Actoprotective activity research of 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetates

A. A. Safonov, A. V. Nevmyvaka

Zaporizhzhia State Medical University, Ukraine

Severe fatigue can occur due to overwork, lack of exercise, depression, insomnia, etc. It should be understood that fatigue, weakness, both emotional and physical, is not a disease. Often, actoprotective substances are used to reduce fatigue. To search for new substances with a different spectrum of pharmacological activity, 1,2,4-triazole derivatives have proven themselves well.

The aim of work was to investigate actoprotective activity among previously synthesized 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetates.

Materials and methods. The compounds used to study pharmacological activity were synthesized at the Department of Natural Sciences for International Students and Toxicological Chemistry ZSMU. White nonlinear rats weighing 200–260 g of 7 animals per group were used to study the actoprotective activity of 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetates. As a method for the study of pharmacological activity was used the method of forced swimming with a load of 10 % by weight of the rat. Statistical results were calculated using Kolmogorov–Smirnov test and Shapiro-Wilk test.

Results. Compounds Ia, Ib, IIb, IIk, IIj had been found to have a moderate actoprotective effect. But none compound had exceeded the comparison drug. Some conclusions were drawn regarding the dependence of "structure – actoprotective effect": the most active compound was 2-aminoethanol 2-((5-(2-bromophenyl)-4-ethyl-4H-1,2,4-triazole-3-yl)thio)acetate (IIj); conversion to 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetate salts and selection as sodium, potassium, or 2-aminoethanol cations was resulted in to increase the actoprotective effect.

Conclusions. As a result, the actoprotective activity of 18 new 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetates was investigated. Some conclusions were drawn regarding the dependence of "structure – actoprotective effect".
There are many factors that “depress” our body: bad habits, constant stress, lack of sleep, hormonal failure, and even anemia. It should be understood that fatigue, weakness, both emotional and physical, is not a disease. Severe fatigue can occur due to overwork, lack of exercise, depression, insomnia, etc. Often, actoprotective substances are used to reduce fatigue. To search for new substances with a large spectrum of pharmacological activity, 1,2,4-triazole derivatives have proven themselves well [1–4]. New 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetates were studied not sufficiently [5–12].

Aim

That’s why the aim of this work was to investigate actoprotective activity among previously synthesized 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetates.

Materials and methods

The compounds used to study pharmacological activity were synthesized at the Department of Natural Sciences for International Students and Toxicological Chemistry, ZSMU [13].

White nonlinear rats weighing 200–260 g of 7 animals per group were used to study the actoprotective activity of 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetates.

As a method for the study of pharmacological activity was used the method of forced swimming [14] with a load of 10% by weight of the rat.

Loads were fixed at the base of the tail of the animals. After immersing the animals underwater for 10 seconds, the laboratory rats’ swimming time was measured until depletion in seconds. The rats were immersed individually in a large container with a water layer in excess of 60 cm. The water temperature was maintained at 24–27 °C. The tested compounds, as well as the standard of comparison – Riboxin® (manufactured by Kyiv Vitamin Plant) were injected intraperitoneally 20 minutes before the start of immersion of animals at a dose of 100 mg/kg. For comparison, we also used a control group of animals with intraperitoneal injection of saline 20 minutes before immersion.

Gravimetric measurements were performed on laboratory electronic analytical scales model ESI-200–4(US).

Statistical results were calculated using Kolmogorov–Smirnov and Shapiro–Wilk tests.

Results

As a result, the actoprotective activity of 18 new compounds was investigated. Compounds Ia, Ib, IIb, IIk, IIj had been found to have an actoprotective effect. But none compound exceeded the comparison drug. Some conclusions have been made regarding the dependence “structure – actoprotective activity”.

Materials and methods

The compounds used to study pharmacological activity were synthesized at the Department of Natural Sciences for International Students and Toxicological Chemistry, ZSMU [13].

White nonlinear rats weighing 200–260 g of 7 animals per group were used to study the actoprotective activity of 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetates.

As a method for the study of pharmacological activity was used the method of forced swimming [14] with a load of 10% by weight of the rat.

Load were fixed at the base of the tail of the animals. After immersing the animals underwater for 10 seconds, the laboratory rats’ swimming time was measured until depletion in seconds. The rats were immersed individually in a large container with a water layer in excess of 60 cm. The water temperature was maintained at 24–27 °C. The tested compounds, as well as the standard of comparison – Riboxin® (manufactured by Kyiv Vitamin Plant) were injected intraperitoneally 20 minutes before the start of immersion of animals at a dose of 100 mg/kg. For comparison, we also used a control group of animals with intraperitoneal injection of saline 20 minutes before immersion.

