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CHEMISTRY

Novel 1,2,4-Thiadiazoles with an NO-Producing Fragment

I. V. Serkov, A. N. Proshin, L. N. Petrova, and Corresponding Member of the RAS S. O. Bachurin

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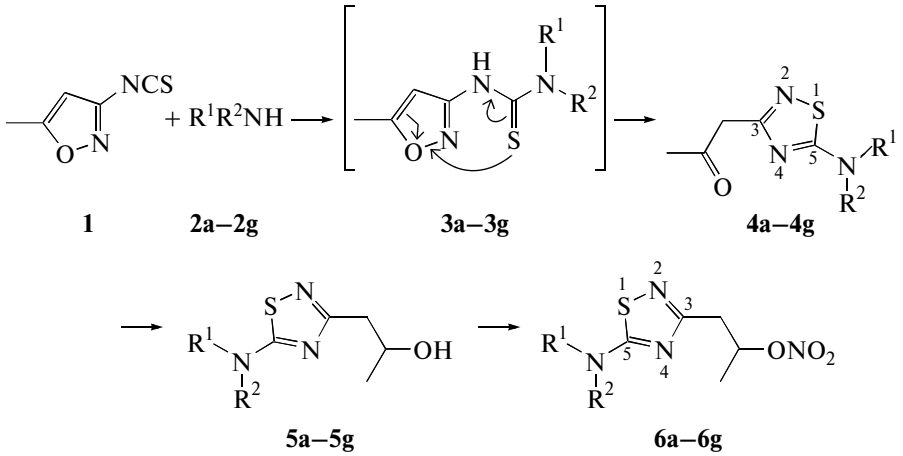
At present, the design of hybrid multitarget pharmaceuticals by introduction of nitric oxide-producing fragment in a molecule of known medicinal agent is one of promising directions of medicinal chemistry [1]. Nitric oxide (NO) is an endogenous signaling molecule with a wide spectrum of biological activity and plays an important role in nervous system functioning. Therefore, NO donors are used for the treatment of different neurodegenerative disorders, Alzheimer disease including [2]. The introduction of an NO-producing fragment into known pharmaceuticals, for example, NO-donor drugs based on Tacrine, is also applied for this purpose [3].

One of promising approaches to search for pharmaceuticals for the treatment of Alzheimer disease and related neurodegenerative disorders is the search for blocking agents for glutamate-mediated influx of Ca^{2+} [4].

In recent time, molecules containing a thiadiazole pharmacophore fragment and showing interesting pharmacological properties, including neuroprotective ones, attract much attention as promising pharmaceuticals [5].



1 **2a–2g** **3a–3g** **4a–4g**

5a–5g **6a–6g**

[R^1 = H (**a**–**e**); R^2 = Ar (**a**–**e**); $R^1 + R^2 = N(CH_2CH_2)_2N$–2-Py (**f**); $N(CH_2)_5$ (**g**)].

Scheme 1.

We have developed a method for the synthesis of unknown *N*,*N*-disubstituted 5-amino-3-(2-oxopropyl)-1,2,4-thiadiazoles (**4a**–**4g**) (Scheme 1). The method is based on the Boulton–Katrizky rearrangement of isoxazole thioureas into 1,2,4-thiadiazoles [6], which consists in the nucleophilic attack of the thiocarbonyl group at the N–O bond of the isoxazole ring, cleavage of the bond followed by recyclization to form a thiadiazole ring. Intermediate thioureas (**3**) necessary for this rearrangement are usually obtained by the reaction of 3-aminoisoxazole with different isothiocyanates. To obtain thioureas **3**, we used for the first time 3-isothiocyanato-5-methylisoxazole (**1**) [7]. The isothiocyanate in dipolar aprotic solvents (DMSO, acetonitrile) was reacted with amines (**2a**–**2g**). This approach allowed us to use secondary amines previously unavailable to this rearrangement and to

Institute of Physiologically Active Substances, Russian Academy of Sciences, Chernogolovka, Moscow oblast, 142432 Russia

Table 1. Effect of the obtained compounds on the influx of $^{45}Ca^{2+}$ in synaptosomes of rat cerebral cortex upon glutamate stimulation

Compound	Hydroxy derivatives **5a–5f**		Nitroxy derivatives **6a–6f**	
	K, % *1	IC_{50}, μM *2	*K*, % *1	IC_{50}, μM *2
a	50.6 ± 9.3	~100	0	2.1
b	101.2 ± 14.4	–	0.9 ± 0.9	0.2
c	84.3 ± 6.1	–	0.2 ± 0.1	14.1
d	51.6 ± 3.7	~100	4.5 ± 4.4	10.0
e	30.1 ± 4.1	63.1	7.5 ± 3.7	10.7
f	110.9 ± 2.2	–	2.6 ± 1.4	16.6

*1 *K* is amount of absorbed $^{45}Ca^{2+}$ in synaptosomes of rat cerebral cortex (control is 100%).

