DRUG SYNTHESIS

CYCLIZATION OF THIOSEMICARBAZIDE DERIVATIVES OF 5-ARYLIDENE-2,4-DIOXOTHIAZOLIDINE-3-ACETIC ACIDS TO 1,3,4-THIADIAZOLES AND THEIR PHARMACOLOGICAL PROPERTIES

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Abstract: By the reaction of (5-arylidene-2,4-dioxothiazolidin-3-yl)acetyl chlorides with 4-phenylthiosemicarbazide, acylthiosemicarbazide derivatives 4-6 were obtained. The cyclization of 4-6 in acid medium led to 1,3,4-thiadiazole derivatives 7-9. The structure of the compounds was confirmed by elemental analysis and IR, 1H NMR, 13C NMR and MS spectra. The effects of compounds 7 and 9 on the central nervous system (CNS) of mice were studied.

Keywords: 5-arylidene-2,4-dioxothiazolidine-3-acetic acid, 1,3,4-thiadiazole, thiosemicarbazides, cyclization, pharmacological screening

Derivatives of 5-arylidene-2,4-dioxothiazolidine-3-acetic acids could be starting products for synthesis of new derivatives with various biological activity (1-4).

There is a lack of literature data about cyclization of thiosemicarbazide derivatives of 5-arylidene-2,4-dioxothiazolidine-3-acetic acids to 1,2,4-triazole and 1,3,4-thiadiazole systems.

Some drugs with 1,2,4-triazole ring are known among 1,2,4-triazole and 1,3,4-thiadiazole derivatives: itraconazole, amdatezole, anastrozole (5-7) and with 1,3,4-thiadiazole system: desaglybuzole, acetazolamide, furidiazine (8-10). Cyclization of acyl derivatives of thiosemicarbazide in alkaline medium led to 1,2,4-triazole derivatives (11-23), whereas in acid medium it gave 1,3,4-thiadiazole derivatives (12,13,15,17-20,22,24). The influence of substituents in the thiosemicarbazide derivatives on the pathway of cyclization is significant. For example, cyclization of thiosemicarbazide derivatives of nicotinic acid in acid and alkaline media led to 1,2,4-triazole derivatives (18). The cyclization of thiosemicarbazide derivatives of fumaric acid in acid medium as well as in alkaline medium led to 1,3,4-thiadiazole derivatives (19). Literature data show that the 2,4-dioxothiazolidine ring is not stable when heated in alkaline medium (25,26). That was confirmed by experiment described in the paper. Hence, acid media were chosen as reaction media for the cyclization.

Thiosemicarbazide derivatives were obtained by the reaction of hydrazides of carboxylic acids with isothiocyanate. In the case of 2,4-dioxothiazolidine derivatives, hydrazides of 5-arylidene-2,4-dioxothiazolidine-3-acetic acids were not obtained from corresponding ethyl esters and hydrazide, because conversion of the 2,4-dioxothiazolidine ring to the pyrazol-3-one system occurred (27). In the present paper thiosemicarbazide derivatives of 5-arylidene-2,4-dioxothiazolidine-3-acetic acids were prepared from corresponding acetyl chlorides and 4-substituted thiosemicarbazide derivatives and then cyclized. These reactions proceeded according to Scheme 1. Compounds 7 and 9 were investigated for their in vivo activity.

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 1H NMR spectra were recorded on a Bruker Avance 300 (Bruker, Germany) apparatus in DMSO-d6 with TMS as internal standard. Chemical shifts are given in ppm (δ-scale). 13C NMR spectra also were recorded on a Bruker Avance 300. MS spectra were recorded on a ThermoFinnigan Trace TSQGC MS apparatus.
Purity of all compounds was checked by TLC on aluminium oxide 60 F254 plates (Merck), in a CHCl3/C2H5OH (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 ± 0.5% of the theoretical values.

1-[(5-Arylidene-2,4-dioxothiazolidin-3-yl)acetyl]-4-phenylthiosemicarbazides (4-6)

General procedure

0.01 mole of (5-arylidene-2,4-dioxothiazolidin-3-yl)acetyl chlorides 1-3 in 5 cm³ of anhydrous dioxane was added to 1.67 g (0.01 mole) of 4-phenylthiosemicarbazide and 1.00 g (0.01 mole) of triethylamine in 5 cm³ of anhydrous dioxane. After 15 min, water was added and the mixture left standing at room temperature for 24 h. The precipitate was filtered off and then recrystallized from acetic acid.

