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SYNTHESIS OF AMIDES OF 2,4-DIOXOTHIAZOLIDIN-5-YL ACETIC ACID WITH 1,2,4-TRIAZOLE SUBSTITUENTS

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Abstract: In the reaction of (2,4-dioxothiazolidin-5-yl)acetyl chloride with 1,2,4-triazole, 4-phenyl-1,2,4-triazolin-5-one and 4-phenyl-1,2,4-triazolin-5-thione, the new corresponding amides (**2-4**) were obtained. For compounds **2** and **4** effects on central nervous system (CNS) of mice were studied.

Keywords: amides of 2,4-dioxothiazolidin-5-yl acetic acid, pharmacological screening

2,4-Dioxothiazolidine derivatives can show different pharmacological activity, such as antidiabetic, anti-inflammatory, antimicrobial and antiviral (1,2). In previous papers, we synthesized and investigated 5-arylidene-2,4-dioxothiazolidin-3-yl acetic acid connecting with 1,2,4-triazole or 1,3,4-thiadiazole systems, which showed antioxidant activity and sedative activity on central nervous system (3, 4). Continuing our research, in present paper 2,4-dioxothiazolidin-5-yl acetic acid was used as a starting material. From the literature data it is known that 2,4-dioxothiazolidin-5-yl acetic acid amides with aryl or thiazol-2-yl substituents showed antimicrobial activity (5). Change for another heterocyclic system in position 5 of 2,4-dioxothiazolidine ring may lead to wideninig the possibility of the pharmacological implementation. One of them can be 1,2,4-triazole system and its derivatives. The obtained earlier 1,2,4-triazole derivatives showed broad spectrum of pharmacological activity such as sedative activity on central nervous system (6-8), anti-cancer (9-12), anti-inflammatory (13-16), antibacterial and virostatic activity (17-19). There is a lack of literature data about investigation of 2,4-dioxothiazolidin-5-yl acetic acid with 1,2,4-triazole derivatives on central nervous system. In this paper, amides of 2,4-dioxothiazolidin-5-yl acetic acid connected with 1,2,4-triazole derivatives in position 1 were obtained and studied how they influence on central nervous system of mice. The reaction proceeded according to Scheme 1.

EXPERIMENTAL

Chemistry

Melting points were determined in Fischer-Johns blocks (Sanyo, Japan) and are not corrected. The IR spectra were recorded in KBr using a Specord IR-75 spectrophotometer (Carl Zeiss, Jena). The ^{1}H NMR spectra were recorded on a Bruker Avance 300 apparatus (Bruker, Germany) in DMSO-d_6 with TMS as internal standard. The mass spectrum was recorded on ThermoFinnigan Trace TSQGC MS apparatus. Purity of all compounds was checked by TLC on aluminium oxide 60 F_{254} plates (Merck), in a $CHCl_3/C_2H_5OH$ (10:2, v/v) solvent system with UV visualization (λ = 254 nm).

Elemental microanalyses for C, H, N performed in the Department of Organic Chemistry of the Lublin Medical University, were within ± 0.4% of the theoretical values.

Synthesis of 2,4-dioxothiazolidin-5-yl acetic acid amides (**2**-**4**) (general procedure)

1.93 g (0.01 mole) of (2,4-dioxothiazolidin-5-yl)acetyl chloride (**1**) in 5 cm^3 of anhydrous dioxane was added to 0.01 mole of corresponding 1,2,4-triazole derivatives and 0.01 mole of triethylamine in 5 cm^3 of anhydrous dioxane. After 15 min, water was added and the mixture was left at room tempereture for 24 h. The precipitate was filtered off and then recrystallized from n-butanol.

