert-butyl-8-[(4^1-methoxybenzylidene)-amino]-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **9 q** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*- [1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **4** and 1.36 g (0.01 M) of 4-methoxybenzaldehyde. Yield 2.28 g (70 %). M.p.= 230–231 °C. Anal. Calc. for $C_{16}H_{18}N_6O_2$. %: N 25.8. Found, %: N 25.9. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.46 (s, 9H, $C(CH_3)_3$), 3.90 (s, 3H, OCH_3), 7.04 and 7.94 (d-d, 4H, C_6H_4, J=8.9 Hz), 8.75 (s,1H, CH), 9.27 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-[(4^1-benzyloxybenzylidene)-amino]-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **9 r** was obtained as compound 5 from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **4** and 2.12 g (0.01 M) of 4-benzyloxybenzaldehyde. Yield 2.93 g (73 %). M.p. = 198–199 °C. Anal. Calc. for $C_{22}H_{22}N_6O_2$. %: N 20.9. Found, %: N 20.6. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.40 (s, 9H, $C(CH_3)_3$), 5.23 (s, 2H, CH_2), 7.22 and 7.93 (d-d, 4H, C_6H_4, J=9.0 Hz), 7.34–7.50 (m, 5H, C_6H_5), 9.17 (s, 1H, CH), 9.18 (s, 1H, CH).

Synthesis of 6-tert-butyl-8-{[4-(4^1-chlorobenzyloxy)-benzylidene]-amino}-8H-[1,2,4] triazolo[4,3-b][1,2,4]triazin-7-one **9 s** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4] triazin-7-one **4** and 2.47 g (0.01 M) of 4-(41-chlorobenzyloxy)-benzaldehyde. Yield 3.36 g (77 %). M.p. = 199–200 °C. Anal. Calc. for $C_{22}H_{21}ClN_6O_2$. %: N 19.2. Found, %: N 19.4. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.40 (s, 9H, $C(CH_3)_3$), 5.23 (s, 2H, OCH_2), 7.21 and 7.93 (d-d, 4H, C_6H_4, J=8.8 Hz), 7.47 and 7.52 (d-d, 4H, C_6H_4, J=8.5 Hz),9.17 (s, 1H, CH), 9.19 (s, 1H, CH).

Synthesis of 2-{4-[(6-tert-butyl-7-oxo-7H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-8-ylimino)-methyl]-phenoxy}-N-isopropyl-N-phenylacetamide **9 t** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4] triazolo[4,3-b] [1,2,4]triazin-7-one **4** and 2.97 g (0.01 M) of 2-(41-formylphenoxy)-N-isopropyl-N-phenylacetamide. Yield 3.75 g (77 %). M.p. = 151–152 °C. Anal. Calc. for $C_{26}H_{29}N_7O_3$. %: N 20.1. Found, %: N 20.3. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.08 (d, 6H, $CH(CH_3)_2$), 1.40 (s, 9H, $C(CH_3)_3$), 4.36 (s, 2H, CH_2), 4.80 (m, 1H, $CH(CH_3)_2$), 6.94 and 7.87 (d-d, 4H, C_6H_4, J=8.7 Hz), 7.41–7.54 (m, 5H, Ph), 9.16 (s, 1H, CH), 9.17 (s, 1H, CH).

Synthesis of 8-[(benzo[1,3]dioxol-5-ylmethylene)-amino]-6-tert-butyl-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **10** was obtained as compound **5** from 2.08 g (0.01 M) 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4] triazin-7-one **4** and 1.50 g (0.01 M) of benzo[1,3]dioxol-5-carbaldehyde. Yield 2.93 g (73 %). M.p. = 221–222 °C. Anal. Calc. for $C_{16}H_{16}N_6O_3$. %: N 24.7. Found, %: N 24.6. ^{1}H NMR (360 MHz, DMSO-d6), δ (ppm): 1.40 (s, 9H, $C(CH_3)_3$), 6.18 (s, 2H, CH_2), 7.12 (d, 1H, C_6H_3), 7.47 (d, 1H, C_6H_3), 7.50 (s, 1H, C_6H_3), 9.16 (s, 1H, CH), 9.18 (s, 1H, CH).