*2 IC_{50} was determined for active compounds.

synthesize unique 5-*N*,*N*-disubstituted thiadiazoles (**4a**–**4g**) in 75–95% yield [8].

The structure of 1,2,4-thiadiazole **4** contain a readily modified oxo group. The presence of this group provides an opportunity for further modification of the parent molecule by introduction of different pharmacophore groups, including an NO-producing fragment. The reduction of the oxo group of thiadiazole (**4a**–**4g**) with sodium borohydride leads to previously unknown hydroxy derivatives **5a**–**5g**, whose nitration results in derivatives **6a**–**6g**. We used 100% nitric acid as a nitrating agent. Thus, we have obtained N,N-disubstituted 5-amino-1,2,4-thiadiazoles containing a nitroxy group as the NO-producing fragment.

We have studied the inhibition of glutamate-mediated influx of $^{45}Ca^{2+}$ in synaptosomes of rat cerebral cortex and showed that the introduction of the NO-producing fragment into aminothiadiazole derivatives leads to a sharp increase in their inhibiting activity. Thus, hydroxy derivative **5b** did not affect the influx of $^{45}Ca^{2+}$ in synaptosomes, whereas nitroxy derivative **6b** showed a considerable inhibiting effect (table).

Thus, we can state that 5-amino-1,2,4-thiadiazoles containing nitroxy group show high blocking activity toward glutamate-mediated influx of Ca^{2+} and, therefore, they are promising compounds for designing neuroprotectors of new generation on their basis.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Bruker CXP-200 spectrometer (Germany), chemical shifts are given on the δ scale and referenced to Me_4Si. Melting points were determined with a Boetius hot-stage apparatus and were not corrected. Solutions were concentrated with a rotary evaporator in a vacuum of a water-jet pump.

Procedure for the synthesis of 5-amino-3-(2-nitroxypropyl)-1,2,4-thiadiazoles (6a–6g). A solution of 1.4 g (0.01 mol) of 3-isothiocyanato-5-methylisoxazole in 10 mL of acetonitrile was added dropwise to a solution of 0.01 mol of secondary amine **2** and 50 mg of *p*-toluenesulfonic acid in 20 mL of acetonitrile with stirring. After addition completed, the mixture was heated to reflux and allowed to stand at ambient temperature until precipitate of 5-amino-3-(2-oxopropyl)-1,2,4-thiadiazole **4** formed, which was separated by filtration. If no precipitation occurred, the reaction mixture was concentrated and the resultant oil was triturated with diethyl ether. Thiadiazole **4** thus obtained (0.01 mol) was suspended in 30 mL of methanol and heated to 50°C, and 0.38 g (0.01 mol) of sodium borohydride was added in portions with vigorous stirring. The precipitate dissolved as sodium borohydride was added and reaction proceeded. After reaction completed, the methanol was removed, 50 mL of methylene chloride was added, the solution was washed with water (2 × 50 mL), and the organic layer was separated and dried with sodium sulfate. The drying agent was separated by filtration, the filtrate was concentrated to give 5-amino-3-(2-hydroxypropyl)-1,2,4-thiadiazole **5**. The prepared thiadiazole **5** (0.001 mol) was dissolved in 1 mL of methylene chloride and added with stirring to a cooled to 0°C solution of concentrated nitric acid (500 μL) in 5 mL of methylene chloride. The reaction mixture was stirred for 40 min and washed with water (3 × 10 mL), and the organic layer was dried with sodium sulfate. The drying agent was separated by filtration, and the filtrate was concentrated to give 5-amino-3-(2-nitroxypropyl)-1,2,4-thiadiazole **6**.