Gravimetric measurements were performed on laboratory electronic analytical scales model ESI-200–4(US).

Statistical results were calculated using Kolmogorov–Smirnov and Shapiro–Wilk test.

Results

As a result, the actoprotective activity of 18 new compounds was investigated. Compounds Ia, Ib, IIb, IIk, IIj had been found to have an actoprotective effect. But none compound exceeded the comparison drug. Some conclusions have been made regarding the dependence “structure – actoprotective activity”.

Materials and methods

The compounds used to study pharmacological activity were synthesized at the Department of Natural Sciences for International Students and Toxicological Chemistry, ZSMU [13].

White nonlinear rats weighing 200–260 g of 7 animals per group were used to study the actoprotective activity of 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetates.
Discussion

It should be noted that the most active compound was 2-((5-(2-bromophenyl)-4-methyl-4H-1,2,4-triazole-3-yl)thio)acetate acid among 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetate acids (Table 1).

It increase actoprotective activity to 8.27 % compared to control (Table 2).

The replacement of the methyl radical with ethyl or phenyl by the fourth position of the 1,2,4-triazole in the 2-((5-(2-bromophenyl)-4-R-4H-1,2,4-triazole-3-yl)thio)acetate acid molecule resulted in the disappearance and reduction of the actoprotective effect (Fig. 1).

Considering the actoprotective activity of 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetate salts, the compounds IIb, IIk, IIj exhibited actoprotective effect 20.45 %, 13.48 %, 22.88 %, respectively. The most active compound was 2-aminoethanol 2-((5-(2-bromophenyl)-4-ethyl-4H-1,2,4-triazole-3-yl)thio)acetate (IIj) (Table 3).

The introduction of the phenyl radical in the fourth position of the 1,2,4-triazole of 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetate salts led to a decrease in the actoprotective effect.

The most active salt among 2-((5-(2-bromophenyl)-4-phenyl-4H-1,2,4-triazole-3-yl)thio)acetates was potassium salt. The change cation led to a decrease in actoprotective activity (Table 4).

Table 1. “Structure – activity” dependence between 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetate acids

| Substance | R            | Effect |
|-----------|--------------|--------|
| Ia        | CH₃          | ↑      |
| Ib        | C₂H₅         | ↔      |
| Ic        | C₆H₅         | ↔      |

Table 2. The actoprotective activity of 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetate acids

|        | mean | std | Me   | (Q1:Q3) | M ± m | % to mean control | % to Me control | KS-test | Shapiro |
|--------|------|-----|------|---------|-------|------------------|----------------|---------|---------|
| Control| 245.43 | 33.69 | 246  | (226.0:251.0) | 245.43 ± 2.81 | 0                | 0              | P < 0.001 | P > 0.05 |
| Riboxine| 315.28 | 66.70 | 292  | (278.5:359.5) | 315.29 ± 5.56 | 28.46            | 18.7           | P < 0.001 | P > 0.05 |
| Ia      | 265.71 | 109.70 | 254  | (204.0:360.5) | 265.71 ± 9.14 | 8.27             | 3.25           | P < 0.001 | P > 0.05 |
| Ib      | 238   | 80.44 | 231  | (187.5:283.5) | 238.0 ± 6.7  | -3.03            | -6.1           | P < 0.001 | P > 0.05 |
| Ic      | 230.85 | 74.10 | 221  | (182.5:273.5) | 230.86 ± 6.18 | -5.94            | -10.16         | P < 0.001 | P > 0.05 |

Fig. 1. The actoprotective activity of 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetate acids.

Fig. 2. Actoprotective activity of 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetate salts.
Table 3. "Structure – activity" dependence between 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetates

| Substance | R   | X          | Effect |
|-----------|-----|------------|--------|
| Ila       | CH₃ | K          | ↑      |
| IIb       | CH₃ | Na         | ↑↑     |
| IIc       | CH₃ | (CH₃)₂NH   | ↓      |
| IID       | CH₃ | morpholine | ↓      |
| IIe       | CH₃ | 2-aminoethanol | ↑ |
| IIf       | CH₃ | K          | ↑      |
| IIg       | CH₃ | Na         | ↔      |
| IIh       | CH₃ | (CH₃)₂NH   | ↓      |
| III       | CH₃ | morpholine | ↔      |
| IIj       | CH₃ | 2-aminoethanol | ↑↑ |
| IIk       | CH₃ | K          | ↑      |
| III       | CH₃ | Na         | ↔      |
| IIII      | CH₃ | (CH₃)₂NH   | ↓      |
| IIIl      | CH₃ | morpholine | ↑      |
| IIIo      | CH₃ | 2-aminoethanol | ↔  |

Among 2-((5-(2-bromophenyl)-4-methyl-4H-1,2,4-triazole-3-yl)thio)acetates, the most active was compound IIb, which contained sodium cation.

