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ORIGINAL PAPER

Synthesis and antinociceptive activity of 4,4′-bis(1-substituted-semicarbazidyl)diphenylmethane and 4,4′-bis(5-substituted-2,4-dihydro-3-oxo-3*H*-1,2,4-triazol-4-yl)diphenylmethane derivatives

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Abstract In the reaction of 4,4′-diphenylmethane diisocyanate with carboxylic acid hydrazides, 4,4′-bis(1-substituted-semicarbazidyl)diphenylmethane derivatives were obtained. Depending on their chemical nature, cyclization of these compounds in alkaline medium led to the formation of two groups of compounds: bis(2,4-dihydro-3*H*-1,2,4-triazol-3-one) derivatives or carboxylic acids. The pharmacological effects of the selected compounds on the central nervous system in mice were investigated. Strong antinociceptive properties of some derivatives, at a wide range of doses, were observed.

Keywords CNS activity · Semicarbazide · 1,2,4-Triazol-3-one

Introduction

Many compounds containing five-membered heterocyclic rings, such as triazoles, oxadiazoles, thiadiazoles, and imidazoles, have been intensively synthesized and evaluated for their biological activities [1–4]. During the last few decades, considerable attention has been devoted to the synthesis of 1,2,4-triazole derivatives possessing diverse pharmacological properties such as antimicrobial [5, 6], anti-inflammatory [7], analgesic [8], antitumor [9], anticonvulsant, and antiviral activities [10]. Some of them were tested for antinociceptive activity and showed promising results [11, 12]. The 1,2,4-triazole system is a structural element of many drugs that have anticonvulsant or antidepressant activity such as nefazodone hydrochloride [13] or amdatezole [14].

One of the methods of preparing compounds containing this nucleus is a cyclization reaction of acylsemicarbazide derivatives in alkaline medium [15, 16]. In a previous paper some 4,4′-bis(5-substituted-2,4-dihydro-3-oxo-3*H*-1,2,4-triazol-4-yl)diphenylmethane derivatives (**3a**–**3d**) were synthesized in the reaction of the corresponding bis-semicarbazide derivatives **2a**–**2d**. The X-ray structure analysis of **3a** revealed that the asymmetric part of the unit cell contains two independent molecules, both existing in the keto tautomeric form [17].

In this paper, an investigation of the cyclization reaction of new 4,4′-bis(1-substituted-semicarbazidyl)diphenylmethanes is presented. Depending on the chemical nature of the substituent in the starting compounds, the reaction led to the formation of different derivatives. One of them is composed of two 1,2,4-triazol-3-one systems connected by a diphenylmethylene group. Additionally, we propose an alternative method for synthesis of these compounds through the reaction of appropriate amidrazone hydrochlorides with 4,4′-diphenylmethane diisocyanate. Selected compounds were investigated pharmacologically to determine their effect on the central nervous system (CNS) in mice.

Results and discussion

Semicarbazides **2a**–**2d** were synthesized in the reaction of appropriate carboxylic acid hydrazides with 4,4′-diphenylmethane diisocyanate (MDI) in anhydrous diethyl ether at room temperature [15]. New semicarbazides **2e**–**2g** were

