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RESEARCH ARTICLE

Synthesis and anticholinesterase activities of novel 1,3,4-thiadiazole based compounds

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Abstract

In the present study, new (1,3,4-thiadiazol-2-yl)benzene-1,3-diol based compounds have been synthesized and their potential anticholinesterases properties have been investigated using the modified of Ellman's spectrophotometric method. The compounds were obtained by the reaction of hydrazides or thiosemicarbazides with aryl-modified sulfinylbis[(2,4-dihydroxyphenyl)methanethione]s. Their chemical structures were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR and EI-MS spectral data and elemental analyses. Most of the compounds acted as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitors *in vitro*, with IC_{50} values ranging from >500 to 0.053 μM and from >500 to 0.105 μM, respectively. The most potent compound **9** (IC_{50} = 0.053 μM) proved to be selective toward AChE, exhibiting selectivity ratios versus BuChE of *ca.* 950. The kinetic studies showed that it is a mixed-type of AChE inhibitor. Another compound (**2**) was active against both enzymes with IC_{50} values in the low nM range. The structure-activity relationships (SARs) of the compounds under consideration were discussed.

Keywords: 1,3,4-Thiadiazole, synthesis, acetylcholinesterase, butyrylcholinesterase, inhibitor

Introduction

Acetylcholinesterase (AChE) inhibition is a major pharmacological approach to the treatment of Alzheimer's disease (AD[1,2]). A common method for the treatment of cognitive and behavioral symptoms of AD is the use of structurally diverse group of cholinesterase inhibitors (ChEIs) with an amine moiety and distinct modes of action. Available ChEI drugs are tacrine (Cognex[3]), donepezil (Aricept[4]) galanthamine (Reminyl) - reversible inhibitors[5] and rivastigmine (Exelon[6]) which acts as a pseudo-irreversible ChEI, and differs in selectivity toward AChE and butyrylcholinesterase (BuChE).

Two major evidences highlight the central role of AChE in AD: (i) inhibitors, which increase synaptic levels of available ACh by preventing its degradation and temporarily retard the loss of cognitive function, (ii) AChE induces the expression of the β-amyloid (Aβ) precursor protein in glia and activates glial cells[7]. The second class of compounds are the only approved drugs for the symptomatic treatment of AD[8]. Recent research has revealed that in severe AD brains the levels of AChE are considerably reduced, whereas BuChE activity increases. BuChE may then act as a compensatory mechanism for ACh metabolism, aggravating the toxicity of Aβ[9].

To obtain more metabolically stable cholinomimetic ligands, it is possible to replace the ester group with a series of five-membered rings like oxadiazoles, thiadiazoles, triazoles and tetrazoles[10]. Some researchers have studied thiadiazoles extensively and the compounds bearing thiadiazole moiety have been reported to exhibit significant anticholinesterase activity[11,12].

The antioxidant and muscarinic receptor binding properties of 3-(thiadiazolyl)pyridine 1-oxides were reported by Martinez et al[13]. as potential acetylcholinesterase inhibitors. Sarkadi et al. studied 1-benzyl-4-[2-(5-phenyl-1,3,4-thiadiazol-2-yl) aminoethyl]piperidines[14]. The thiadiazolidin-3,5-dione (TDZD) derivatives were reported as the first non-ATP competitive inhibitors of glycogen synthase kinase 3β (GSK-3β) which is one of the most attractive therapeutic

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targets for the development of selective inhibitors of Alzheimer's disease treatment[15]. Misra and co-wokers described thiadiazoloquinazolone derivatives as AChEI[12,16]. Recently N-(benzothiazol-2-yl)-2-[(5-amino/methyl-1,3,4-thiadiazol-2-yl)thio]acetamides have been presented as AChEI with IC_{50} values in the low μM range[17].

In the previous studies, we found a series of 4-(5-phenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diols substituted in the phenyl ring as *in vitro* AChE and BuChE inhibitors of high potency[18]. Some of them were efficient against both enzymes, others were characterized by high selectivity to AChE over to BuChE. With the aim of gaining insights into the structure–activity relationships and possibly identifying new leads in the AChE/BuChE inhibitors, in the present work we synthesized some new compounds from the (1,3,4-thiadiazol-2-yl)benzene-1,3-diol group. We included derivatives with 5-heterocyclic, aryl and alkyl substituents attached directly to the 1,3,4-thiadiazole ring or by -NH- group as well as the compounds with modified resorcinol moiety. The inhibition mechanism for the chosen compounds was studied.