The substitution of sodium cation with potassium cation or 2-aminoethanol resulted in a nearly 2-fold reduction in the actoprotective effect.

The introduction of a cation of morpholine or dimethylammonium into 2-((5-(2-bromophenyl)-4-methyl-4H-1,2,4-triazole-3-yl)thio)acetates molecule resulted in an anti-actoprotective effect (Fig. 2).

As a result of the research, the conversion to 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetate salts and the selection as cations of sodium, potassium or 2-aminoethanol led to an increase in the actoprotective effect.

Conclusions

As a result, the actoprotective activity of 18 new 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetates was investigated.

Compounds Ia, Ib, IIb, III, I1j had been found to have a moderate actoprotective effect. But none compound exceeded the comparison drug.

Some conclusions were drawn regarding the dependence of “structure – actoprotective effect”:

Table 4. The actoprotective activity of 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetate salts

|                | mean | std  | Me  | (Q1:Q3) | M ± m (25.0:251.0) | % to mean control | % to Me control | KS-test | Shapiro |
|----------------|------|------|-----|---------|-------------------|-------------------|-----------------|---------|---------|
| Control        | 245.43 | 33.69 | 246 | (226.0:251.0) | 245.43 ± 2.81 | 0 | 0 | P < 0.001 | P > 0.05 |
| Riboxine      | 315.28 | 66.70 | 292 | (278.5:359.5) | 315.29 ± 5.56 | 28.46 | 18.7 | P < 0.001 | P > 0.05 |
| Ila           | 174.85 | 104.42 | 150 | (123.0:168.0) | 174.86 ± 8.7 | -28.75 | -39.02 | P < 0.001 | P < 0.01 |
| IIb           | 216.14 | 105.51 | 174 | (131.5:314.0) | 216.14 ± 8.79 | -11.93 | -29.27 | P < 0.001 | P > 0.05 |
| IIc           | 263.00 | 136.91 | 251 | (153.5:393.5) | 263.0 ± 11.41 | 7.16 | 2.03 | P < 0.001 | P > 0.05 |
| IID           | 241.71 | 40.58 | 240 | (218.5:264.0) | 241.71 ± 3.38 | 0 | 0 | P < 0.001 | P > 0.05 |
| Ile           | 304.00 | 60.76 | 337 | (240.0:353.0) | 304.0 ± 5.06 | 25.77 | 40.42 | P < 0.001 | P < 0.05 |
| IIf           | 196.71 | 91.31 | 209 | (114.5:271.5) | 196.71 ± 7.61 | -18.62 | -12.92 | P < 0.001 | P > 0.05 |
| IIg           | 262.71 | 89.47 | 319 | (183.5:322.5) | 262.71 ± 7.46 | 8.69 | 32.92 | P < 0.001 | P > 0.05 |
| IIh           | 274.28 | 78.83 | 295 | (214.5:325.0) | 274.29 ± 6.57 | 13.48 | 22.92 | P < 0.001 | P > 0.05 |
| III           | 240.00 | 81.65 | 208 | (181.5:305.0) | 240.0 ± 6.8 | -0.71 | -13.33 | P < 0.001 | P > 0.05 |
| Control       | 241.00 | 52.83 | 253 | (228.0:266.5) | 241.0 ± 4.4 | 0 | 0 | P < 0.001 | P > 0.05 |
| Riboxine      | 310.42 | 61.69 | 317 | (277.0:348.0) | 310.43 ± 3.47 | 28.81 | 25.3 | P < 0.001 | P > 0.05 |
| Ila           | 270.85 | 113.67 | 310 | (185.5:356.5) | 270.86 ± 9.47 | 12.39 | 22.53 | P < 0.001 | P > 0.05 |
| IIb           | 290.28 | 125.50 | 302 | (216.5:398.5) | 290.29 ± 10.46 | 20.45 | 19.37 | P < 0.001 | P > 0.05 |
| IIf           | 266.00 | 76.04 | 303 | (243.5:313.0) | 266.0 ± 6.34 | 10.37 | 19.76 | P < 0.001 | P > 0.05 |
| IIg           | 222.71 | 89.54 | 202 | (165.5:257.0) | 222.71 ± 7.46 | -7.59 | -20.16 | P < 0.001 | P > 0.05 |
| IIh           | 296.14 | 106.07 | 340 | (228.0:367.0) | 296.14 ± 8.84 | 22.88 | 34.39 | P < 0.001 | P > 0.05 |
| Control       | 250.14 | 33.78 | 252 | (233.0:258.5) | 250.14 ± 2.82 | 0 | 0 | P < 0.001 | P > 0.05 |
| Riboxine      | 318.42 | 60.19 | 312 | (283.0:354.0) | 318.43 ± 5.02 | 27.3 | 23.81 | P < 0.001 | P > 0.05 |
| Ila           | 244.28 | 77.03 | 222 | (179.0:309.0) | 244.29 ± 6.42 | -2.34 | -11.9 | P < 0.001 | P > 0.05 |
| IIb           | 272.85 | 82.63 | 275 | (245.0:322.5) | 272.86 ± 8.89 | 9.08 | 9.13 | P < 0.001 | P > 0.05 |
| IIg           | 206.14 | 62.37 | 227 | (173.5:241.5) | 206.14 ± 5.2 | -17.59 | -9.92 | P < 0.001 | P > 0.05 |
the most active compound was 2-aminoethanol 2-((5-(2-bromophenyl)-4-ethyl-4H-1,2,4-triazole-3-yl)thio) acetate (II)
conversion to 2-((5-(2-bromophenyl)-4-substituted-4H-1,2,4-triazole-3-yl)thio)acetate salts and selection as sodium, potassium, or 2-aminoethanol cations was resulted in to increase the actoprotective effect.