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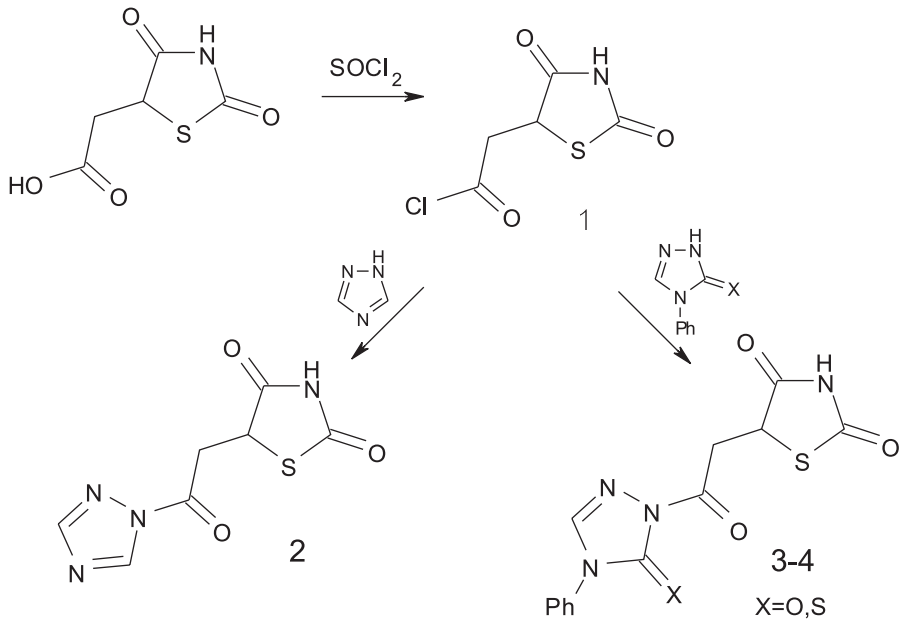

Scheme 1.

1-[(2,4-Dioxothiazolidin-5-yl)acetyl]-1,2,4-triazole (**2**)

Yield 1.24 g (55 %), m.p. 205-207°C. IR (KBr, cm^{-1}): 3252 (NH); 2949, 1410 ($CH_{al.}$); 1736, 1680 (C=O); 1606 (C=N); 1485 (C-N); 687 (C-S-C). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.77 (dd, 1H, *J* = 18.9 Hz and *J* = 8.7 Hz, CH_2); 3.97 (dd, 1H, *J* = 18.9 Hz and *J* = 3.6 Hz, CH_2); 4.83 (dd, 1H, *J* = 8.7 Hz and *J* = 3.6 Hz, CH); 8.09, 8.62 (2s, 2H, triazole); 12.02 (s, 1H, NH).

1-[(2,4-Dioxothiazolidin-5-yl)acetyl]-5-oxo-4-phenyl-4,5-dihydro-1,2,4-triazole (**3**)

Yield 1.90 g (60 %), m.p. 220-221°C. IR (KBr, cm^{-1}): 3250 (NH); 3041 ($CH_{ar.}$); 2943, 1412 ($CH_{al.}$); 1731, 1687 (C=O); 1602 (C=N); 1489 (C-N); 690 (C-S-C). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.70 (dd, 1H, *J* = 18.9 Hz and *J* = 9.3 Hz, CH_2); 4.03 (dd, 1H, *J* = 18.9 Hz and *J* = 3.6 Hz, CH_2); 4.81 (dd, 1H, *J* = 9.3 Hz and *J* = 3.6 Hz, CH); 7.50-7.62 (m, 5H, Ph); 8.64 (s, 1H, CH=); 12.10 (s, 1H, NH).

1-[(2,4-Dioxothiazolidin-5-yl)acetyl]-4-phenyl-5-thioxo-4,5-dihydro-1,2,4-triazole (**4**)

Yield 1.93 g (58%), m.p. 212-215°C. IR (KBr, cm^{-1}): 3249 (NH); 3047 ($CH_{ar.}$); 2948, 1411 ($CH_{al.}$); 1730, 1682 (C=O); 1604 (C=N); 1548, 1379 (C=S); 1490 (C-N); 692 (C-S-C). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.73 (dd, 1H, *J* = 18.9 Hz and *J* = 9.0 Hz, CH_2); 4.06 (dd, 1H, *J* = 18.9 Hz and *J* = 3.3 Hz, CH_2); 4.86 (dd, 1H, *J* = 9.0 Hz and *J* = 3.3 Hz, CH); 7.52-7.62 (m, 5H, Ph); 8.93 (s, 1H, CH=); 12.13 (s, 1H, NH). MS m/z (%): 334 (M, 1); 176 (100); 157 (3); 135 (6); 129 (6); 118 (3); 91 (13); 77 (51).