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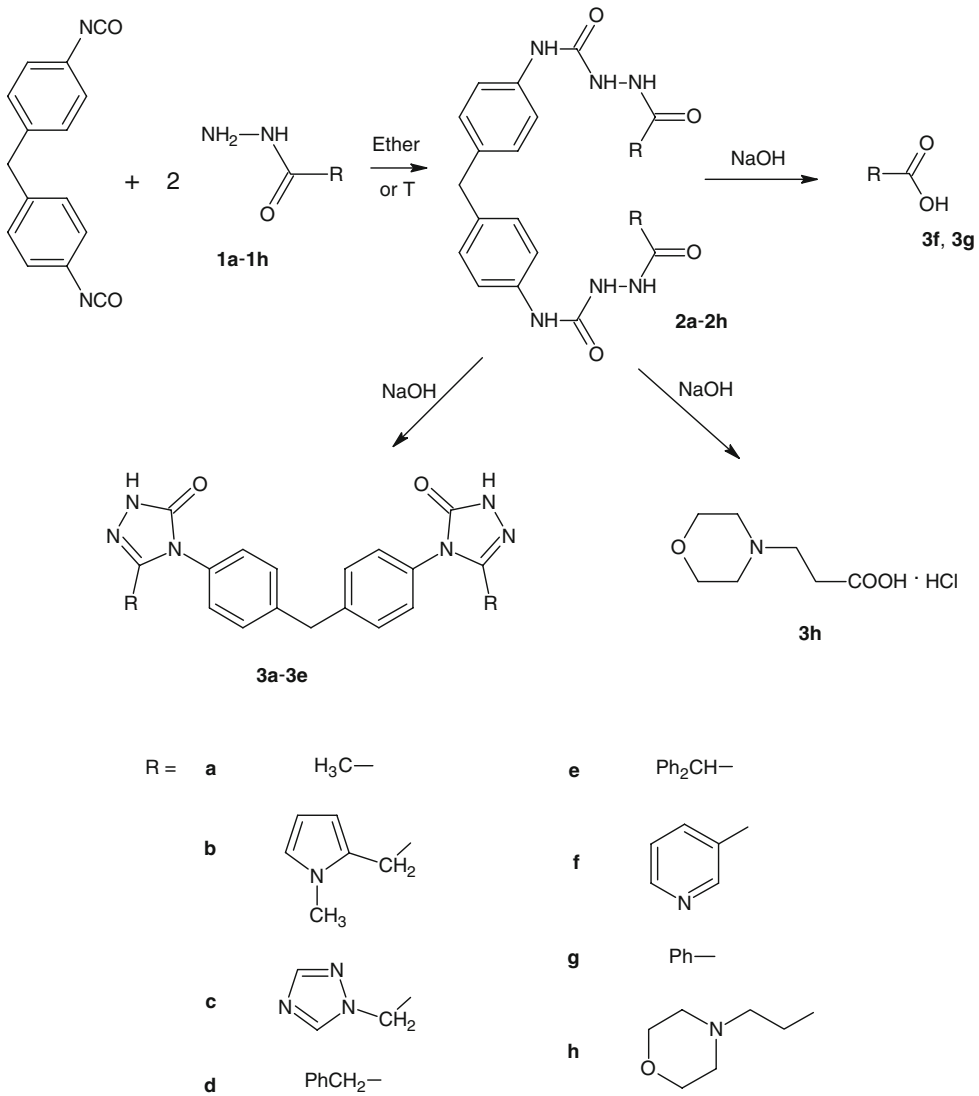
obtained by heating the substrates in the melt. The reaction conditions were established experimentally. Next, bis-semicarbazides **2a**–**2g** were heated in 2% aqueous NaOH solution. The products of these reactions depended on the substituent in the starting material. In the case of 4,4′-bis(1-substituted-semicarbazidyl)diphenylmethanes **2a**–**2e**, 4,4′-bis(5-substituted-2,4-dihydro-3-oxo-3*H*-1,2,4-triazol-4-yl)diphenylmethane derivatives **3a**–**3e** were obtained (**3a**–**3d** were described earlier [17]). The alkaline cyclization of semicarbazides **2f**–**2h** afforded quite different products. From the reaction of **2f** and **2g** the corresponding carboxylic acids were formed. The structures of these compounds were confirmed by ^{1}H NMR spectra in comparison with the Spectral Database for Organic Compounds (SDBS) spectral library and MS, and in case of benzoic acid additionally by X-ray analysis. Similarly, in case of bis[(4-morpholinylpropionyl)semicarbazide] **2h**, 4-(2-carboxyethyl)-4-morpholinium chloride was obtained [18] (Scheme 1).

In continuation of the experiments the reaction of amidrazone salts with 4,4′-diphenylmethane diisocyanate was investigated. In this alternative synthetic method amidrazone hydrochlorides were used as starting materials. Appropriate amidrazones were prepared by a method described earlier [19, 20].

The condensation of hydrochlorides of acetamidrazone or N^1-phenylbenzamidrazone with MDI was carried out by heating the substrates in the melt for 20 h. The conditions of the reaction were established experimentally. Based on the results of elemental analysis and the spectral data (IR, ^{1}H NMR) it was revealed that the reaction leads to formation of a cyclic five-membered ring system by instantaneous reaction on the N^1 nitrogen atom of the hydrazine moiety and the N^3 nitrogen atom of the amide moiety. Probably, the course of this reaction includes the formation of intermediate semicarbazone derivatives which cyclize spontaneously to the 1,2,4-triazole system.

Scheme 1



In the reaction of acetamidrazone hydrochloride and MDI, 4,4′-(methylenedi-4,1-phenylene)bis(2,4-dihydro-5-methyl-3*H*-1,2,4-triazol-3-one) (**3a**) was obtained in 71% yield. This is an alternative method because this compound was also obtained by the cyclization of 4,4′-bis(semicarbazidyl)diphenylmethane **2a** in alkaline medium [17]. Mixed melting points have not shown any depression. The IR and ^{1}H NMR spectra of these compounds were also identical. When we used N^{1}-phenylbenzamidrazone hydrochloride, 4,4′-(methylenedi-4,1-phenylene)bis(2,4-dihydro-2,5-diphenyl-3*H*-1,2,4-triazol-3-one) (**4**) was obtained (Scheme 2).