1-[(5-(4-Methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetyl]-4-phenylthiosemicarbazide (4)

Yield 3.23 g (73 %), m.p. 172-173°C. IR (KBr, cm⁻¹): 3343 (NH); 3051 (CH ar.); 2949, 1408 (CH al.); 2839 (OCH3); 1733, 1688 (C=O); 1552, 1382 (C=S); 1510 (C=N); 828 (p-substituted benzene); 684 (C-S-C). ¹H NMR (DMSO-d6) δ (ppm): 3.84 (s, 3H, OCH3); 4.51 (s, 2H, CH2); 7.15-7.77 (m, 9H, 4-H, 4-MeO-C6H4 and 5H, Ph); 7.95 (s, 1H, =CH); 9.68, 9.84, 10.51 (3s, 3H, 3NH).

1-[(5-(4-Methylbenzylidene)-2,4-dioxothiazolidin-3-yl)acetyl]-4-phenylthiosemicarbazide (5)

Yield 3.79 g (89 %), m.p. 166-167°C. IR (KBr, cm⁻¹): 3351 (NH); 3053 (CH ar.); 2944, 1411 (CH al.); 1737, 1687 (C=O); 1536, 1382 (C=S); 1489 (C-N); 813 (p-substituted benzene); 681 (C-S-C). ¹H NMR (DMSO-d6) δ (ppm): 2.37 (s, 3H, CH3); 4.51 (s, 2H, CH2); 7.15-7.77 (m, 4H, 4-Me-C6H4 and 5H, Ph); 7.95 (s, 1H, =CH); 9.66, 9.82, 10.49 (3s, 3H, 3NH).

1-[(5-(4-Bromobenzylidene)-2,4-dioxothiazolidin-3-yl)acetyl]-4-phenylthiosemicarbazide (6)

Yield 3.63 g (74 %), m.p. 183-185°C. IR (KBr, cm⁻¹): 3340 (NH); 3049 (CH ar.); 2949, 1409 (CH al.); 1733, 1692 (C=O); 1547, 1383 (C=S); 1489 (C-N); 821 (p-substituted benzene); 683 (C-S-C). ¹H NMR (DMSO-d6) δ (ppm): 4.52 (s, 2H, CH2); 7.15-7.77 (m, 9H, 4-Br-C6H4, Ph); 7.98 (s, 1H, =CH); 9.68, 9.84, 10.51 (3s, 3H, 3NH). MS m/z (%): 492 (M+1, 17); 398 (21); 356 (15); 285 (100); 256 (4); 211 (11); 179 (9).
Cyclization of thiosemicarbazide derivatives of...

General procedure

0.01 mole of thiosemicarbazide derivative 4-6 was refluxed in 20 cm³ of 25% HCl for 2 h or was dissolved in concentrated sulfuric acid (10 cm³) and the solution was kept at room temperature for 24 h. In the first case, after cooling the precipitate was filtered off. In the second case, reaction mixture was poured onto crushed ice. The precipitate was filtered off and crystallized from DMF: water (1:1) in both cases.

5-(4-Methoxybenzylidene)-2,4-dioxo-3-[(5-phenylamine-1,3,4-thiadiazol-2-yl)methyl]thiazolidine (7)

Yield 3.09 g (73%) in the first case and 3.04 g (72%) in the second case, m.p. 261-263°C. IR (KBr, cm⁻¹): 3254 (NH); 3052 (CH ar.); 2953, 1412 (CH al.); 2838 (OCH₃); 1747, 1697 (C=O); 1611 (C=N); 1505 (p-substituted benzene); 744 (Ph); 689 (C-S-C). ¹H NMR (DMSO-d₆) δ (ppm): 3.84 (s, 3H, OCH₃); 5.11 (s, 2H, CH₂); 7.00-7.64 (m, 9H, 4-MeO-C₆H₄, Ph); 7.97 (s, 1H, =CH); 10.42 (s, 1H, NH).