Pharmacology

The experiments were carried out on male Albino-Swiss mice (20-24 g). The compounds **2** and **4** were administered intraperitoneally (i.p.) as suspensions in a 1% Tween 80 solution in the constant volume of 0.1 mL/10 g per mice. The control group received the same volumes of the solvent. Compounds **2** and **4** were administered in doses equivalent to 0.1 and 0.0125 of their LD_{50}. Each experimental group consisted of 10 animals. The experiments were approved by the Ethics Committee of the Medical University of Lublin.

Chimney test. 30 min after i.p. administration of compounds **2** and **4** at a dose of 0.1 of their LD_{50} the motor coordination of mice were evaluated with the chimney test of Boissier et al. (20). Briefly, mice had to climb up backwards in a plastic tube (inner diameter 3 cm, length 25 cm). Mice unable to perform the task within 60 s were considered to display motor impairment. Motor impairment was quantified as the percentage of animals that failed to complete the test.

Body temperature. The rectal temperature was measured with a thermometer (Ellab, Copenhagen) before the administration of com-

Table 1. Analgesic activity of the investigated derivatives **2** and **4** in the "writhing syndrome" test in mice (n = 10).

Compound	Part of LD_{50}	Mean writhing number ($\bar{X}$ ± SEM)	Inhibition of pain reactivity (%)
Control	–	25,5 ± 3,2	–
2	0,025	19,9 ± 3,3	22,2
Control	–	42,4 ± 4,0	–
2	0,05	18,3 ± 4,5*	56,8*
Control	–	36,9 ± 7,8	–
2	0,1	12,1 ± 2,3*	67,2*
Control	–	42,4 ± 4,0	–
4	0,025	34,5 ± 3,1	18,6
Control	–	36,9 ± 7,8	–
4	0,05	14,4 ± 3,5*	61,0*
4	0,1	5,4 ± 1,5*	85,4*

% of inhibition of pain reactivity obtained by comparison with control group
* $p < 0.001$ vs. the control group

Table 2. The effect of the investigated derivatives on the head twitch responses induced by L-5-hydroxytryptophan in mice.

Compound	Part of LD_{50}	Number of head twitch episodes (X ± SEM)
Control	–	10,5 ± 2,9
2	0,025	8,1 ± 2,8
2	0,05	2,9 ± 0,5*
2	0,1	3,1 ± 1,7*

* $p < 0.001$ vs. the control group

pounds **2** or **4** at a dose of 0.1 of their LD_{50} and 15, 30, 45, 60, 90 or 120 min after it.

"**Four plate**" **test.** 30 min after the administration of both compounds at a dose of 0.1 of their LD_{50} the anxiolytic activity was measured by the "four plate" test in mice according to Aron et al. (21). The number of punished crossings was counted for 1 min.

Forced swimming test. Antidepresive activity was measured by the "forced swimming" test in mice according to Porsolt et al. (22). 30 min after i.p. administration of compounds **2** or **4** at a dose of 0.1 of their LD_{50} the mice were individually placed in a glass cylinder (27 × 16 cm) containing 15 cm of water (25°C) and forced to swim. A mouse was considered immobile when it floated in the water in an upright position and made only small movements to keep its head above the water. The duration of immobility of mice was measured during the last 4 min of the 6-min test.

Thiopental-induced sleep. Thiopental in a dose of 65 mg/kg i.p. was given 30 min after i.p. injection of compound **2** or **4** at a dose of 0.1 of their LD_{50}. The sleeping time of mice (from disappearance to return of the righting reflex) was measured.

Pain reactivity. Analgesic properties of both investigated compounds were measured by the "writhing syndrome" test (23) in mice. 30 min after i.p. administration of compounds **2** or **4** in doses of 0.0125 to 0.1 of their LD_{50} the animals were injected with 0.6% acetic acid i.p. and the number of writhing episodes was counted for 30 min.