Materials and methods

Chemistry

The IR spectra were recorded with a Perkin-Elmer FT-IR 1725X spectrophotometer (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, England) (in KBr) or Varian 670 FT-IR spectrometer (ATR). The spectra were made in the range of 600–4000 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 using a Varian Mercury 400 or a Bruker DRX 500 (Bruker Daltoncs, Inc. Billerica, MA) instrument. Chemical shifts (δ, ppm) were given in relation to tetramethylsilane (TMS). The spectra MS (EI, 70 eV) were recorded using the apparatus AMD-604 (Intectra GmbH, Harpstedt, Germany). Elemental analyses (C, H, N) were conducted using a Perkin-Elmer 2400 instrument (Perkin Elmer, Waltham, MA) and were found to be in good agreement (±0.4%) with the calculated values. The melting point (mp) was determined using a Büchi B-540 (Flawil, Switzerland) melting point apparatus.

The purity of the compounds was examined by a liquid chromatograph Knauer (Berlin, Germany) with a dual pump, a 20-μL simple injection valve and a UV–visible detector (330 nm). The Hypersil Gold C18 (1.9 μm, 100 × 2.1 mm) column was used as the stationary phase. The mobile phase included different contents of methanol and acetate buffer (pH 4, 20 nM) as the aqueous phase. The flow rate was 0.5 mL min^{-1} at room temperature. The retention time of an unretained solute (t_0) was determined by the injection of a small amount of acetone dissolved in water. Log *k* values for 70% of methanol (v/v) in the mobile phase are presented. Log k values were calculated as $\log k = \log (t_R - t_o)/t_o$, where: t_R = retention time of a solute, t_o = retention time of an unretained solute.

Synthesis of compounds

*4-(5-Propyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**1**)*

A mixture of butyrohydrazide (0.025 mol) (Alfa Aesar) and STB (0.025 mol) in methanol (120 mL) was refluxed (3 h). The hot mixture was filtered, water (40 mL) was added to the filtrate and the mixture was left at room temperature (24 h). Recrystallization from MeOH/H_2O (4:3) (70 mL) afforded **1**.

Yield: 65%; HPLC: log k = –0.26; m.p.: 243–245°C; anal. calc. for $C_{11}H_{12}N_2O_2S$ (236.29): C, 55.91; H, 5.12; N, 11.86; found: C, 56.02; H, 5.10; N, 11.80; ^1H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.04 (s, 1H, HO-C(3)), 9.97 (s, 1H, HO-C(1)), 7.94 (m, 1H, H-C(5)), 6.47 (m, 1H, H-C(2)), 6.42 (m, 1H, H-C(6)), 3.04 (t, *J* = 7.46 Hz, 2H, CH_2), 1.76 (sextet, *J* = 7.43 Hz, 2H, CH_2), 0.97 (t, *J* = 7.35 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 168.2, 162.6, 160.8, 156.2, 129.0, 108.4, 108.1, 102.4, 30.7 (CH_2), 22.8 (CH_2), 13.4 (CH_3); IR (ATR, cm^{-1}): 3162 (OH), 2962 (H-Ar), 1626 (C=N), 1598, 1552, 1529 (C=C), 1468, 1421, 1330, 1250, 1228, 1171 (C-O), 1133, 1054 (N=C-S-C=N), 986, 967, 845, 801, 745, 690 (C-S-C), 674; EI-MS (m/z, %): 236 (58), 235 (5), 221 (18), 210 (5), 209 (10), 208 (100), 207 (5), 184 (4), 167 (6), 153 (16), 136 (14), 135 (10), 80 (4), 69 (4), 52 (4), 41 (4).

*4-[5-(Naphthalen-2-yl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**3**)*

A mixture of naphthalene-2-carbohydrazide (0.005 mol) (Alfa Aesar) and STB (0.0037 mol) in methanol (35 mL) was refluxed (1.5 h). The hot mixture was filtered, water (50 mL) was added to the filtrate and the mixture was left at room temperature (24 h). Recrystallization from MeOH/H_2O (1:1) (25 mL) afforded **3**.

Yield: 74%; HPLC: log k = 0.514; m.p.: 248–250°C; anal. calc. for $C_{18}H_{12}N_2O_2S$ (320.37): C, 67.48; H, 3.78; N, 8.74; found: C, 67.41; H, 3.80; N, 8.70; ^1H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.14 (s, 1H, HO-C(3)), 10.09 (s, 1H, HO-C(1)), 8.58 (s, 1H, H-Ar), 8.19 (dd, *J* = 8.58 Hz and *J* = 1.78 Hz, 1H, H-Ar), 8.14 (m, 3H, H-Ar), 8.02 (m, 1H, H-C_{Ar}), 7.64 (m, 2H, H-Ar), 6.56 (d, *J* = 2.3 Hz, 1H, H-C(2)), 6.50 (dd, *J* = 8.66 Hz and *J* = 2.31 Hz, 1H, H-C(5)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 166.3, 162.9, 161.4, 156.5, 133.8, 132.9, 129.0, 128.9, 128.6, 127.8, 127.7, 127.5, 127.4, 127.1, 123.9, 108.5, 108.4, 102.2; IR (KBr, cm^{-1}): 3397, 3151 (OH), 1631 (C=N), 1600, 1527, 1473 (C=C), 1431, 1334, 1257, 1182 (C-O), 1140, 1052 (N=C-S-C=N), 1022, 986, 968, 939, 898, 852, 806, 743, 682 (C-S-C), 640, 611; EI-MS (m/z, %): 320 (M^+, 100), 186 (4), 185 (24), 171 (16), 167 (42), 160 (5), 154 (11), 153 (30), 149 (4), 140 (6), 139 (4), 135 (7), 127 (25), 126 (9), 119 (4), 107 (6), 86 (4), 77 (5), 69 (4), 63 (4), 52 (6), 51 (5), 40 (9), 39 (6).