Screening of compound's antioxidant activity (AOA) was studied *in vitro* by their ability to inhibit lipoperoxide formation, induced by $FeSO_4$ in yolk lipoproteids emulsion. [8, 9]. Ascorbic acid and ionol were taken as comparison compounds. The test was conducted by the following method. Each of compounds as 10 μM/ml solution diluted in DMSO, 0,5 ml 0,7 % $FeSO_4 \cdot 7H_2O$ solution and

3 ml potassium-phosphate buffer were added to yolk emulsion. After for 60 min at 37 °C incubation, solution was cooled and used for determination of lipid peroxidation products, which react with thiobarbituric acid (TBA-active products), particularly, malonic dialdehyde (MDA). For this, to solution, obtained after incubation, 2 ml of cooled 20 % trichloroacetic acid and 0,05 ml trilon B (50 mg/l) were added. After centrifugation, 2 ml 1 % of freshly prepared tiobarbituric acid was added to each supernatant and incubated for 35 min at 95 °C. After cooling, 4 ml of buthanol-1 was added, and optical density of buthanol extracts was measured, using KFK-2 photo colorimeter at wave length of 540 nanometers. Each determination was repeated three times.

AOA of test and control samples was evaluated by formula:

$$AOA = \frac{D_c - D_t}{D_c} \cdot 100\ \%, \text{ where}$$

D_c – optical density at 540 nm of control sample (DMSO without test compound);

D_t – optical density at 540 nm of test sample (DMSO with test compound);

Results and discussion. According to the data obtained (table), it is possible to note certain dependences of the test substances structures with their antioxidant properties.

Thus, the introduction of electron-withdrawing substituents (F, Cl, Br and COOMe) into the benzene ring leads to decrease of an antioxidant properties as to the base molecule 6-tert-butyl-8-(benzylideneamino)-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 a** without substitutes.

Replacing the benzene ring with heterocyclic thiophene **6** and furan **5** residues results in very strong prooxidant properties. Thus, 6-tert-butyl-8-[(furan-2-ylmethylene)-amino]-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **5** has the highest value of prooxidant activity: -110.9 % in relation to ascorbic acid and -134.1 % in relation to ionol.

It has been shown that antioxidant properties of the molecule depend significantly on the position of the hydroxyl group in the benzene ring. Thus, the highest activity value in the 2-OH **(8 i)**, 3-OH **(8 k)** i 4-OH **(9 p)** series is registered for compound 6-tert-butyl-8-[(41-hydroxybenzylidene)-amino]-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **9 p**. The lowest is for the compound **(8 k)** with the presence of a hydroxyl group in the third position of the benzene ring. It should be noted that this pattern persists with the introduction into the ring of electron-donating OCH_3 and OC_2H_5 substituents (compounds **8 l**, **8 m**, **8 n** and **8 o)** that enhance the antioxidant properties of the parent molecule. In the case of condensation of compound **4** with salicylic aldehyde and with 2-hydroxynaphthalene-1-carbaldehyde substances **8 i** and **7** are formed, respectively.

Analysing their antioxidant properties, we can conclude that condensed derivatives with a hydroxyl group have a higher AOA than their corresponding benzene derivatives with values of 48.08 % and 35.90 % **(7** and **8 i)** respectively. For substances **7**, **8 a** antioxidant properties were observed at the level of ascorbic acid, and substances **8 l**, **8 o** showed higher results than ascorbic acid.

Thus, a new series of *8H*-[1,2,4]triazolo[4,3-в][1,2,4]triazin-7-ones derivatives has been proposed, which may form the basis for the creation of new antioxidants.

