1,2,4-Thiadiazoles as promising multifunctional agents for treatment of neurodegenerative diseases

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Detailed studies of properties of new 3-substituted 5-anilino-1,2,4-thiadiazoles containing different substituents at position 3 of the thiadiazole ring were carried out, in particular, their esterase profile and antioxidant properties. It was found that the presence in the molecule of 2-aminopropyl fragment determines an efficient and selective inhibition of butyrylcholinesterase as compared to acetylcholinesterase and carboxylesterase, with radical-scavenging activity being weak. The compounds containing a 2-aminopropenyl fragment possess a high radical-scavenging activity, weakly inhibit cholinesterases, and exhibit anticarboxylesterase activity. A wide spectrum of activity of 3-substituted 5-anilino-1,2,4-thiadiazoles as inhibitors of cholinesterases and highly efficient scavengers of free radicals gives a basis for the optimization of structure and development in this series of original agents for therapy of neurodegenerative diseases.

Key words: 1,2,4-thiadiazole, 3-substituted 5-anilino-1,2,4-thiadiazoles, acetylcholinesterase, butyrylcholinesterase, carboxylesterase, antioxidants, neurodegenerative diseases, Alzheimer´s disease.

Among a wide range of different neurodegenerative diseases, a special place in its negative value to society takes Alzheimer´s disease (AD) characterizing by a gradual steady development of disorder of memory and higher cortical functions of the brain. A promising approach to the treatment of such complex, multi-factor diseases[1] is the development of multifunctional agents possessing cognitive-stimulating (inhibitors of cholinesterases) and neuroprotective properties.[2—5]

Inhibitors of acetylcholinesterase (AChE, EC 3.1.1.7) improve cognitive functions at dementias of different origins,[6,7] increasing concentration of neurotransmitter acetylcholine in the brain synapses. Inhibitors of butyrylcholinesterase (BChE, EC 3.1.1.8) also improve cognitive functions,[8,9] which is especially important at AD progression, when activity of AChE is decreased and its function in hydrolysis of acetylcholine is taken over by BChE.[9—11] Apart from that, BChE inhibitors do not have such a dangerous side effects as acute cholinergic toxicity, as well as other unwanted effects characteristic of AChE inhibitors.[12]

Disorders in neurotransmitting and metabolic neuron processes are one of the key pathological changes in neurodegenerative diseases. The processes of neuron metabolism disorder first of all include oxidation stress, arising as a result of a sharp enhancement of oxidation processes in the brain cells when the functions of the antioxidant protection system is insufficient.[13,14] In this connection, the compounds possessing an ability to quench free radicals can be used as neuroprotectors.[15—17]

1,2,4-Thiadiazole derivatives are of great interest also because of their very wide spectrum of biological activity.[18,19] In particular, the compounds containing a 1,2,4-thiadiazole fragment are described as potential agents of therapy of AD and other neurodegenerative disorders. Thus, it is known[20] that 1,2,4-thiadiazole *N*-benzylpiperidine derivatives are efficient inhibitors of AChE. 3-(1,2,4-Thiadiazolyl)pyridine 1-oxides are found to have an ability to efficiently bind to muscarinic cholinergic receptors and exhibit antioxidant activity in the DPPH-assay.[21] The neuroprotective properties and the ability to quench free radicals were demonstrated for 1,2,4-thiadiazolylnitrones.[22] Among 1,2,4-thiadiazole derivatives, inhibitors of glycogen synthase kinase 3β,[23] inhibitors of beta-secretase,[24] and modulators of gamma-secretase[25] were found. To sum up, the analysis of the literature data shows that the 1,2,4-thiadiazole scaffold is promising for the development of multifunctional agents for treatment of neurodegenerative disorders.

The present work is aimed on the directed synthesis of 3-substituted 5-anilino-1,2,4-thiadiazoles containing different substituents at position 3 of the thiadiazole ring, studies of their esterase profile and antioxidant activity.

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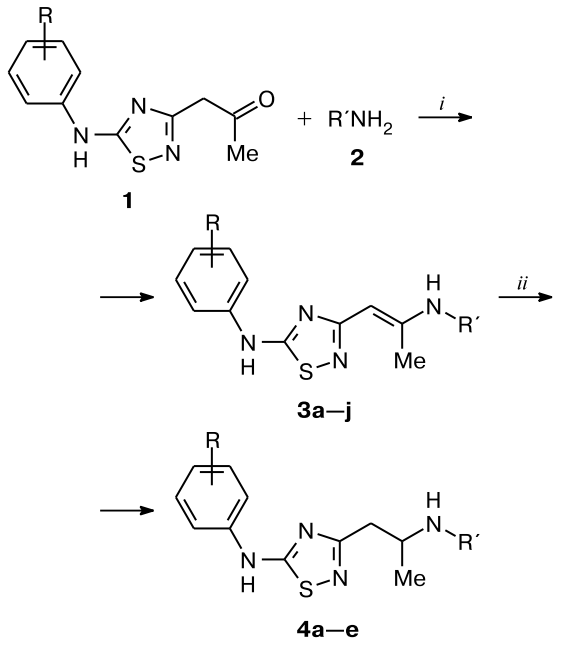
The studies of esterase profile includes evaluation of the inhibitory activity of compounds against AChE, BChE, and structurally close enzyme carboxylesterase (CaE, EC 3.1.1.1).[26—29] The enzyme CaE hydrolyzes multitude of therapeutic agents containing ester groups[30] and the inhibition of this enzyme with anticholinesterase compounds used for a long time by a patient for treatment of AD can be the reason for unwanted drug-drug intereactions.[31] Antioxidant activity of compounds was determined based on their ability to quench free radicals in DPPH- and ABTS-assays.[32,33]