*4-[5-(1,3-Benzodioxol-5-yl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**4**)*

A mixture of 1,3-benzodioxole-5-carbohydrazide (0.005 mol) (Alfa Aesar) and STB (0.0037 mol) in methanol (30 mL) was refluxed (3 h). The hot mixture was filtered,

water (30 mL) was added to the filtrate and the mixture was left at room temperature (24 h). Recrystallization from MeOH/H_2O (2:1) (30 mL) afforded **4**.

Yield: 82%; HPLC: log k = 0.118; m.p.: 308–310°C; anal. calc. for $C_{15}H_{10}N_2O_4S$ (314.32): C, 57.32; H, 3.21; N, 8.91; found: C, 57.40; H, 3.19; N, 8.95; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.06 (s, 1H, HO-C(3)), 10.04 (s, 1H, HO-C(1)), 8.02 (d, *J* = 8.67 Hz, 1H, H-C(5)), 7.55 (d, *J* = 1.74 Hz, 1H, H-C(4')), 7.50 (dd, *J* = 8.07 Hz and *J* = 1.78 Hz, 1H, H-C(6')), 7.07 (d, *J* = 8.10 Hz, 1H, H-C(7')), 6.51 (d, *J* = 2.32 Hz, 1H, H-C(2)), 6.47 (dd, *J* = 8.68 Hz and *J* = 2.32 Hz, 1H, H-C(6)), 4.16 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.8, 162.2, 161.2, 156.2, 149.4, 148.1, 128.8, 124.2, 122.3, 109.0, 108.4, 108.3, 106.7, 102.3, 101.8; IR (KBr, cm^{-1}): 3484, 3394, 3047 (OH), 3047 (H-Ar), 2921 (CH), 1612 (C=N), 1509 (C=C), 1448, 1321, 1285, 1263, 1229, 1178 (C-O), 1128, 1099, 1038, 1006, 986, 971, 937, 876, 858, 805, 725, 670 (C-S-C); EI-MS (m/z, %): 314 (M^+, 100), 285 (4), 179 (14), 178 (21), 167 (36), 165 (16), 153 (8), 148 (7), 147 (16), 146 (15), 135 (9), 134 (5), 121 (8), 119 (4), 108 (5), 107 (9), 106 (4), 80 (5), 79 (4), 75 (5), 69 (7), 65 (5), 63 (15), 62 (7), 52 (9), 51 (6), 39 (8), 38 (4).

4,4'-[5,5'-methylenedi-(1,3,4-thiadiazole-2-yl)] di(benzene-1,3-diol)

A mixture of propanedihydrazide (0.01 mol) (Alfa Aesar) and STB (0.015 mol) in methanol (80 mL) was refluxed (4 h). The removed compound was filtered and washed with water. Recrystallization from MeOH/H_2O (1:1) (40 mL) afforded **5**.

Yield: 84%; HPLC: log k = –0.446; m.p.: 277–279°C; anal. calc. for $C_{17}H_{12}N_4O_4S_2$ (400.43): C, 50.99; H, 3.02; N, 13.99; found: C, 51.18; H, 3.00; N, 14.05; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.02 (s, 2H, HO-C(3)), 10.09 (s, 2H, HO-C(1)), 8.02 (d, 2H, *J* = 8.71 Hz, H-C(5)), 6.49 (d, 2H, *J* = 2.32 Hz, H-C(2)), 6.45 (dd, 2H, *J* = 8.70 Hz and *J* = 2.31 Hz, H-C(6)), 5.03 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 163.9 (2C), 161.2 (2C), 156.2 (2C), 128.8 (2C), 123.1 (2C), 108.3 (2C), 108.2 (2C), 102.3 (2C), 29.3 (CH_2); IR (KBr, cm^{-1}): 3257 (OH), 1630 (C=N), 1522, 1470 (C=C), 1414, 1327, 1281, 1246, 1209, 1188 (C-O), 1136, 1108 (C_{Ar}-H), 986, 969, 852 (C_{Ar}-H), 799, 749, 730, 669 (C-S-C), 627, 601; EI-MS (m/z, %): 400 (M^+, 100), 371 (4), 238 (4), 234 (13), 233 (16), 232 (12), 208 (4), 207 (7), 204 (5), 167 (12), 154 (4), 153 (43), 136 (12), 135 (45), 108 (14), 107 (12), 106 (7), 97 (9), 84 (8), 80 (10), 79 (7), 69 (12), 66 (5), 65 (6), 63 (6), 52 (17), 50 (4), 45 (5), 39 (13).