Selected compounds were screened for their influence on the central nervous system. The pharmacological behavioral tests showed that both 4,4′-bis(1-substituted-semicarbazidyl)diphenylmethanes **2a**–**2d** and 4,4′-bis-(5-substituted-2,4-dihydro-3-oxo-3*H*-1,2,4-triazol-4-yl)diphenylmethanes **3a** and **3b** displayed antinociceptive activity (Table 1). A significant decrease in the number of writhing episodes was observed at a wide range of doses. Compound **2c** at a dose of 1.5625 mg/kg i.p. significantly decreased the number of writhing episodes induced in mice. A similar activity was observed by compound **2d** having a benzyl substituent. Among the cyclic derivatives, 4,4′-(methylenedi-4,1-phenylene)bis[2,4-dihydro-5-[(1-methyl-2-pyrrolyl)methyl]-3*H*-1,2,4-triazol-3-one] (**3a**) significantly decreased the number of writhing episodes induced in mice, whereas for 4,4′-(methylenedi-4,1-phenylene)bis(2,4-dihydro-5-methyl-3*H*-1,2,4-triazol-3-one) (**3a**) a relation between dose and effect could be seen. In the remaining pharmacological tests none of the compounds **2a**–**2d**, **3a**, and **3b** at a dose of 0.1 of their LD_{50} produced significant effects on the CNS of mice. In other behavioral tests the investigated compounds had no effect on the central nervous system of mice.

In conclusion, it was shown that the tested compounds have antinociceptive properties, which deserve further investigation of the action in rodents.

Experimental

Melting points were determined in a Fisher-Johns block. Elemental analyses (C, H, N) were conducted using the Elemental Analyser CHN, and results were found to be in good agreement with calculated values. IR spectra were recorded from KBr discs using a Specord IR-75 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 300F instrument (300 MHz) in DMSO-d_6 with TMS as an internal standard. The mass spectra were obtained with an AMD-604 mass spectrometer using a 70-eV electron beam. Chemicals were purchased from Lancaster or Fluka Ltd. The purity of obtained compounds was checked by TLC on aluminium oxide 60 F_{254} plates (Merck) in a $CHCl_3/C_2H_5OH$ (10:1 and 10:2) solvent system with UV or iodine visualization.

The hydrazides were synthesized by a published method [21]. Compounds **2a**–**2d** and **3a**–**3d** were synthesized and characterized earlier [17].

Diphenylacetic acid 1,1′-[2,2′-[methylenebis(4,1-phenyleneiminocarbonyl)]dihydrazide] (**2e**, $C_{43}H_{38}N_6O_4$)
A mixture of 4.52 g diphenylacetic acid hydrazide (**1e**, 20 mmol) and 2.5 g 4,4′-diphenylmethane diisocyanate (10 mmol) was heated in an oil bath at 120 °C for 15 h. The formed product was washed first with diethyl ether, then with hot water. Subsequently it was dried and crystallized from ethanol to give 4.98 g **2e** (71%). M.p.: 250-252 °C; IR (KBr): $\bar{\nu}$ = 3,216, 3,028, 2,930, 1,667, 1,411 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.78