Conclusions

1. A series of new derivatives of *8H*-[1,2,4]triazolo[4,3-в][1,2,4]triazin-7-ones has been synthesized and their structure has been proved by modern physicochemical methods. An antioxidant properties of new compounds were evaluated in screening test *in vitro* as compared with ionol and ascorbic acid.
2. The regularities of the chemical structure – pharmacological action are established. It is shown that the introduction of electron-withdrawing substituents (F, Cl, Br and COOMe) into the benzene ring leads to a decrease of the antioxidant properties as to the base molecule 6-tert-butyl-8-

Table

Antioxidant activity (AOA) of derivatives of 8H-[1,2,4]triazolo[4,3-в] [1,2,4]triazin-7-ones

Compound, substituents		Optical density (540 nm)	AOA, %
The control – DMSO		0.312	
The control – Ionol		0.053	83,01
The control – ascorbic acid		0.148	52,56
4		0.250	19,87
5	2-furyl	0.658	-110,90
6	2-thiophenyl	0.382	-22,44
7		0.162	48,08
8 a	H	0.163	47,76
8 b	4-Me	0.335	-7,37
8 c	4-F	0.338	-8,33
8 d	4-Cl	0.272	12,82
8 e	4-Br	0.282	9,62
8 f	4-NMe_2	0.288	7,69
8 g	4-COOMe	0.262	16,03
8 h	2-Cl	0.249	20,19
8 i	2-OH	0.200	35,90
8 j	2-OCH_3	0.267	14,42
8 k	3-OH	0.322	-3,21
8 l	2-OH, 3-OCH_3	0.122	60,90
8 m	4-OH, 3-OCH_3	0.228	26,92
8 n	3-OH, 4-OCH_3	0.258	17,31
8 o	4-OH, 3-OC_2H_5	0.121	61,22
9 p	4-OH	0.178	42,95
9 q	4-OCH_3	0.225	27,88
9 r	4-OCH_2Ph	0.336	-7,69
9 s	4-$OCH_2C_6H_4Cl$	0.337	-8,01
9 t		0.332	-6,41
10	3,4-CH_2OCH_2	0.302	3,21

(benzylideneamino)-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 a** without substitutes. The most active were derivatives of 8-(benzylideneamino)-6-~{tert}-butyl-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-ones, which had a hydroxyl group and an additional oxyalkyl fragment in the third position of the benzene ring **(8 l, 8 o).**

3. The data obtained substantiate the feasibility of further studying the derivatives of 6-tert-butyl-8-(benzylideneamino)-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-ones as potential antioxidant agents.

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Y. N. Novodvorskyi, O. Y. Bahlai, I. V. Komarov, A. M. Demchenko

Synthesis and antioxidant activity of derivatives of *8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-ones

Oxidative stress is usually connected with many severe diseases, such as atherosclerosis, hypertension, Alzheimer's disease etc. Therefore, the search for new antioxidants is a very important pharmacological task.

The aim of the study – to synthesize new derivatives of 8-(4[1]-hydroxy-3R-benzylidenamino)-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazine-7-ones and to evaluate their antioxidant properties in screening test *in vitro* as compared with ionol and ascorbic acid.

By interaction of equimolar amounts of 8-amino-6-tert-butyl-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one with the corresponding aromatic and heterocyclic aldehydes in ethanol, a series of Schiff bases with the key fragment *8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one were synthesized. The structure and purity of all the substances obtained were confirmed by ^{1}H NMR spectroscopy.

Screening of compound's antioxidant activity (AOA) was studied *in vitro* by their ability to inhibit lipoperoxide formation, induced by $FeSO_4$ in yolk lipoproteins emulsion.

It is shown, that, depending on the structure, the test substances exhibit both antioxidant and prooxidant properties. The regularities of the chemical structure – pharmacological action are established. It is shown that the introduction of electron-withdrawing substituents (F, Cl, Br and COOMe) into the benzene ring leads to a decrease in the antioxidant properties as to the base molecule 6-tert-butyl-8-(benzylideneamino)-*8H*-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-one **8 a** without substitutes. The most active were derivatives of 8-(benzylideneamino)-6-~{tert}-butyl-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-ones, which had a hydroxyl group and an additional oxyalkyl fragment in the third position of the benzene ring (**8 l**, **8 o**).

The data obtained substantiate the feasibility of further studying the most active derivatives of 6-tert-butyl-8-(benzylideneamino)-8H-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7-ones as potential antioxidant agents.