4,4'-{[5,5'-(ethane-1,2-diyl)]di(1,3,4-thiadiazol-2-yl)} di(benzene-1,3-diol)

A mixture of succinic dihydrazide butanedihydrazide (0.01 mol) (Aldrich) and STB (0.015 mol) in methanol (80 mL) was refluxed (2 h). The removed compound was filtered and washed with water. Recrystallization from MeOH/H_2O (1:1) (50 mL) afforded **6**.

Yield: 89%; HPLC: log k = 0.802; m.p.: 316–319°C; anal. calc. for $C_{18}H_{14}N_4O_4S_2$ (414.46): C, 52.16; H, 3.40; N, 13.52; found: C, 52.25; H, 3.42; N, 13.48; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 10.97 (s, 2H, HO-C(3)), 10.01 (s, 2H, HO-C(1)), 7.95 (d, 2H, *J* = 8.69 Hz, H-C(5)), 6.48 (d, 2H, *J* = 2.31 Hz, H-C(2)), 6.42 (dd, 2H, *J* = 8.68 Hz and *J* = 2.32 Hz, H-C(6)), 3.60 (s, 4H, CH_2); ^{13}C NMR (500 MHz, DMSO-d_6, ppm) δ: 166.4, 163.3, 161.0, 156.2, 128.9, 108.4, 108.2, 102.3, 28.6 (2C, CH_2); IR (KBr, cm^{-1}): 3556 (OH), 3038, 2942 (C_{Ar}-H), 1605 (C=N, C=C), 1521, 1475 (C=C), 1432, 1343, 1319, 1277, 1214, 1184 (C-O), 1122 (C_{Ar}-H), 1084, 987, 970, 867 (C_{Ar}-H), 813, 723, 708, 663 (C-S-C), 618; EI-MS (m/z, %): ESI-MS (m/z): 413.1 $[M-H]^-$, 827.1 $[2M-H]^-$.

*4-(5-(4-(Trifluoromethyl)phenylamino)-1,3,4-thiadiazol-2-yl) benzene-1,3-diol (**8**)*

A mixture of 4-(4-(trifluoromethyl)phenyl)-3-thiosemicarbazide (0.004 mol) (Alfa Aesar) and STB (0.003 mol) in methanol (30 mL) was refluxed (3h). The hot mixture was filtered, water (50 mL) was added to the filtrate and it was left at room temperature (24 h). Recrystallization from MeOH/H_2O (2:1) (30 mL) afforded **8**.

Yield: 72%; HPLC: log k = 0.292; m.p.: 234–236°C; anal. calc. for $C_{15}H_{10}F_3N_3O_2S$ (353.32): C, 50.99; H, 2.85; N, 11.89; found: C, 51.08; H, 2.86; N, 11.81; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 10.89 (s, 1H, HO-C(3)), 10.68 (s, 1H, HO-C(1)), 9.23 (s, 1H, NH), 7.86 (m, 3H, H-C_{AR}), 7.71 (m, 2H, H-C_{AR}), 6.46 (d, *J* = 2.39 Hz, 1H, H-C(2)), 6.41 (dd, *J* = 8.62 Hz and *J* = 2.39 Hz, 1H, H-C(6)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 162.9, 160.4, 155.6, 155.4, 144.2, 128.5, 126.3, 126.2, 121.3, 121.0, 116.7, 116.6, 108.4, 108.1, 102.4; IR (KBr, cm^{-1}): 3400, 3255 (OH, NH), 3024 (C_{AR}-H), 1619 (C=N, C=C), 1573 (C=C), 1476, 1440, 1413, 1330 (CF_3), 1247, 1216, 1168 (C-O), 1061 (N=C-S-C=N), 1012, 987, 970, 937, 832, 805, 736, 679 (C-S-C); EI-MS (m/z, %): 353 (M^+, 100), 352 (9), 334 (4), 219 (4), 218 (34), 198 (7), 191 (4), 153 (5), 150 (4), 145 (5), 136 (5), 94 (6), 66 (4).