Pentetrazole seizures. 30 min after administration of **2** or **4** in doses equivalent to 0.025, 0.05 and 0.1 LD_{50} mice were injected with pentetrazole (100 mg/kg s.c.). Immediately after it, the mice were

observed for 30 min and the number of clonic and tonic seizures as well as mortality were recorded in that period.

Head-twitches. The investigated compounds **2** or **4** were given in doses equivalent to 0.025, 0.05 and 0.1 LD_{50}. 30 min before L-5-hydroxytryptophan (L-5-HTP, 180 mg/kg i.p.). The number of head-twitch episodes of mice was counted during 60 min after the injection of L-5-HTP.

Statistics. Statistical analysis was carried out using the Student's t-test and the Chi square test (for convulsions).

RESULTS AND DISCUSSION

Chemistry

(2,4-Dioxothiazolidin-5-yl)acetyl chloride (**1**) was used as a starting material which by the reaction of 2,4-dioxothiazolidin-5-yl acetic acid with thionyl chloride was obtained by the method described earlier (5). By the reaction of (2,4-dioxothiazolidin-5-yl)acetyl chloride with 1,2,4-triazole, 4-phenyl-1,2,4-triazolin-5-one and 4-phenyl-1,2,4-triazolin-5-thione new amides of 2,4-dioxothiazolidin-5-yl acetic acid were obtained. These reactions were carried out in anhydrous 1,4-dioxane with the presence of triethylamine and led to derivatives substituted in position 1 of 1,2,4-triazole ring. Such substitution in position 1 was confirmed by investigation of nucleophilic substitution of derivatives with 1,2,4-triazole system (24). 1,2,4-Triazole was obtained by the reaction by triformylaminomethane with hydrazine sulfate (25), whereas 4-phenyl-1,2,4-triazolin-5-one and 4-phenyl-1,2,4-triazolin-5-thione were obtained by the cyclization in alkaline media from 4-phenyl-1-formylsemicarbazide and 4-phenyl-1-formyl-thiosemicarbazide, respectively, by the method described earlier (26-29).

The structure of newly obtained compounds (**2**-**4**) was confirmed by elemental analysis, IR, ^{1}H NMR spectra and mass spectrum for compound **4**.

In the IR spectra of compounds (**2**-**4**) the following characteristic absorption bands were observed: 1680-1736 cm^{-1} (C=O) and 3249-3252 cm^{-1} (NH). Absorption bands of the C=S group for compound **4** were observed at 1379 and 1548 cm^{-1}. In the ^{1}H NMR spectra all compounds show proton signal group typical for ABX system in 3.70-3.77 and 3.97-4.06 ppm range for CH_2 group and in 4.81-4.86 ppm range for CH group. All derivatives with 1,2,4-triazole, 4-phenyl-1,2,4-triazolin-5-one and 4-phenyl-1,2,4-triazolin-5-thione system (**2**-**4**) show proton signal of the CH group in the 8.09-8.93 ppm range.

Pharmacology

Preliminary pharmacological studies showed that the tested compounds **2** and **4** weakly affected the CNS of mice. Neither compound at doses equivalent to 0.1 LD_{50} had neurotoxic properties as they did not affect the motor coordination in the chimney test. Both investigated compounds in doses of 0.05 and 0.1 LD_{50} displayed analgesic activities in the "writhing syndrome" test, significantly decreasing the number of writhing episodes induced by 0.6% acetic acid in mice (Table 1). Compound **2** (1-[(2,4-dioxothiazolidin-5-yl)acetyl]-1,2,4-triazole) was more active, because it produced antiserotoninergic effects in mice. Compound **2** given in doses of 0.05 and 0.1 LD_{50} significantly decreased the number of head twitch responses induced by L-5-HTP as compared with control group (Table 2). In the remaining tests all the compounds were inactive.

The results presented above show that compound **4** (1-[(2,4-dioxothiazolidin-5-yl)acetyl]-4-phenyl-5-thioxo-4,5-dihydro-1,2,4-triazole) has weaker pharmacological activity than compound **2**.