Key words: antioxidant activity, ascorbic acid, ionol, derivatives of 8H-[1,2,4]triazolo[4,3-в][1,2,4]triazin-7-ones

Є. М. Новодворський, О. Ю. Баглай, І. В. Комаров, А. М. Демченко

Синтез та антиоксидантна активність похідних *8H*-[1,2,4]триазоло [4,3-в][1,2,4]триазин-7-ону

Багато серйозних захворювань, такі як атеросклероз, гіпертензія, хвороба Альцгеймера, діабет та ін. пов'язані з розвитком окиснювального стресу. Тому пошук нових антиоксидантів є дуже актуальною задачею фармакології.

Мета дослідження – синтезувати нові похідні *8H*-[1,2,4]триазоло [4,3-в][1,2,4]триазин-7-ону та вивчити їхню антиоксидантну активність у скринінговому дослідженні *in vitro* порівняно з іонолом та аскорбіновою кислотою.

Взаємодією еквімолярних кількостей 8-аміно-6-трет-бутил-*8H*-[1,2,4] триазоло[4,3-в][1,2,4] триазин-7-ону з відповідними ароматичними та гетероциклічними альдегідами в етанолі синтезовано низку основ Шиффа з ключовим фрагментом *8H*-[1,2,4]триазоло[4,3-в][1,2,4]триазин-7-ону. Будову та чистоту всіх отриманих речовин підтверджено даними ЯМР ^{1}H спектроскопії.

Антиоксидантну активність (АОА) похідних *8H*-[1,2,4]триазоло [4,3-в] [1,2,4]триазин-7-ону оцінювали в дослідах *in vitro* за їхньою здатністю пригнічувати утворення продуктів ліпопереокиснення, індуковане $FeSO_4$ у ліпопротеїдах курячого яйця.

Показано, що досліджувані речовини залежно від будови проявляють як антиоксидантні, так і

прооксидантні властивості. Встановлені закономірності хімічна будова – біологічна активність.

Отримані дані обґрунтовують доцільність подальшого вивчення найактивніших похідних *8H*-[1,2,4] триазоло[4,3-в] [1,2,4]триазин-7-ону як нових потенційних антиоксидантних засобів.

Ключові слова: антиоксидантна активність, аскорбінова кислота, іонол, похідні 8H-[1,2,4] триазоло[4,3-в][1,2,4]триазин-7-онів

Е. Н. Новодворский, А. Ю. Баглай, И. В. Комаров, А. М. Демченко

Синтез и антиоксидантная активность производных *8H*-[1,2,4]триазоло[4,3-в] [1,2,4]триазин-7-онов

Многие серьезные заболевания, такие как атеросклероз, гипертензия, болезнь Альцгеймера, диабет и др. сопряжены с развитием окислительного стресса. Поэтому поиск новых антиоксидантов является актуальной задачей фармакологии.

Цель исследования – синтезировать новые производные *8H*-[1,2,4]триазоло[4,3-в][1,2,4]триазин-7-она и изучить их антиоксидантную активность в скрининговом тесте *in vitro* по сравнению с ионолом и аскорбиновой кислотой.

Взаимодействием эквимолярных количеств 8-амино-6-трет-бутил-*8H*-[1,2,4]триазоло[4,3-в] [1,2,4]триазин-7-она с соответствующими ароматическими и гетероциклическими альдегидами в этаноле синтезирован ряд оснований Шиффа с ключевым фрагментом *8H*-[1,2,4]триазоло [4,3-в] [1,2,4]триазин-7-она. Строение и чистота всех полученных веществ подтверждены данными ЯМР ^{1}H спектроскопии.

Антиоксидантную активность (АОА) производных *8H*-[1,2,4] триазоло[4,3-в][1,2,4]триазин-7-она оценивали *in vitro* по их способности ингибировать образование продуктов липопереокисления, индуцированное $FeSO_4$ в липопротеинах куриного яйца.

Показано, что исследуемые вещества в зависимости от строения проявляют как антиоксидантные, так и прооксидантные свойства. Установлены закономерности химическое строение – биологическая активность.

Полученные данные обосновывают целесообразность дальнейшего изучения наиболее активных производных *8H*-[1,2,4]триазоло [4,3-в][1,2,4]триазин-7-она в качестве новых потенциальных антиоксидантных средств.

Ключевые слова: антиоксидантная активность, аскорбиновая кислота, ионол, производные 8H-[1,2,4]триазоло[4,3-в][1,2,4]триазин-7-онов

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