*4-{5-[5-(4-Bromophenyl)-1,2-oxazol-3-yl]-1,3,4-thiadiazol-2-yl}benzene-1,3-diol (**10**)*

A mixture of 5-(4-bromophenyl)isoxazole-3-carboxylic acid hydrazide (0.01mol) (Alfa Aesar) and STB (0.01 mol) in methanol (45 mL) was refluxed (3 h). The removed solid was filtered and washed with water. Recrystallization from MeOH (40 mL) afforded **10**.

Yield: 62%; HPLC: log k = 0.692; m.p.: 345–347°C; anal. calc. for $C_{17}H_{10}BrN_3O_3S$ (416.25): C, 49.05; H, 2.42; N, 10.09; found: C, 49.25; H, 4.43; N, 10.06; ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 11.37 (s, 1H, HO-C(3)), 10.18 (s, 1H, HO-C(1)), 8.16 (d, 1H, *J* = 8.62 Hz, H-C(5)), 7.98 (m, 2H, H-C(2', 6')), 7.83 (s, 1H, H-isoxazole), 7.80 (m, 2H, H-C(3', 5'), 6.54 (d, 1H, *J* = 2.28 Hz, H-C(2)), 6.49 (dd, *J* = 8.71 and 2.27 Hz, 1H, H-C(6)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 169.7, 163.8, 161.9, 156.9, 156.8, 154.9, 132.4 (2C), 129.1, 127.9 (2C), 125.4, 124.5, 108.6, 107.9, 102.3, 99.2; IR (ATR, cm^{-1}): 3358 (OH), 2916 (H-Ar), 2850 (CH), 1605 (C=N), 1525 (C=C), 1474, 1444, 1421, 1392, 1351, 1272, 1219, 1187 (C-O), 1107, 1039 (N=C-S-C=N),

1008, 947, 933, 870, 831, 797, 685 (C-S-C); EI-MS (m/z, %): 416 (M^+, 21), 415 (100), 235 (6), 208 (6), 207 (5), 185 (39), 183 (40), 167 (18), 157 (10), 155 (10), 153 (10), 135 (15), 108 (4), 107 (5), 89 (13), 75 (5), 69 (4), 52 (5), 39 (74).

*4-{5-[5-(4-Fluorophenyl)thiophen-2-yl]-1,3,4-thiadiazol-2-yl} benzene-1,3-diol (**11**)*

A mixture of 5-(4-fluorophenyl)thiophene-2-carboxylic acid hydrazide (0.01mol) (Alfa Aesar) and STB (0.01 mol) in methanol (55 mL) was refluxed (3 h). The removed solid was filtered and washed with water. Recrystallization from MeOH (40 mL) afforded **11**.

Yield: 65%; HPLC: log k = 0.609; m.p.: 312–314°C; anal. calc. for $C_{18}H_{11}FN_2O_2S_2$ (370.42): C, 58.36; H, 2.99; N, 7.56; found: C, 58.51; H, 3.01; N, 7.54; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.17 (s, 1H, HO-C(3)), 10.09 (s, 1H, HO-C(1)), 8.06 (d, 1H, *J* = 8.71 Hz, H-C(5)), 7.82 (m, 2H, H-C(2′, 6′), 7.76 (d, 1H, *J* = 3.89 Hz, H-C(thioph)), 7.60 (d, 1H, *J* = 3.89 Hz, H-C(thioph)), 7.31 (m, 2H, H-C(3′, 5′)), 6.53 (d, 1H, *J* = 2.29 Hz, H-C(2)), 6.46 (dd, *J* = 8.69 and 2.29 Hz, 1H, H-C(6)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 162.1, 161.4, 161.2, 160.1, 156.4, 144.5, 131.4, 130.7, 129.5, 128.9, 127.8, 127.7, 124.9, 116.3, 116.1, 108.5, 108.2, 102.3; IR (ATR, cm^{-1}): 3157 (OH), 3097 (C_{AR}-H), 2917 (CH), 2850 (CH), 1636 (C=N), 1601, 1547 (C=C), 1468, 1445, 1411, 1352, 1249, 1176 (C-O), 1098, 967, 912, 874, 823, 792, 681 (C-S-C); EI-MS (m/z, %): 370 (M^+, 100), 234 (8), 221 (7), 203 (9), 167 (8), 153 (4), 133 (6), 69 (5).

*4-{5-[5-(4-Bromophenyl)thiophen-2-yl]-1,3,4-thiadiazol-2-yl} benzene-1,3-diol (**12**)*

A mixture of 5-(4-bromophenyl)thiophene-2-carboxylic acid hydrazide (0.01mol) (Aldrich) and STB (0.01 mol) in methanol (40 mL) was refluxed (3 h). The removed solid was filtered and washed with water. Recrystallization from MeOH (40 mL) afforded **12**.