5-(4-Methylbenzylidene)-2,4-dioxo-3-[(5-phenylamine-1,3,4-thiadiazol-2-yl)methyl]thiazolidine (8)

Yield 3.26 g (80%) in the first case and 3.14 g (77%) in the second case, m.p. 247-248°C. IR (KBr, cm⁻¹): 3259 (NH); 3045 (CH ar.); 2951, 1436 (CH al.); 1740, 1689 (C=O); 1606 (C=N); 819 (p-substituted benzene); 748 (Ph); 683 (C-S-C). ¹H NMR (DMSO-d₆) δ (ppm): 2.37 (s, 3H, CH₃); 5.12 (s, 2H, CH₂); 7.02-7.63 (m, 9H, 4-Me-C₆H₄, Ph); 7.98 (s, 1H, =CH); 10.40 (s, 1H, NH). MS m/z (%): 409 (M+, 100); 178 (11); 138 (8); 90 (9).

5-(4-Bromobenzylidene)-2,4-dioxo-3-[(5-phenylamine-1,3,4-thiadiazol-2-yl)methyl]thiazolidine (9)

Yield 3.50 g (74%) in the first case and 3.17 g (67%) in the second case, m.p. 234-236°C. IR (KBr, cm⁻¹): 3269 (NH); 3052 (CH ar.); 1745, 1695 (C=O); 1608 (C=N); 1500 (C-N); 815 (p-substituted benzene); 746 (Ph); 704, 688 (C-S-C). ¹H NMR (DMSO-d₆) δ (ppm): 5.12 (s, 2H, CH₂); 7.02-7.78 (m, 9H, 4-Br-C₆H₄, Ph); 7.99 (s, 1H, =CH); 10.40 (s, 1H, NH). ¹³C NMR δ (ppm): 42.6 (CH₂); 116.7; 127.5; 128.6; 140.3 (6Car., Ph); 121.5; 124.0; 131.5; 132.0 (6C ar., 4-Br-C₆H₄); 121.7 (C-C(O)-N); 132.3 (CH=); 151.9 (N=C(S-)–NH); 164.4 (CH₂-C(S–)=N); 165.5 (C=C(O)-N); 166.1 (N=C(O)-S). MS m/z (%): 473 (M, 8); 462 (17); 298 (14); 284 (100); 256 (34); 242 (6); 213 (5); 181 (2); 157 (3).

Pharmacology

The experiments were carried out on Albino-Swiss mice of both sexes (22-26 g). The animals were housed in colony cages with free access to tap water and food (standard laboratory pellets – Bacutil, Motycz, Poland) and maintained in the 12/12 h light-dark cycle (light on from 7 a.m. to 7 p.m.). The experimental and the control group consisted of 8 animals. The experiments were performed between 8 a.m. and 3 p.m. Compound 7 was administrated intraperitoneally (i.p.) in doses of 25, 50 and 100 mg/kg (equivalent to 0.025, 0.05 and 0.1 of their LD₅₀) and compound 9 in doses of 17.5, 35 and 70 mg/kg i.p. (equivalent to 0.025, 0.05 and 0.1 of their LD₅₀) as suspensions in 1% Tween 80 in the constant volume of 10 cm³/kg. Control animals received the equivalent volume of solvent.

The experiments were performed in accordance with ethical requirements. The following behavioral tests were performed:

Chimney test. The effects of compounds 7 and 9 in a dose of 0.1 of their LD₅₀ on motoric impairment was quantified with the chimney test (29). Briefly, mice had to climb up backwards in a plastic tube (3 cm inner diameter, 25 cm long). Mice unable to perform the task within 60 s were considered to

<table>
<thead>
<tr>
<th>Compound</th>
<th>Part of LD₅₀</th>
<th>Dose mg/kg i.p.</th>
<th>Inhibition of pain reactivity, %*</th>
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<tbody>
<tr>
<td>Control</td>
<td>-</td>
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<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0.025</td>
<td>25</td>
<td>50.5*</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>50</td>
<td>70.3*</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>100</td>
<td>80.0*</td>
</tr>
<tr>
<td>9</td>
<td>0.025</td>
<td>17.5</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>35</td>
<td>68.3*</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>70</td>
<td>87.1*</td>
</tr>
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</table>

* % of inhibition of pain reactivity obtained by comparison with control group.

*p < 0.001 vs the control group
display motoric impairment. It was quantified as the percentage of animals that failed to complete the test.

Body temperature. The rectal body temperature in mice (Ellab thermometer) was recorded 15, 30, 45, 60, 90 and 120 min after the administration of the investigated compounds in a dose of 0.1 of their LD_{50}. The mice were then observed for 30 min and the number of punished crossings was counted for 1 min.

“Four-plate” test (30). Anxiolytic activity was investigated 30 min after the injection of the compounds in a dose of 0.1 of their LD_{50}. The number of writhing episodes was counted for 30 min.