The target 1,2,4-thiadiazole derivatives were synthesized according to the procedure developed by us earlier, namely, by the reaction of 5-amino-3-(2-oxopropyl)-1,2,4-thiadiazoles (**1**) with primary amines (**2**). The enamines (**3a—e**) formed in the first step were reduced with sodium borohydride at 40—50 °C to obtain the target 1,2,4-thiadiazole amino derivatives (**4a—e**).

Scheme 1



i. MeOH, ~20 °C, 2—5 h; *ii*. MeOH, $NaBH_4$, 40—50 °C, 2 h.

Compound	R	R′	Compound	R	R′
3a, 4a	3-Cl-4-F	Bn	**3f**	3-F-4-Cl	C_2H_4OH
3b, 4b	3-Cl-4-F	4-$MeC_6H_4CH_2$	**3g**	4-CF_3	$C_2H_4ONO_2$
3c, 4c	4-Cl	*cyclo*-C_6H_{11}	**3h**	4-Cl	$C_2H_4ONO_2$
3d, 4d	3,4-Cl_2	*cyclo*-C_7H_{13}	**3i**	4-Ac	$C_2H_4ONO_2$
3e, 4e	4-Cl	C_2H_4OH	**3j**	3-F-4-Cl	$C_2H_4ONO_2$

The studies of inhibitory activity of compounds against three structurally close serine esterases and analysis of the esterase profile showed that all the studied 3-substituted 5-anilino-1,2,4-thiadiazoles in a concentration of 20 μmol L^{-1} virtually do not inhibit AChE (Table 1). The

Table 1. Esterase profile of 3-substituted 5-anilino-1,2,4-thiadiazoles

Compound	Inhibitory activity of compounds against serine esterases, IC_{50}/μmol L^{-1} (% of inhibition of enzyme activity by compound in a concentration of 20 μmol L^{-1}, $n = 3$)		
	AChE	BChE	CaE
3a	>20 (3.1)	81±7	25±3
4a	>20 (18.9)	5.6±0.4	110±9
3b	—*	—*	36±3
4b	>20 (11.2)	7.4±0.5	130±13
3c	—*	>20 (23.9)	8.6±0.8
4c	>20 (21.6)	1.7±0.1	>20 (5.8)
3d	—*	20±2	4.2±0.4
4d	>20 (6.1)	1.4±0.1	>20 (11.6)
3e	>20 (7.3)	311±34	11.8±1.3
4e	>20 (18.5)	265±27	>20 (24.5)
3f	>20 (8.0)	>20 (29.8)	10±1
3g	>20 (11.8)	>20 (13.3)	41.0±3.9
3h	>20 (6.0)	>20 (10.4)	11.2±0.9
3i	>20 (9.3)	>20 (12.8)	3.12±0.02
3j	>20 (13.5)	>20 (14.7)	26.7±1.9

* No activity.

inhibition degree of BChE and CaE, as it is seen from Table 1, depends on the structure of substituents at position 3 of the thiadiazole ring, in particular, it is considerably determined by the presence of 2-aminopropyl or 2-aminopropenyl fragments. Compounds **4a** and **4b** containing benzyl substituents in the 2-aminopropyl fragment selectively and efficiently inhibit BChE with the IC_{50} values in the micromolar region (5.6±0.4 and 7.4±0.5 μmol L^{-1}, respectively). Conversely, the compounds with the same benzyl substituents in the 2-aminopropenyl fragment inhibit BChE weakly (**3a**, IC_{50} = 81±7 μmol L^{-1}) or do not inhibit at all (**3b**). The replacement in compounds bearing 2-aminopropyl fragment of benzyl groups with the cyclohexyl and cycloheptyl substituents leads to the enhancement of the inhibitory activity against BChE. Thus, compounds **4c** and **4d** efficiently and selectively inhibit BChE with the values IC_{50} = 1.7±0.1 and 1.4±0.1 μmol L^{-1}, respectively, while similar compounds **3c,d** containing a 2-aminopropenyl fragment inhibit weakly or virtually do not inhibit BChE.