Yield: 62%; HPLC: log k = 0.899; m.p.: 253–254°C; anal. calc. for $C_{18}H_{11}BrN_2O_2S_2$ (431.33): C, 50.12; H, 2.57; N, 6.49; found: C, 50.30; H, 2.59; N, 6.44; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.21 (s, 1H, HO-C(3)), 10.12 (s, 1H, HO-C(1)), 8.06 (d, 1H, *J* = 8.70 Hz, H-C(5)), 7.78 (d, 1H, *J* = 3.91 Hz, H-C(thioph)), 7.73 (m, 2H, H-C(2′, 6′), 7.67 (m, 3H, H-C(3′, 5′, thioph)), 6.55 (d, 1H, *J* = 2.26 Hz, H-C(2)), 6.46 (dd, *J* = 8.70 and 2.27 Hz, 1H, H-C(6)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 162.1, 161.4, 159.0, 156.3, 144.4, 132.2 (2C), 132.1, 131.8, 130.8, 128.9, 127.6 (2C), 125.6, 121.6, 108.4, 108.2, 102.3; IR (ATR, cm^{-1}): 3352 (OH), 2946 (C_{AR}-H), 2835 (CH), 1660, 1630 (C=N), 1603, 1529 (C=C), 1462, 1450, 1415, 1226, 1035, 685 (C-S-C); EI-MS (m/z, %): 431 (M^+, 20), 430 (100), 297 (12), 295 (11), 283 (13), 281 (10), 266 (5), 265 (21), 264 (5), 263 (17), 216 (6), 199 (4), 172 (5), 171 (5), 167 (30), 158 (7), 153 (11), 140 (10), 135 (4), 107 (4), 69 (10), 40 (10).

*4-(5-Benzyl-1,3,4-thiadiazol-2-yl)-6-chlorobenzene-1,3-diol (**13**)*

A mixture of phenylacetic hydrazide (0.016 mol) (Aldrich) and SClTB (0.012 mol) in methanol (80 mL) was refluxed (3 h). The removed solid was filtered, washed with water. Recrystallization from MeOH/H_2O (3:1) (10 mL) afforded **13**.

Yield: 60%; HPLC: log k = 0.087; m.p.: 189–190°C; anal. calc. for $C_{15}H_{11}ClN_2O_2S$ (318.78): C, 56.52; H, 3.48; N, 8.79; found: C, 56.59; H, 3.46; N, 8.76; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.31 (s, 1H, HO-C(3)), 10.76 (s, 1H, HO-C(1)), 8.05 (s, 1H, H-C(5)), 7.35 (d, 4H, *J* = 4.42 Hz, H-C(Ar′)), 7.27 (m, 1H, H-C(Ar′)), 6.68 (s, 1H, C(2)), 4.45 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 168.6, 162.1, 156.1, 154.6, 138.0, 128.9 (2C), 128.8 (2C), 127.8, 127.1, 111.8, 109.5, 103.6, 34.7 (CH_2); IR (ATR, cm^{-1}): 3100 (OH), 3028 (C_{Ar}-H), 2870, 2851 (CH), 1625 (C=N), 1604, 1587 (C=C), 1480, 1467, 1442, 1426, 1407, 1391, 1384, 1371, 1352, 1296, 1256, 1223, 1191 (C-O), 1162, 1095, 971, 871, 843 (C_{Ar}-H), 805, 730, 700, 678 (C-S-C); EI-MS (m/z, %): 318 (M^+, 59), 317 (10), 169 (9), 150 (12), 149 (100), 148 (6), 141 (5), 122 (22), 121 (9), 117 (7), 116 (9), 91 (37), 89 (7), 78 (5), 69 (7), 65 (12), 63 (5), 51 (10), 39 (7).

*4-Chloro-6-(5-(4-fluorophenylamino)-1,3,4-thiadiazol-2-yl) benzene-1,3-diol (**14**)*

A mixture of 4-fluorophenyl-3-thiosemicarbazide (0.003 mol) (Alfa Aesar) and SClTB (0.002 mol) in methanol (25 mL) was refluxed (3 h). The removed solid was filtered and washed with water. Recrystallization from MeOH (10 mL) afforded **14**.