“Writhing syndrome” test (31). Compounds 7 and 9 were administrated in doses of 0.025, 0.05 and 0.1 of their LD_{50}, and after 30 min the animals were injected 0.6% acetic acid i.p. The number of writhing episodes was counted for 30 min.

Pentetrazole-induced convulsions. Pentetrazole (115 mg/kg s.c.) was given 30 min after the administration of compound 7 in doses of 0.05 and 0.1 of their LD_{50}. Compound 9 in a dose of 0.1 of their LD_{50}. The mice were then observed for 30 min and the number of animals developing clonic and tonic seizures as well as mortality was recorded during that period.

Head twitches (32). Compounds 7 and 9 were given in a dose of 0.1 of their LD_{50} 30 min before 5-hydroxy-L-tryptophan (5-L-HP, 190 mg/kg i.p.). The number of head twitch episodes in mice was counted for 60 min after the injection of 5-L-HP. Statistical analysis was carried out using Student’s t-test.

RESULTS AND DISCUSSION

Chemistry

The starting materials were thiosemicarbazide derivatives of 5-arylidene-2,4-dioxothiazolidine-3-acetic acids, which were obtained in the reaction of corresponding (5-arylidene-2,4-dioxothiazolidin-3-yl)acetyl chlorides with 4-phenylthiosemicarbazide (Scheme 1). (5-Arylidene-2,4-dioxothiazolidin-3-yl)acetyl chlorides were obtained by the reaction of corresponding acetic acid with thionyl chloride (28). Cyclization of the obtained new thiosemicarbazide derivatives was carried out in acid media. 25% HCl and conc. H_{2}SO_{4} were chosen as reaction media for the cyclization. In both of them, derivatives of 1,3,4-thiadiazole 7-9 were obtained. Mixed melting points have not shown any depression. The 1H NMR spectra of the compounds were identical.

New 2,4-dioxothiazolines with methylene group connecting 1,3,4-thiadiazole ring were obtained. Their structures were confirmed by elemental analysis, IR, 1H NMR, 13C NMR spectra for compound 9 and MS for compounds 6, 8 and 9.

In the IR spectra of thiosemicarbazide derivatives 4-6 the following characteristic absorption bands were observed: 1687-1735 cm\(^{-1}\) (C=O) and 3340-3351 cm\(^{-1}\) (NH). In compounds 7-9 containing 1,3,4-thiadiazole ring, the absorption of the C=N group appeared at 1493-1505 cm\(^{-1}\) and those of the C=N group at 1606-1611 cm\(^{-1}\). Absorption bands of the NH group were observed at 3254-3269 cm\(^{-1}\).

1H NMR spectra of thiosemicarbazide derivatives 4-6 show three signals typical of the NH group in the 9.66-10.51 ppm δ range. In the 1H NMR spectra of 1,3,4-thiadiazole derivatives 7-9 the signals typical of the NH group were at 10.40-10.42 ppm. All compounds were obtained only as Z isomers, their 1H NMR spectra showed only one signal attributable to the resonance of the methylidene proton in the range 7.93-7.99 ppm. These data were correlated with reports by another authors (2,4).

Pharmacology

Previous investigations have shown that the LD_{50} values after i.p. injection of new compounds 7 and 9 were in mice higher than 1000 mg/kg or 700 mg/kg, respectively.

The present study showed that both compounds: 5-(4-methoxybenzylidene)-2,4-dioxo-3-[(5-phenylamine-1,3,4-thiadiazol-2-yl)methyl]thiazolidine (7) and 5-(4-bromobenzylidene)-2,4-dioxo-3-[(5-phenylamine-1,3,4-thiadiazol-2-yl)methyl]thiazolidine (9) displayed analgesic action. Compound 7 at doses of 0.025 to 0.1 LD_{50} significantly decreased the number of writhing episodes induced by 0.6% acetic acid in mice. Only doses of 0.1 and 0.05 LD_{50} of compound 9 exerted analgesic activity. It indicates that the kind of 4-methoxybenzylidene group has an influence on the strength of the analgesic action.

In other screening tests both the investigated derivatives administered at a dose of 0.1 LD_{50} were inactive. In conclusion, we found that CNS effects are weak and only analgesic activity of 7 and 9 are interesting and should be examined in more detail.

REFERENCES

Cyclization of thiosemicarbazide derivatives of...


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