5-(3',4'-Dichlorophenylamino)-[3-(2-nitroxypropyl)]-1,2,4-thiadiazole (6a: R^1 = H, R^2 = 3,4-$Cl_2C_6H_3$). Light brown crystals, T_m 118–120°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 8.55 (br s, 1H, NH), 7.28 (m, 3H, H_{arom}), 5.59 (m, 1H, CH), 3.10 (m, 2H, CH_2), 1.45 (d, 3H, J = 6.2 Hz, CH_3).

5-(3'-Methylphenylamino)-[3-(2-nitroxypropyl)]-1,2,4-thiadiazole (6b: R^1 = H, R^2 = 3-MeC_6H_4). Yellow crystals, T_m 102–104°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 8.12 and 7.31 (m, 4H, H_{arom}), 5.65 (m, 1H, CH), 3.16 (m, 2H, CH_2), 2.66 (s, 3H, CH_3), 1.45 (d, 3H, J = 6.2 Hz, CHC$\underline{H}_3$).

5-Phenylamino-[3-(2-nitroxypropyl)]-1,2,4-thiadiazole (6c: R^1 = H, R^2 = Ph). T_m 72–74°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 11.02 (s, 1H, NH), 8.26 (d, 2H, J = 9.2 Hz, H_{arom}), 7.73 (d, 2H, J = 9.2 Hz, H_{arom}), 7.36 (s,

1H, H_{arom}), 5.71 (m, 1H, CH), 3.22 (m, 2H, CH_2), 1.54 (d, 3H, $J = 6.2$ Hz, $CHC\underline{H}_3$).

5-(2'-Chloro-5'-trifluoromethylphenylamino)-[3-(2-nitroxypropyl)]-1,2,4-thiadiazole (6d: R^1 = H, R^2 = 2-Cl-5-$CF_3C_6H_3$). Light yellow crystals, T_m 96–97 C. ^{1}H NMR ($CDCl_3$, δ, ppm): 8.28 (m, 1H, H_{arom}), 8.06 (s, 1H, NH), 7.49 (dm, 1H, $J = 8.4$ Hz, H_{arom}), 7.27 (m, 1H, H_{arom}), 5.60 (m, 1H, CH), 3.15 (m, 2H, CH_2), 1.48 (d, 3H, $J = 6.4$ Hz, $CHC\underline{H}_3$).

5-(2'-Methyl-3'-chlorophenylamino)-[3-(2-nitroxypropyl)]-1,2,4-thiadiazole (6e: R^1 = H, R^2 = 2-Me-3-ClC_6H_3). Dark yellow oil. ^{1}H NMR ($CDCl_3$, δ, ppm): 9.56 (s, 1H, NH), 7.81 (m, 1H, H_{arom}), 7.22 (m, 2H, H_{arom}), 5.58 (m, 1H, CH), 3.18 (m, 2H, CH_2), 1.47 (d, 3H, $J = 6.4$ Hz, $CHC\underline{H}_3$).

1'-[3-(2-Nitroxypropyl)-1,2,4-thiadiazol-5-yl]-(4'-pyridin-2-yl)piperazine (6f: $R^1 + R^2 = N(CH_2CH_2)_2N$–2-Py). Dark yellow oil. ^{1}H NMR ($CDCl_3$, δ, ppm): 8.28 (m, 1H, H_{arom}), 7.56 (m, 1H, H_{arom}), 6.77 (m, 1H, H_{arom}), 6.70 (m, 1H, H_{arom}), 5.55 (m, 1H, CH), 3.73 (m, 4H, $N(C\underline{H}_2CH_{2)2}N$–2-Py), 3.63 (m, 4H, $N(CH_2C\underline{H}_{2})_2N$–2-Py), 3.14 (m, 2H, CH_2), 1.44 (d, 3H, $J = 6.4$ Hz, $CHC\underline{H}_3$).

1'-[3-(2-Nitroxypropyl)-1,2,4-thiadiazol-5-yl]piperidine (6g: $R^1 + R^2 = N(CH_2)_5$). Yellow oil. ^{1}H NMR ($CDCl_3$, δ, ppm): 5.63 (m, 1H, CH), 3.48 (br s, 4H, $2 \times NCH_2$), 3.11 (dd, 1H, $J = 6.8$ Hz, $J = 14.9$ Hz, $C\underline{H}H$), 2.94 (dd, 1H, $J = 6.6$ Hz, $J = 14.9$ Hz, $CH\underline{H}$), 1.70 (br s, 6H, $CH_2CH_2CH_2$), 1.44 (d, 3H, $J = 6.4$ Hz, $CHC\underline{H}_3$).

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