Concerning inhibition of CaE, the presence in the molecule of 2-aminopropyl or 2-aminopropenyl fragment has the opposite influence. The compounds with 2-aminopropyl fragment and benzyl substituents at position 3 of the thiadiazole ring **4a** and **4b** weakly inhibit CaE (IC_{50} = = 110±9 and 130±13 μmol L^{-1}, respectively), whereas similar compounds with 2-aminopropenyl fragment **3a** and **3b** inhibit CaE stronger (IC_{50} = 25±3 and 36±3 μmol L^{-1}, respectively). The anti-CaE activity of compounds with 2-aminopropenyl fragment increases upon introduction

of cyclic aliphatic substituents at position 3 of the thiadiazole ring (**3c** and **3d**, IC_{50} = 8.6±0.8 and 4.2±0.4 μmol L^{-1}, respectively). Such a selectivity of action against BChE in compounds with 2-aminopropyl fragment and preferred inhibition of CaE by compounds with 2-aminopropenyl fragment should be taken into account when developing medicinal agents based on 3-substituted 5-anilino-1,2,4-thiadiazoles, since inhibition of CaE by these compounds taken by a patient for a long time for treatment AD can be a reason for unwanted drug-drug intereactions.

As it is seen from Table 1, the compounds with 2-aminopropenyl fragment **3g—j** containing a nitroxyl radical and their precursors, the corresponding alcohols **3e**, **3f**, and **4e**, weakly inhibit BChE and moderately CaE. The maximal anti-CaE activity among them is possessed by compound **3i** with IC_{50} = 3.12±0.02 μmol L^{-1}, that, as it was mentioned above, can be the reason of unwanted drug-drug interactions.

The studies of the mechanism of BChE inhibition with 3-substituted 5-anilino-1,2,4-thiadiazoles showed that these compounds are mixed-type reversible inhibitors. Figure 1 exemplifies the data on the dependence of residual activity of BChE on the concentration of inhibitor (compound **4b**) at different concentrations of the substrate given in Lineweaver-Burk reciprocal plots. The intersection of lines of dependencies $1/V = f(1/S)$ in the left upper quadrant indicates a mixed type of the BChE inhibition by the test compound. The inhibition constants of BChE by compound **4b** are as follows: K_i = 1.84±0.17 μmol L^{-1} (competitive component) and αK_i = 6.80±0.74 μmol L^{-1} (noncompetitive component).

The studies of antioxidant properties of 3-substituted 5-anilino-1,2,4-thiadiazole derivatives in the assays with model radicals DPPH and ABTS (Table 2) showed that in compounds with 2-aminopropyl fragment **4a—e**, the radical-scavenging activity is virtually completely absent in

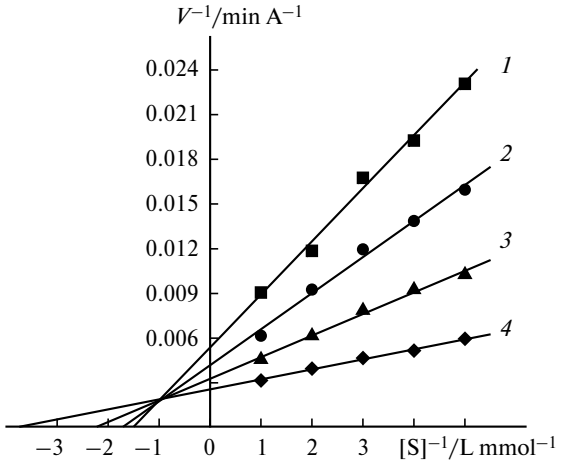


Fig. 1. Kinetics of inhibition of BChE in the presence of compound **4b** in a concentration of 9 (*1*), 6 (*2*), and 3 μmol L^{-1} (*3*), as well as in the absence of the inhibitor (*4*).

Table 2. Antiradical activity of 3-substituted 5-anilino-1,2,4-thiadiazoles in DPPH- and ABTS-assays

Compound	Antiradical activity of compounds[a]		
	DPPH, % of binding	ABTS	
		% of binding	IC_{50}
3a	51.0±4.2	96.0±3.8	35±2.4
4a	0	19.2±4.8	—[b]
3b	58.0±1.6	96.0±2.3	29±3.2
4b	0	34.0±5.2	—[b]
3c	52.6±3.2	96.2±4.3	31.8±2.3
4c	3.1±4.2	16.8±3.8	—[b]
3d	48.6±2.8	95.3±3.3	26.4±2.8
4d	2.1±1.8	20.4±3.8	—[b]
3e	50.6±3.5	92.8±4.5	36.5±2.4
4e	2.2±3.2	18.3±3.6	—[b]
3f	46.8±3.2	89.6±4.3	42.6±3.4
3g	37.1±2.1	78.0±4.2	67.2±6.8
3h	52.2±3.4	91.3±1.76	26.9±2.45
3i	59.8±3.2	96.0±4.2	20.3±2.54
3j	50.3±2.6	95.0±3.8	33.5±2.85
Trolox	100±1.2	100±2.2	12.3±1.6
Ascorbic acid	100±1.8	100±2.7	21.4±2.25