Funding
The research is carried out within the SRW of Zaporizhzhia State Medical University 'Synthesis, physical-chemical and biological properties of 3,4-disubstituted 3(5)-thio-1,2,4-triazole with antioxidant, antihypoxic, antimicrobial, cardio and hepatoprotective activity' state registration number 0118U007143.

Conflicts of interest: authors have no conflict of interest to declare.

Information about authors:
Safonov A. A., PhD, Associate Professor of the Department of Natural Sciences for Foreign Students and Toxicological Chemistry, Zaporizhzhia State Medical University, Ukraine.
Nevmyvaka A. V., Senior Laboratory Assistant of the Department of Pharmacology, Pharmacognosy and Botany, Zaporizhzhia State Medical University, Ukraine.

References
[1] Shcherbak, M. A., Kaplauchenko, A. G., Maletsky, N. N., & Sharaya, Ye. A. (2014). Issledovaniya po sozdaniyu lekarstvennoi formy na osnove 3-(4-nitrofenil)-5-(nonilfenil)-1,2,4-triazolo-4-amina [The research on creating the dosage form based on 3-(4-nitrophenyl)-5-(nonylphenyl)-1,2,4-triazole-4-amine]. Zaporozhye Medical Journal, (4), 82-85. [in Russian]. https://doi.org/10.14739/2310-1210.2014.4.27449
[2] Kaplauchenko, A. H. (2013). Doslidzhennya zi stvorennia novoho oryhnalnogo vitchyznainoho likarskoho zasobu na osnovi 1,2,4-triazolo [The research of creating a new original domestic drug based on 1,2,4-triazole]. Naukovyi zhurnal MOZ Ukrainy, (2), 115-121. [in Ukrainian].
[3] Shcherbyna, R. O. (2014) Analiz farmakolohichni aktyvnosti pokhidnykh 1,2,4-triazolu [Analysis of pharmacological activity of 1,2,4-triazole derivatives]. Farmatsevtychnyi chasopys, (4), 145-150. [in Ukrainian].
[4] Li, Y. S., Tian, H., Zhao, D. S., Hu, D. K., Liu, X. Y., Jin, H. W., Song, G. P., & Cui, Z. N. (2016). Synthesis and bioactivity of pyrazole and triazole derivatives as potential PDE4 inhibitors. Biogenic & Medicinal Chemistry Letters, 26(15), 3632-3635. https://doi.org/10.1161/bmcl.2016.06.002
[5] Shcherbyna, R. O., Panasenko, O. I., & Knyshe, Ye. H. (2016). Vyvchennia antyoksydantnoi aktyvnosti soli 2-((4-R-3-(morfolinometinyl)-4H-1,2,4-triazol-5-il)atsetatnykh kyslot [The studying of antioxidant activity of salts 2-((4-R-3-(morpholinomethyl)-4H-1,2,4-triazole-5-yl)acetic acids]. Ukrainian Biopharmaceutical Journal, (1), 37-40. [in Ukrainian].
[6] Shcherbyna, R. O., Kapeljanovych, Ye. V., Pruhlo, Ye. S., Panasenko, O. I., & Knyshe, Ye. H. (2014). Doslidzhennia aktoprotektornoi aktyvnosti pokhidnykh 4-R-3-(morfolinometinyl)-1,2,4-triazol-5-ilu [The studying of actoprotective action of 4-R-3-(morpholinomethyl)-1,2,4-triazole-5-thiole derivatives]. Odeskyi medychnyi zhurnal, (6), 19-22. [in Ukrainian].