Scheme 2

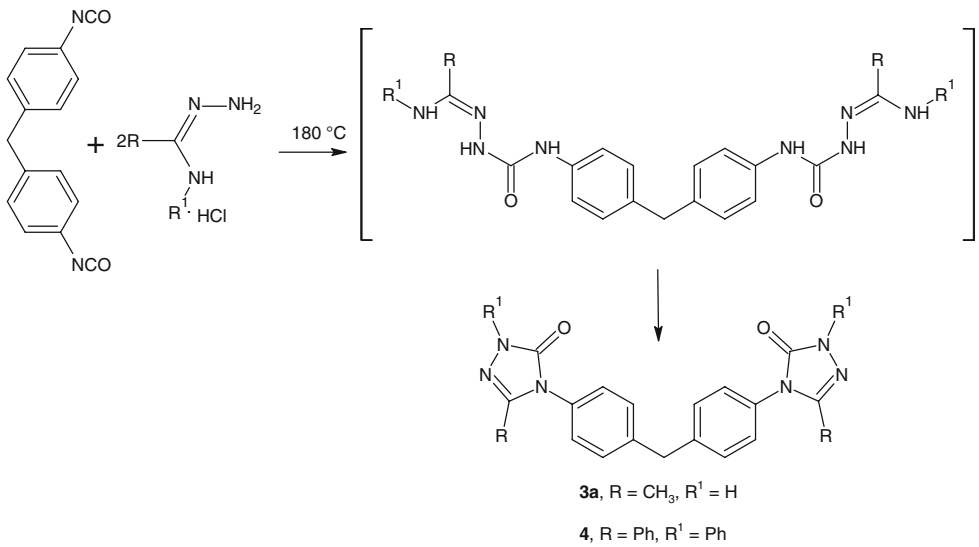


Table 1 Antinociceptive activity of investigated compounds in the writhing syndrome test in mice ($n = 10$)

Compound	Treatment (mg/kg i.p.)	Fraction of LD_{50}	Inhibition (%)
Control	–	–	0
2a	6.25	0.00625	17
	12.5	0.0125	71**
	25.0	0.025	76**
	50.0	0.05	95**
	100.0	0.1	89**
2b	6.25	0.00625	21
	12.5	0.0125	66*
	25.0	0.025	91**
	50.0	0.05	80**
	100.0	0.1	99**
2c	1.5625	0.00156	47**
	3.125	0.003125	19**
	6.25	0.00625	34**
	12.5	0.0125	61**
	25.0	0.025	72**
	50.0	0.05	88**
	100.0	0.1	87**
2d	1.5625	0.00156	9
	3.125	0.003125	44**
	6.25	0.00625	47**
	12.5	0.0125	71*
	25.0	0.025	96**
	50.0	0.05	93**
	100.0	0.1	94*
3a	3.125	0.003125	10
	6.25	0.00625	27*
	12.5	0.0125	45**
	25.0	0.025	84**
	50.0	0.05	72**
	100.0	0.1	88**
3b	3.125	0.003125	36*
	6.25	0.00625	38*
	12.5	0.0125	70**
	25.0	0.025	72**
	50.0	0.05	84**
	100.0	0.1	97**

Percentage inhibition obtained compared with control group

* $p < 0.05$ versus control group, ** $p < 0.01$ versus control group

(s, 2H), 4.18 (s, 2CH), 6.83–7.39 (m, phenyl-H), 8.05 (s, 2H, NH), 8.58 (s, 2H, NH), 9.79 (s, 2H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 48.60$ (CH_2), 117.18, 126.92, 127.14, 127.29, 127.52, 133.75, 136.14, 138.48, 138.85, 153.92 (38× C_{arom}), 169.21 (C=O), 169.71 (C=O) ppm.

3-Pyridinecarboxylic acid 1,1′-[2,2′-[methylenebis (4,1-phenyleneiminocarbonyl)]dihydrazide] (**2f**, $C_{22}H_{20}N_7O_4$)

To 2.74 g 3-pyridinecarboxylic acid hydrazide (**1f**, 20 mmol) dissolved in 40 cm^3 *N*,*N*-dimethylacetamide, 2.5 g 4,4′-diphenylmethane diisocyanate (10 mmol) was added. The mixture was refluxed in a water bath for 20 h. The solvent was evaporated under reduced pressure. The residue was extracted with anhydrous ethanol and recrystallized from ethanol to give 3.46 g **2f** (71%). M.p.: 285–286 °C; IR (KBr): $\overline{\nu} = 3{,}206$, 3,015, 2,959, 1,672, 1,419 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.80$ (s, 2H), 7.08–7.57 (m, phenyl-H), 8.25–9.07 (m, 3-pyridyl), 8.25 (s, 2H, NH), 8.81 (s, 2H, NH), 10.47 (s, 2H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 59.53$ (CH_2), 113.77, 117.08, 117.63, 133.35, 133.96, 146.77, 147.27, 150.39, 154.30 (22× C_{arom}), 163.37 (C=O), 163.83 (C=O) ppm.

Benzoic acid 1,1′-[2,2′-[methylenebis(4,1-phenyleneiminocarbonyl)]dihydrazide] (**2g**, $C_{23}H_{21}N_6O_4$)

To 2.72 g benzoic acid hydrazide (20 mmol) dissolved in 40 cm^3 *N*,*N*-dimethylacetamide, 2.5 g 4,4′-diphenylmethane diisocyanate (10 mmol) was added. The mixture was kept at room temperature at 24 h. Then 50 cm^3 water was added. The precipitate was filtered and crystallized from ethanol to give 3.02 g **2g** (68%). M.p.: 329–330 °C; IR (KBr): $\overline{\nu} = 3{,}299, 3{,}028, 2{,}360, 1{,}410, 1{,}659$ cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.78$ (s, 2H), 7.07-8.06 (m, phenyl-H), 8.12 (s, 2H, NH), 8.85 (s, 2H, NH), 10.26 (s, 2H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 50.19$ (CH_2), 112.79, 117.31, 126.13, 126.88, 127.44, 127.73, 145.16 (24× C_{arom}), 154.25 (C=O), 164.33 (C=O) ppm.