[a] Percentage of quenching of DPPH- and ABTS-radicals by compound in a concentration of 100 μmol L^{-1} or IC_{50}/μmol L^{-1}, n = 3.
[b] Not determined.

the DPPH-assay and weak activity in the ABTS-assay is observed. At the same time, virtually all the studied compounds containing 2-aminopropenyl fragment (**3a—j**) exhibit pronounced antiradical activity in the DPPH-assay (~50% binding at a concentration of 100 μmol L^{-1}) and high radical-scavenging activity in the ABTS-assay, which is comparable with the activity of standard antioxidants, ascorbic acid and trolox (see Table 2). The radical-scavenging ability of the most active (**3i**, IC_{50} = 20.3±2.54 μmol L^{-1}) and least active (**3g**, IC_{50} = 67.2±6.8 μmol L^{-1}) compounds differ only by a factor of 3, *i.e.*, the structure of substituent at position 3 of 5-anilino-1,2,4-thiadiazoles weakly affects their antiradical activity. The presence in the molecule of these compounds of 2-aminopropenyl fragment is a determining factor for exhibiting the ability to quench free radicals.

In conclusion, the present studies of the esterase profile and antioxidant properties of 3-substituted 5-anilino-1,2,4-thiadiazole derivatives showed that depending on the presence in the molecule of 2-aminopropyl or 2-aminopropenyl fragment, these compounds exhibit specific biological activity. The presence of 2-aminopropyl fragment determines an efficient and selective inhibition of BChE as compared to AChE and CaE, with their radical-scavenging activity being weak. At the same time, compounds containing 2-aminopropenyl fragment possess high radical-scavenging activity, but weakly inhibit cholinesterases

and exhibit anti-CaE activity. Thus, a number of 3-substituted 5-anilino-1,2,4-thiadiazole derivatives were found to possess properties of selective inhibitors of BChE or highly efficient free radical scavengers, that makes promising the further search in this series for the agents for the neurodegenerative diseases therapy.

Experimental

1,2,4-Thiadiazole derivatives **3a—j** and **4a—e** were synthesized according to the procedures described earlier.[34] ^{1}H NMR spectra were recorded on a Bruker CXP-200 spectrometer (200 MHz, Germany), chemical shifts are given in δ-scale relative to Me_4Si. Melting points were determined on a Boetius heating stage without correction. Elemental analysis was carried out using a Carlo-Erba C,H,N-analyzer. Mass spectra were recorded on an Exactive mass spectrometer (ThermoFisher Scientific, Germany).

***N*-(3-Chloro-4-fluorophenyl)-3-[2-(benzylamino)prop-1-enyl]-1,2,4-thiadiazol-5-amine (3a).** The yield was 92%. A yellowish powder, m.p. 155—157 °C. Found (%): C, 57.95; H, 4.42; N, 14.35. $C_{18}H_{16}ClFN_4S$. Calculated (%): C, 57.57; H, 4.30; N, 14.95. MS, *m/z*: 375.9 $[M + H]^+$. ^{1}H NMR ($CDCl_3$), δ: 2.02 (s, 3 H, CC<u>H</u>$_3$); 4.53 (d, 2 H, ArC<u>H</u>$_2$, J = 6.3 Hz); 5.19 (s, 1 H, HC=C); 7.17 (m, 2 H, H_{arom}); 7.36 (m, 6 H, H_{arom}); 8.77 (t, 1 H, N<u>H</u>CH$_2$, J = 6.3 Hz).

***N*-(3-Chloro-4-fluorophenyl)-3-[2-(benzylamino)propyl]-1,2,4-thiadiazol-5-amine (4a).** The yield was 65%. A white powder, m.p. 82—84 °C. Found (%): C, 58.93; H, 4.37; N, 14.72. $C_{18}H_{18}ClFN_4S$. Calculated (%): C, 58.68; H, 4.67; N, 14.41. MS, *m/z*: 377.9 $[M + H]^+$. ^{1}H NMR ($CDCl_3$), δ: 1.21 (d, 3 H, CH$_2$CHC<u>H</u>$_3$, J = 6.5 Hz); 2.87 (dd, 1 H, C<u>H</u>HCHMe, J_1 = 6.0 Hz, J_2 = 14.4 Hz); 3.01 (dd, 1 H, CH<u>H</u>CHMe, J_1 = 6.7 Hz, J_2 = 14.2 Hz); 3.27 (sextet, 1 H, CH$_2$C<u>H</u>Me, J = 6.3 Hz); 3.79, 3.91 (both d, 2 H, ArC<u>H</u><u>H</u>, J = 13.0 Hz); 7.22 (m, 7 H, H_{arom}); 7.42 (m, 1 H, H_{arom}).