[7] Akyonova, I. I., Shcherbyna, R. O., Panasenko, O. I., Knyshe, Ye. H., & Akyonov, I. V. (2014). Doslidzhennia riststymuliuiuchoi aktyvnosti pokhidnykh 1,2,4-triazolu na przykli nasinina soniashnyka protoho [The investigation of growth-stimulating activity of derivatives of 1,2,4-triazole on seeds of sunflower simple] Ukrainiam Biopharmaceutical Journal, (6), 78-82. [in Ukrainian].
[8] Murty, M. S. R., Ram, K. R., Rao, V. Y., Yadav, J. S., Rao, J. V., Pamanji, R. R., & Velatoru, L. R. (2012). Synthesis of New S-alkylated-3-mercapto-1,2,4-triazole Derivatives Bearing Cyclic Amine Moiety as Potent Anticancer Agents. Letters in Drug Design & Discovery, 9(3), 276-281. https://doi.org/10.2174/157018012799129882
[9] Pillai, R. R., Karrouchi, K., Fettach, S., Armakovic, S., Armakovic, S. J., Brik, Y., Taoufik, J., Radi, S., Faouzi, M. E., & Ansar, M. (2019). Synthesis, spectroscopic characterization, reactive properties by DFT calculations, molecular dynamics simulations and biological evaluation of Schiff bases tethered 1,2,4-triazole and pyrazole rings. Journal of Molecular Structure, 1177, 47-54. https://doi.org/10.1016/j.molstruc.2018.09.037
[10] Shcherbyna, R. O., Panasenko, O. I., Knyshe, Ye. H., & Venymskyy, B. O. (2014). Syntez i fizyko-khimichni vlastnosti 2-((4-R-3-(morfolinometinyl)-4H-1,2,4-triazol-5-il)atsetatnykh kyslot [Synthesis and physical-chemical properties of 2-((4-R-3-(morpholinomethyl)-4H-1,2,4-triazole-5-thiole)cysiacid]. Current Issues in Pharmacy and Medicine: Science and Practice, (3), 18-21. [in Ukrainian]. https://doi.org/10.14739/2409-2932.2014.3.30016
[11] El-Sherief, H. A. M., Youssif, B. G. M., Bukhari, S. N. A., Abdelazeem, A. H., Abdel-Aziz, M., & Abdel-Rahman, H. M. (2018). Synthesis, anticancer activity and molecular modeling studies of 1,2,4-triazole derivatives as EGFR inhibitors. European Journal of Medicinal Chemistry, 156, 774-789. https://doi.org/10.1016/j.ejmech.2018.07.024
[12] Samelyuk, Yu. H., & Kaplauchenko, A. H. (2015). Hostra toksychnist alkyl-ethyl-4H-1,2,4-triazolo-5-ilatsetatnykh kyslot [The studying 1,2,4-triazole-5-thiole derivatives]. Current Issues in Pharmacy and Medicine: Science and Practice, (3), 57-60. [in Ukrainian]. https://doi.org/10.14739/2409-2932.2015.3.52680
[13] Safonov A. A., Nevmyvaka A. V. (2020). Synthesis of novel 3-(2-bromophenyl)-4-substituted-1H-1,2,4-triazole(4H)-thiones derivatives. Current Issues in Pharmacy and Medicine: Science and Practice, 13(1), 11-16. https://doi.org/10.14739/2409-2932.2020.1.198087
[14] Stefanov, O. V. (Ed.). (2001). Doklinichni doslidzhennia likarskykh zasobiv [Preclinical studies of medicinal products: methodical recommendations]. Kyiv: Avicena. [in Ukrainian].