4,4′-(Methylenedi-4,1-phenylene)bis[5-(diphenylmethyl)-2,4-dihydro-3H-1,2,4-triazol-3-one] (**3e**, $C_{43}H_{34}N_6O_2$)

A mixture of 5.5 g bis-semicarbazide **2e** (10 mmol) and 50 cm^3 2% aqueous sodium hydroxide solution was refluxed for 20 h. After cooling, the solution was neutralized with dilute hydrochloric acid. The precipitate was filtered off and then crystallized from ethanol. Yield 4.33 g (65%); m.p.: 95–97 °C; IR (KBr): $\overline{\nu} = 3{,}312$, 3,027, 2,361, 1,674, 1,452 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.93$ (s, 2H), 5.22 (s, 2H), 7.02–7.32 (m, phenyl-H), 11.78 (s, 2H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 53.47$ (CH_2), 105.48, 112.90, 126.91, 138.89, 145.22, 154.07, 163.01 (38× C_{arom}), 169.41 (C=O), 169.86 (C=O) ppm.

*General procedure for the synthesis of bis(1,2,4-triazol-3-one)s **3a** and **4** from amidrazone hydrochlorides*

A mixture of 2.18 g acetamidrazone hydrochloride (20 mmol) or 4.94 g N^1-phenylbenzamidrazone hydrochloride

(20 mmol) and 2.5 g 4,4′-diphenylmethane diisocyanate (10 mmol) was heated in an oil bath at 180 °C for 20 h. After cooling, the mixture was refluxed with 15 cm^3 anhydrous ethanol for 1 h. Next, the solid was filtered off. The solvent was cooled and the precipitated compound was filtered off and dried.

*4,4′-(Methylenedi-4,1-phenylene)bis(2,4-dihydro-5-methyl-3H-1,2,4-triazol-3-one)(**3a**)*
Yield 2.56 g (71%); m.p.: 307–309 °C (Ref. [17] 308–310 °C).

4,4′-(Methylenedi-4,1-phenylene)bis(2,4-dihydro-2,5-diphenyl-3H-1,2,4-triazol-3-one) (**4**, $C_{41}H_{30}N_6O_2$)
Yield 3.89 g (61%); m.p.: 153–154 °C; IR (KBr): $\overline{\nu}$ = 3,374, 3,042, 2,922, 1,717, 1,449 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 4.08$ (s, 2H), 7.05-8.02 (m, phenyl-H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 45.40$ (CH_2), 114.12, 117.01, 117.54, 122.23, 127.37, 127.50, 127.80, 136.31, 150.28, 151.26, 153.81 (38× C_{arom}), 164.67 (C=O), 165.36 (C=O) ppm; MS: *m/z* (%) = 638 (M^+, 30).

Pharmacology

The experiments were carried out on male Albino Swiss mice (20–24 g) kept at room temperature of 18–20 °C under natural day-night cycle with free access to food and water ad libitum. Compounds **2a**–**2d**, **3a**, and **3b** were administered intraperitoneally (i.p.) as suspensions in 1% solution of Tween 80. Control animals received the same volumes (0.1 cm^3 of the solvent per 10 g of mouse weight). In all experiments the compounds were used in doses starting from 0.1 of their LD_{50}, decreasing gradually until there was no further pharmacological activity. Each experimental group consisted of ten animals. Permission for the animal tests and experiments was given by the Ethical Board of the Medical University of Lublin.

Screening of CNS activity in mice was performed in a series of tests described below. The results obtained are presented as means and were evaluated statistically using Student's *t* test or Fisher exact test.

Motor coordination was quantified with the chimney test [22]. The rectal body temperature in mice was measured by Ellab thermometer. Anxiolytic activity was measured by the four-plate test in mice according to Aron et al. [23]. Antidepressive properties were assessed by the forced swimming test [24]. Pain reactivity was measured by the writhing syndrome test [25]. Antiepileptic effects were tested by reduction of pentetrazole (90 mg/kg sc)-induced seizures. Antiserotoninergic effects were determined by the Corne test [26].

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