REFERENCES

1. The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals, 13th ed. Merck & Co., New York 2001.
2. Singh S.P., Parmar S.S., Raman K., Stenberg V.I.: Chem. Rev. 81, 175 (1981).
3. Trotsko N., Dobosz M., Lukianchuk V.: Acta Pol. Pharm. Drug Res. 63, 47 (2006).
4. Trotsko N., Dobosz M., Jagiełło-Wójtowicz E.: Acta Pol. Pharm. Drug Res. 64, 227 (2007).
5. Zimenkovskii B.S., Kutsyk R.V., Lesyk R.B., Matyichuk V.S., Obushak N.D., Klyufinska T.I.: Khim. Farm. Zh. 40, 13 (2006).
6. Pachuta-Stec A., Dobosz M.: Acta Pol. Pharm. Drug Res. 58, 307 (2001).
7. Dobosz M., Pachuta-Stec A., Rękas J.: Acta Pol. Pharm. Drug Res. 50, 225 (1993).
8. Dobosz M., Rękas J., Pachuta-Stec A.: Acta Pol. Pharm. Drug Res. 48, 29 (1991).
9. Demirbas N., Ugurluoglu R., Demirbas A.: Bioorg. Med. Chem.10, 3717 (2002).
10. Demirbas N., Ugurluoglu R.: Turk. J. Chem. 28, 559 (2004).
11. Ikizler A.A., Ikizler A., Serdar M., Yildirim N.: Acta Pol. Pharm. Drug Res. 54, 363 (1997).
12. Shivarama Holla B., Veerendra B., Shivananda M.K., Boja Poojary.: Eur. J. Med. Chem. 38, 759 (2003).

13. Labanauskas L., Udrenaite E., Gaidelis P., Brukstus A.: Farmaco 59, 255 (2004).
14. Labanauskas L., Kalcas V., Udrenaite P., Brukstus A., Dauksas V.: Pharmazie 56, 617 (2001).
15. Sahin G., Pulaska E., Kelicen P., Demirdamar R., Altmok G.: Arzneim. Forsch. 51, 478 (2001).
16. Tozkoporan B., Gokhan N., Atkay G., Yesilada E., Ertan M.: Eur. J. Med. Chem. 35, 743 (2000).
17. Colanceska-Ragenovic K., Dimova V., Kakurinov V., Molnar D.G., Buzarovska A.: Molecules 6, 815 (2001).
18. Demirbas N., Karaoglu S.A., Demirbas A., Sancak K.: Eur. J. Med. Chem. 39, 793 (2004).
19. Wujec M., Pitucha M., Dobosz M., Kosikowska U., Malm A.: Acta Pharm. 54, 251 (2004).
20. Boissier J.R., Tardy J., Diverres J.C., Med. Exp. (Basel) 3, 81 (1960).
21. Aron C., Simon P., Larousse C., Boissier J.R.: Neuropharmacology 10, 459 (1971).
22. Porsolt R.D., Bertin A., Deniel N., Jalfre M.: Arch. Int. Pharmacodyn. Ther. 229, 327 (1977).
23. Witkin L., Heubner G., Galdi F., O'Keefe E., Spitaletta F., Plumer A.: J. Pharmacol. Exp. Ther. 132, 400 (1951).
24. Wujec M., Pitucha M., Dobosz M., Kosikowska U., Malm A.: Acta Pol. Pharm. Drug Res. 60, 451 (2003).
25. Dobosz M.: Ann. UMCS Lublin, Sectio AA 34, 151 (1979).
26. Dobosz M., Sikorska M.: Acta Pol. Pharm. Drug Res. 51, 369 (1994).
27. Dobosz M., Sikorska M.: Acta Pol. Pharm. Drug Res. 51, 377 (1994).
28. Dobosz M., Pachuta-Stec A.: Acta Pol. Pharm. Drug Res. 53, 123 (1996).
29. Dobosz M., Struga M.: Acta Pol. Pharm. Drug Res. 54, 313 (1997).

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