Yield: 64%; HPLC: log k = 0.146; m.p.: 261–262°C; anal. calc. for $C_{14}H_9ClFN_3O_2S$ (337.76): C, 49.78; H, 2.69; N, 12.44; found: C, 49.84; H, 2.71; N, 12.38; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.03 (s, 1H, HO-C(1)), 10.64 (s, 1H, HO-C(3)), 10.27 (s, 1H, NH), 7.92 (s, 1H, H-C(5)), 7.66 (m, 2H, C_{AR}-H), 7.19 (m, 2H, C_{Ar}-H), 6.67 (s, 1H, H-C(2)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 163.6, 160.4, 157.9, 155.7, 154.8, 137.4, 128.5, 108.9, 108.8, 115.7, 115.5, 111.6, 109.9, 103.6; IR (ATR, cm^{-1}): 3374 (OH), 3020 (H-Ar), 1617 (C=N), 1567 (C=C), 1444, 1432, 1411, 1240, 1211, 1164 (C-O), 1059 (N=C-S-C=N), 1010, 988, 975, 942, 912, 828, 801, 726, 674 (C-S-C), 612, 587. EI-MS (m/z, %): 337 (M^+, 100), 336 (9), 189 (6), 187 (13), 184 (5), 170 (8), 169 (18), 168 (65), 155 (8), 154 (8), 141 (20), 136 (19), 130 (8), 128 (20), 121 (5), 114 (9), 110 (16), 100 (15), 95 (20), 83 (20), 75 (14), 69 (15), 63 (5), 57 (6), 51 (8), 40 (16), 39 (5).

*4-Chloro-6-(5-(3-chlorophenylamino)-1,3,4-thiadiazol-2-yl) benzene-1,3-diol (**15**)*

A mixture of 4-(3-chlorophenyl-3-thiosemicarbazide (0.01 mol) (Alfa Aesar) and SClTB (0.01 mol) in methanol (50 mL) was refluxed (3 h). The hot mixture was filtered and water (50 mL) was added to the filtrate. The formed solid was filtered off and crystallized from methanol (4:1) (50 mL). The formed solid was boiled in chloroform (30 mL) and filtered. Recrystallization from MeOH/H_2O (1:1) (50 mL) afforded **15**.

Yield: 60%; HPLC: log k = 0.392; m.p.: 300–302°C; anal. calc. for $C_{14}H_9C_{12}O_2S$ (354.21): C,47.47; H, 2.56; N, 11.86; found: C, 47.58; H, 2.54; N, 11.89; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.10 (s, 1H, HO-C(1)), 10.68 (s, 1H,

HO-C(3)), 10.49 (s, 1H, NH), 7.98 (m, 1H, H-C(5)), 7.95 (t, 1H, *J* = 1.93 Hz, H-C(2')), 7.47 (m, 1H, H-C(6')), 7.37 (t, 1H, *J* = 8.10 Hz, H-C(5')), 7.04 (m, 1H, H-C(4')), 6.70 (s, 1H, H-C(2)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 163.9, 155.4, 153.9, 152.9, 142.1, 133.5, 130.6, 127.3, 121.0, 116.6, 115.7, 111.7, 109.8, 103.6; IR (ATR, cm^{-1}): 3369 (OH), 3018 (Ar-H), 1619 (C=N), 1569 (C=C), 1440, 1438, 1421, 1235, 1220, 1168 (C-O), 1057 (N=C-S-C=N), 1009, 997, 985, 937, 922, 838, 810, 731, 664 (C-S-C), 620, 614, 594, 578; EI-MS (m/z, %): 354 (M$^+$, 25), 353 (100), 321 (5), 319 (14), 189 (5), 187 (14), 184 (69), 171 (7), 170 (10), 169 (11), 159 (12), 157 (10), 155 (5), 152 (7), 149 (23), 130 (5), 128 (14), 127 (5), 122 (7), 113 (5), 111 (12), 100 (9), 90 (4), 69 (7).

*4-Chloro-6-(5-(3,4-dihydroxyphenyl)-1,3,4-thiadiazol-2-yl) benzene-1,3-diol (**16**)*

A mixture of 3,4-dihydroxybenzhydrazide (0.015 mol) (Alfa Aesar) and SClTB (0.015 mol) in methanol (75 mL) was refluxed (3 h). The hot mixture was filtered, water (40 mL) was added to the filtrate and it was left at room temperature (24 h). Recrystallization from MeOH (40 mL) afforded 1**6**.

Yield: 62%; HPLC: log k = 0.11; m.p.: 280–282°C; anal. calc. for $C_{14}H_9ClN_2O_4S$ (336.75): C, 49.93; H, 2.69; N, 8.32; found: C, 50.03; H, 2.68; N, 8.36; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.30 (s, 1H, HO-C(1)), 10.83 (s, 1H, HO-C(3)), 9.58 (s, 1H, HO-C(3')), 9.41 (s, 1H, HO-C(4')), 8.10 (s, 1H, C(5)), 7.43 (d, *J* = 2.18 Hz, 1H, H-C(2')), 7.26 (dd, *J* = 8.18 Hz, *J* = 2.16 Hz, 1H, H-C(6')), 6.87 (d, *J* = 8.20, 1H, H-C(5')), 6.77 (s, 1H, H-C(2)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 166.6, 160.2, 156.4, 154.8, 148.5, 145.8, 127.7, 121.4, 119.5, 116.3, 114.1, 111.8, 109.5, 104.0; IR (ATR, cm^{-1}): 3350 (OH), 3093 (CH), 2851 (CH), 1626 (C=N), 1605 (C=C), 1502 (C=C), 1461, 1450, 1400, 1385, 1319, 1297, 1280, 1253, 1231, 1187 (C-O), 1157, 1110, 978, 903, 875, 852, 827, 791, 721, 684 (C-S-C); EI-MS (m/z, %): 336 (M$^+$, 100), 303 (8), 302 (8), 201 (19), 187 (6), 168 (6), 167 (18), 153 (12), 136 (5), 135 (9), 121 (4), 63 (4).