***N*-(3-Chloro-4-fluorophenyl)-3-[2-(4-methylbenzylamino)-prop-1-enyl]-1,2,4-thiadiazol-5-amine (3b).** The yield was 91%. Colorless crystals, m.p. 164—166 °C. Found (%): C, 58.36; H, 4.32; N, 14.81. $C_{19}H_{18}ClFN_4S$. Calculated (%): C, 58.68; H, 4.67; N, 14.41. MS, *m/z*: 389.9 $[M + H]^+$. ^{1}H NMR ($CDCl_3$), δ: 2.03 (s, 3 H, CH_3); 2.38 (s, 3 H, ArC<u>H</u>$_3$); 4.49 (d, 2 H, ArC<u>H</u>$_2$, J = 6.3 Hz); 5.21 (s, 1 H, HC=C); 7.20 (m, 6 H, H_{arom}); 7.42 (m, 1 H, H_{arom}); 8.71 (t, 1 H, N<u>H</u>CH$_2$, J = 6.3 Hz).

***N*-(3-Chloro-4-fluorophenyl)-3-[2-(4-methylbenzylamino)-propyl]-1,2,4-thiadiazol-5-amine (4b).** The yield was 67%. A white powder, m.p. 90—92 °C. Found (%): C, 58.22; H, 5.26; N, 14.37. $C_{19}H_{20}ClFN_4S$. Calculated (%): C, 58.38; H, 5.16; N, 14.33. MS, *m/z*: 391.9 $[M + H]^+$. ^{1}H NMR ($CDCl_3$), δ: 1.21 (d, 3 H, CH$_2$CHC<u>H</u>$_3$, J = 6.3 Hz); 2.33 (s, 3 H, ArC<u>H</u>$_3$); 2.90 (dd, 1 H, C<u>H</u>HCHMe, J_1 = 6.0 Hz, J_2 = 14.4 Hz); 3.01 (dd, 1 H, CH<u>H</u>CHMe, J_1 = 6.7 Hz, J_2 = 14.2 Hz); 3.28 (sextet, 1 H, CH$_2$C<u>H</u>Me, J = 6.3 Hz); 3.77, 3.89 (both d, 2 H, ArC<u>H</u><u>H</u>, J = 13.0 Hz); 7.17 (m, 5 H, H_{arom}); 7.29 (m, 1 H, H_{arom}); 7.41 (m, 1 H, H_{arom}).

***N*-(3,4-Dichlorophenyl)-3-[2-(cyclohexylamino)prop-1-enyl]-1,2,4-thiadiazol-5-amine (3c).** The yield was 90%. A white powder, m.p. 186—188 °C. Found (%): C, 53.35; H, 5.32; N, 14.61. $C_{17}H_{20}Cl_2N_4S$. Calculated (%): C, 53.27; H, 5.26; N, 14.62. MS, *m/z*: 384.3 $[M + H]^+$. ^{1}H NMR ($CDCl_3$), δ: 1.35 (m, 6 H, $(CH_2)_3$); 1.68 (m, 2 H, C<u>H</u>HCHC<u>H</u>H); 1.80 (m, 2 H, CH<u>H</u>CH-CH<u>H</u>); 2.04 (s, 3 H, Me); 3.37 (m, 1 H, NCH); 5.05 (s, 1 H, HC=C); 7.16 (dd, 1 H, H_{arom}, J_1 = 8.8 Hz, J_2 = 2.3 Hz); 7.45 (d, 1 H, H_{arom}, J = 8.8 Hz); 7.48 (d, 1 H, H_{arom}, J = 2.3 Hz); 8.42 (d, 1 H, CHN<u>H</u>, J = 9.3 Hz); 10.19 (br.s, 1 H, ArNH).

***N*-(3,4-Dichlorophenyl)-3-[2-(cyclohexylamino)propyl]-1,2,4-thiadiazol-5-amine (4c).** The yield was 67%. A yellow powder, m.p. 177—179 °C. Found (%): C, 53.10; H, 5.82; N, 14.54. $C_{17}H_{22}Cl_2N_4S$. Calculated (%): C, 52.99; H, 5.75; N, 14.54. MS, *m/z*: 386.3 $[M + H]^+$. ^{1}H NMR ($CDCl_3$), δ: 1.03 (d, 3 H, Me, J = 5.6 Hz); 0.85—1.35 (m, 6 H, $(CH_2)_3$); 1.61 (m, 2 H, C<u>H</u>H-CHC<u>H</u>H); 1.80 (m, 2 H, CH<u>H</u>CHCH<u>H</u>); 2.48 (m, 1 H, NCH); 2.80 (d, 2 H, CH_2); 3.30 (d, 1 H, NCH); 7.11 (dd, 1 H, H_{arom}, J_1 = 8.8 Hz, J_2 = 2.2 Hz); 7.39 (d, 1 H, H_{arom}, J = 8.8 Hz); 7.43 (d, 1 H, H_{arom}, J = 2.2 Hz).

***N*-(4-Chlorophenyl)-3-[2-(cycloheptylamino)prop-1-enyl]-1,2,4-thiadiazol-5-amine (3d).** The yield was 88%. A white powder, m.p. 154—156 °C. Found (%): C, 59.66; H, 6.50; N, 15.49. $C_{18}H_{23}ClN_4S$. Calculated (%): C, 59.57; H, 6.39; N, 15.44. MS, *m/z*: 363.9 $[M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 1.60 (m, 10 H, $(CH_2)_4$, C<u>H</u>HCHC<u>H</u>H); 1.91 (m, 2 H, CH<u>H</u>CHCH<u>H</u>); 1.98 (s, 3 H, Me); 3.55 (m, 1 H, NCH); 4.99 (s, 1 H, HC=C); 7.22 (d, 2 H, H_{arom}, J = 8.7 Hz); 7.54 (d, 2 H, H_{arom}, J = 8.7 Hz); 8.27 (d, 1 H, CHN<u>H</u>, J = 9.75 Hz); 10.46 (br.s, 1 H, ArNH).

***N*-(4-Chlorophenyl)-3-[2-(cycloheptylamino)propyl]-1,2,4-thiadiazol-5-amine (4d).** The yield was 71%. A white powder, m.p. 187—189 °C. Found (%): C, 59.20; H, 6.96; N, 15.29. $C_{18}H_{25}ClN_4S$. Calculated (%): C, 59.24; H, 6.90; N, 15.35. MS, *m/z*: 365.9 $[M + H]^+$. ^{1}H NMR ($CDCl_3$), δ: 1.09 (d, 3 H, Me, J = 6.7 Hz); 1.21—1.70 (m, 10 H, $(CH_2)_4$, C<u>H</u>HCHC<u>H</u>H); 1.81 (m, 2 H, CH<u>H</u>CHCH<u>H</u>); 2.75 (m, 1 H, NCH); 2.85 (m, 2 H, CH_2); 3.28 (m, 1 H, NCH); 7.24 (d, 2 H, H_{arom}, J = 8.7 Hz); 7.36 (d, 2 H, H_{arom}, J = 8.7 Hz).

***N*-(4-Chlorophenyl)-3-[2-(hydroxyethylamino)prop-1-enyl]-1,2,4-thiadiazol-5-amine (3e).** The yield was 86%. A white powder, m.p. 184—186 °C. Found (%): C, 50.24; H, 4.95; N, 18.11. $C_{13}H_{15}ClN_4OS$. Calculated (%): C, 50.24; H, 4.86; N, 18.03. MS, *m/z*: 311.8 $[M + H]^+$. ^{1}H NMR ($CDCl_3$), δ: 1.96 (s, 3 H, Me); 3.75 (m, 4 H, $(CH_2)_2$); 5.02 (s, 1 H, HC=C); 7.17 (d, 2 H, H_{arom}, J = 8.7 Hz); 7.36 (d, 2 H, H_{arom}, J = 8.7 Hz); 8.21 (br.s, 1 H, ArN<u>H</u>); 8.35 (t, 1 H, NH, J = 6.1 Hz).

***N*-(4-Chlorophenyl)-3-[2-(hydroxyethylamino)propyl]-1,2,4-thiadiazol-5-amine (4e).** The yield was 66%. A white powder, m.p. 168—170 °C. Found (%): C, 49.88; H, 5.55; N, 17.99. $C_{13}H_{17}ClN_4OS$. Calculated (%): C, 49.91; H, 5.48; N, 17.91. MS, *m/z*: 313.8 $[M + H]^+$. ^{1}H NMR ($CDCl_3$), δ: 1.09 (d, 3 H, Me, J = 6.7 Hz); 2.91 (m, 4 H, $(CH_2)_2$); 3.28 (m, 1 H, NCH); 3.76 (m, 2 H, CH_2); 7.17 (d, 2 H, H_{arom}, J = 8.8 Hz); 7.37 (d, 2 H, H_{arom}, J = 8.8 Hz).

***N*-(4-Chloro-3-fluorophenyl)-3-[2-(hydroxyethylamino)prop-1-enyl]-1,2,4-thiadiazol-5-amine (3f).** The yield was 90%. A white powder, m.p. 172—174 °C. Found (%): C, 47.56; H, 4.35; N, 17.10. $C_{13}H_{14}ClFN_4OS$. Calculated (%): C, 47.49; H, 4.29; N, 17.04. MS, *m/z*: 329.8 $[M + H]^+$. ^{1}H NMR ($CDCl_3$), δ: 2.04 (s, 3 H, Me); 3.41 (q, 2 H, NCH_2, J = 5.7 Hz); 3.71 (t, 2 H, OCH_2, J = 5.7 Hz); 5.17 (s, 1, HC=C); 7.11 (t, 1 H, H_{arom}, J = 8.8 Hz); 7.40 (m, 1 H, H_{arom}); 7.71 (dd, 1 H, H_{arom}, J_1 = 8.8 Hz, J_2 = 2.4 Hz); 8.35 (t, 1 H, NH, J = 6.1 Hz).

***N*-(4-Trifluoromethylphenyl)-3-[2-(2-nitroxyethylamino)-prop-1-enyl]-1,2,4-thiadiazol-5-amine (3g).** The yield was 90%. Dark brown crystals, m.p. 147—149 °C. Found (%): C, 43.28; H, 3.66; N, 17.91. $C_{14}H_{14}F_3N_5O_3S$. Calculated (%): C, 43.19; H, 3.62; N, 17.99. MS, *m/z*: 390.4 $[M + H]^+$. ^{1}H NMR ($CDCl_3$),

δ: 2.00 (s, 3 H, Me); 3.61 (q, 2 H, NHC$\underline{H}_2$, J = 5.6 Hz); 4.59 (t, 2 H, CH_2ONO_2, J = 5.6 Hz); 5.21 (s, 1 H, CH); 7.58 (s, 4 H, H_{arom}); 8.47 (t, 1 H, N$\underline{H}CH_2$, J = 6.4 Hz).

***N*-(4-Chlorophenyl)-3-[2-(2-nitroxyethylamino)prop-1-enyl]-1,2,4-thiadiazol-5-amine (3h).** The yield was 88%. Brown crystals, m.p. 186—188 °C. Found (%): C, 43.80; H, 4.02; N, 19.63. $C_{13}H_{14}ClN_5O_3S$. Calculated (%): C, 43.88; H, 3.97; N, 19.68. MS, m/z: 356.8 $[M + H]^+$. ^{1}H NMR ($CDCl_3$), δ: 2.02 (s, 3 H, Me); 3.61 (q, 2 H, NHC$\underline{H}_2$, J = 5.7 Hz); 4.60 (t, 2 H, CH_2ONO_2, J = 5.5 Hz); 5.12 (s, 1 H, CH); 7.25 (d, 2 H, H_{arom}, J = 9.0 Hz); 7.53 (d, 2 H, H_{arom}, J = 9.0 Hz); 8.36 (t, 1 H, N$\underline{H}CH_2$, J = 6.5 Hz); 10.57 (s, 1 H, NH).

***N*-(4-Acetylphenyl)-3-[2-(2-nitroxyethylamino)prop-1-enyl]-1,2,4-thiadiazol-5-amine (3i).** The yield was 87%. Yellow crystals, m.p. 170—172 °C. Found (%): C, 49.64; H, 4.77; N, 19.36. $C_{15}H_{17}N_5O_4S$. Calculated (%): C, 49.58; H, 4.72; N, 19.27. MS, m/z: 364.4 $[M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 1.99 (s, 3 H, Me); 2.39 (s, 3 H, Me); 3.61 (q, 2 H, NHC$\underline{H}_2$, J = 5.3 Hz); 4.58 (t, 2 H, CH_2ONO_2, J = 5.5 Hz); 5.10 (s, 1 H, CH); 7.24 (d, 2 H, H_{arom}, J = 8.8 Hz); 7.53 (d, 2 H, H_{arom}, J = 8.8 Hz); 8.34 (t, 1 H, N$\underline{H}CH_2$, J = 6.4 Hz); 10.57 (br.s, 1 H, NH).

***N*-(4-Chloro-3-fluorophenyl)-3-[2-(2-nitroxyethylamino)-prop-1-enyl]-1,2,4-thiadiazol-5-amine (3j).** The yield was 87%. Yellowish brown crystals, m.p. 148—150 °C. Found (%): C, 41.79; H, 3.58; N, 18.81. $C_{13}H_{13}ClFN_5O_3S$. Calculated (%): C, 41.77; H, 3.51; N, 18.74. MS, m/z: 374.8 $[M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 2.06 (s, 3 H, Me); 3.65 (q, 2 H, NHC$\underline{H}_2$, J = 5.7 Hz); 4.63 (t, 2 H, CH_2ONO_2, J = 5.7 Hz); 5.17 (s, 1 H, CH); 7.17 (m, 1 H, H_{arom}); 7.44 (m, 1 H, H_{arom}); 7.83 (m, 1 H, H_{arom}); 8.40 (t, 1 H, N$\underline{H}CH_2$, J = 6.3 Hz); 10.58 (br.s, 1 H, NH).

Biochemical studies. *Studies of the esterase profile of compounds.* Kinetic studies were carried out using commercial agents: human erythrocyte AChE, horse serum BChE, and porcine liver CaE (Sigma, USA). Activity of AChE and BChE was determined by Ellman method[35] (λ = 412 nm), using acetylthiocholine (1 mmol L^{-1}) and butyrylthiocholine (1 mmol L^{-1}) as a substrate, respectively; conditions: 100 m*M* phosphate buffer with pH 7.5, 25 °C. Activity of CaE was determined spectrophotometrically based on release of 4-nitrophenol (λ = 405 nm), using 4-nitrophenyl acetate (1 mmol L^{-1}) as a substrate;[36] conditions: 100 m*M* phosphate buffer with pH 8.0, 25 °C. The measurements were carried out using a BioRad Benchmark Plus microplate spectrophotometer (France). Compounds were dissolved in DMSO, the incubation mixture contained 2% of solvent. The primary evaluation of inhibitory activity of compounds was carried out by determination of inhibition degree of enzymes at a concentration of the compound of 20 μmol L^{-1}. For this, a sample of the corresponding enzyme was incubated with the test compound for 10 min, then the residual activity of the enzyme was measured. Each experiment was carried out in triplicate. The IC_{50} values, a concentration of the inhibitor required to decrease enzyme activity by 50%, were determined for the most active compounds. To determine the IC_{50} of the inhibition of AChE, BChE, and CaE, a sample of the corresponding enzyme was incubated with the test compound in the range of concentrations $1 \cdot 10^{-11}$—$1 \cdot 10^{-4}$ mol L^{-1} for 10 min and then a residual activity of the enzyme was determined. Each experiment was carried out in triplicate. The IC_{50} values were calculated using the Origin 6.1 program.

To clarify the inhibition mechanism of BChE, we studied a dependence of BChE activity on the substrate concentration in the absence and in the presence of the inhibitor in three increasing concentrations. The kinetic data were analyzed in the Lineweaver-Burk plot. The kinetic constants of inhibition K_i (competitive component) and αK_i (noncompetitive component) were calculated using the Origin 6.1 program.

All the measurements were carried out on a BioRad Benchmark Plus microplate spectrophotometer (France).

Studies of antiradical activity of compounds. The antiradical activity of compounds under study was determined using two the spectrophotometric methods: DPPH- and ABTS-assays. The compounds were dissolved in DMSO. The content of DMSO in the reaction mixture was 4%. Ascorbic acid and trolox were used as a positive control. The radical-scavenging activity of compounds was evaluated at their concentration of 100 μmol L^{-1}, the IC_{50} values (a concentration of the compound at which the ABTS-radical is bound by 50%) were determined for the most active compounds in the ABTS-assay.

The reaction with the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma—Aldrich) was carried out as described earlier[32] with insignificant modifications. A solution of DPPH was prepared in 96% ethanol. A concentration of DPPH in the reaction mixture was 100 μmol L^{-1}. A test compound was added to the reaction medium containing a DPPH-radical and 96% ethanol and thoroughly stirred. The reaction was carried out at a temperature of 25 °C in dark, the incubation time was 1 h. A discoloration degree of the solution of DPPH upon addition of the test compounds was determined at a wavelength of 517 nm on a Cary 60 spectrophotometer (Agilent Technologies, Canada). All the measurements were carried out in triplicate.

The antiradical activity of compounds (I) was evaluated using the formula

$$I = [(A_0 - A_i)/A_0] \cdot 100\%,$$

where A_0 and A_i are the optical density of a control solution of DPPH-radical in the absence and after addition of a test compound, respectively.

The reaction with $ABTS^{\cdot+}$-radical was carried out according to the method described earlier[33] with insignificant modifications. The $ABTS^{\cdot+}$ radical was obtained by the reaction of an aqueous solution of ABTS (2,2′-azino-bis(3-ethylbenzthiazolino-6-sulfonic acid (7 mmol L^{-1}) (Sigma—Aldrich)) with a 4.9 m*M* aqueous solution of potassium persulfate (Sigma—Aldrich) taken in equal volumes. The reaction proceeded over 12—16 h at room temperature in dark. Before carrying out the measurements, the resulting solution of $ABTS^{\cdot+}$-radical was diluted with 96% ethanol until the optical density of the sample reached 0.75—0.95 at a wavelength of 734 nm. The test compounds were added to the solution of $ABTS^{\cdot+}$-radical and thoroughly stirred. The reaction was carried out at 30 °C in dark, the incubation time was 1 h. The discoloration degree of the solution of $ABTS^{\cdot+}$-radical was determined at a wavelength of 734 nm using a Cary 60 spectrophotometer (Agilent Technologies, Canada). All the measurements were carried out in triplicate. The antiradical activity of compounds (I) was evaluated as described above for the DPPH-radical. The IC_{50} values were determined for the most active compounds. The range of concentrations of test compounds was $1 \cdot 10^{-6}$—$1 \cdot 10^{-4}$ mol L^{-1}. The IC_{50} values were calculated using the Origin 6.1 program.

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