*3-{5-[4-(Dimethylamino)phenyl]-1,3,4-thiadiazol-2-yl} biphenyl-2,3',4,5',6-pentol (**17**)*

A mixture of 4-dimethylaminobenzhydrazide (0.014 mol) (Aldrich) and 2,3',4,5',6-pentahydroxybiphenyl-3-carbodithioic acid (0.014 mol) in methanol (70 mL) was refluxed (2.5 h). The removed compound was filtered and washed with water. Recrystallization from MeOH (40 mL) afforded **17**.

Yield: 65%; HPLC: log k = -0.26; m.p.: 236–238°C; anal. calc. for $C_{22}H_{19}N_3O_5S$ (437.47): C, 60.40; H, 4.38; N, 9.61; found: C, 60.54; H, 4.36; N, 9.57;^{1}H NMR (500 MHz, DMSO-d_6, δ): 12.66 (s, 1H, HO), 11.33 (s, 1H, HO), 9.80 (s, 1H, HO), 8.98 (s, 2H, HO), 7.82 (d, *J* = 8.90 Hz, 2H, H-C(Ar)), 6.82 (d, *J* = 9.00 Hz, 2H, H-C(Ar)), 6.26 (s, 1H, H-C(Ar)), 6.16 (d, *J* = 2.18 Hz, 2H, H-C(Ar)), 6.12 (t, *J* = 2.15 Hz, 1H, H-C(Ar)), 3.01 (s, 6H, CH_3); IR (ATR, cm^{-1}): 3200 (OH), 2930 (Ar-H), 1640, 1630 (C=N), 1605 (C=C), 1483, 1468, 1461, 1443, 1426, 1413, 1390, 1371, 1334, 1249, 1231, 1200, 1156 (C-O), 1070, 1043 (N=C-S-C=N), 1006, 945, 883, 806, 689 (C-S-C); EI-MS (m/z, %): 437 (M$^+$, 25), 219 (35), 178 (6), 177 (18), 164 (18), 163 (7), 150 (9), 148 (17), 147 (12), 146 (72), 145 (100), 132 (9), 131 (9), 129 (11), 121 (9), 120 (11), 104 (7), 102 (13), 91 (5), 77 (7), 51 (6), 45 (13), 44 (23), 42 (11), 39 (6), 36 (27), 34 (11).

Compounds: 4-(5-heptyl-1,3,4-thiadiazol-2-yl) benzene-1,3-diol (**2**), 4,4'-{[5,5'-(benzene-1,4-diyl)] bis(1,3,4-thiadiazol-2-yl)}di(benzene-1,3-diol) (**7**) and 4-(2,4-dichlorophenoxymethyl)benzene-1,3-diol (**9**) were prepared according to the procedure already described[19,20].

Measurement of ChEs activities

Acetylcholinesterase (AChE, E.C. 3.1.1.7, from the electric eel), butylcholinesterase (BuChE, E.C. 3.1.1.8, from equine serum), acetylthiocholine iodide (ATCh), butylthiocholine iodide (BTCh), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), neostigmine bromide and donepezil hydrochloride monohydrate were purchased from Sigma-Aldrich (Steinheim, Germany). The inhibitory activities against AChE and BuChE of the prepared compounds were performed by means of the method previously developed by Ellman et al., using donepezil and neostigmine as the reference compounds[21]. This is based on the reaction of released thiocholine to give a coloured product with a chromogenic reagent. Seven different concentrations of the synthesized compounds in the range 10^{-3}–10^{-9} M were measured at 412 nm. All the assays were under 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH = 8) using a Varian Cary 50 Spectrophotometer. Enzyme solutions were prepared to give 2 units/mL in 2 mL aliquots.

The assay medium contained phosphate buffer, pH 8.0 (1 mL), 50 µL of 0.01 M DTNB, 10 µL of enzyme, 50 µL of acetylthiocholine iodide (ATCh) and 50 µL of the test compound solution. ATCh was added to the assay medium after 10 min of incubation time. The activity was determined by measuring the increase in absorbance at 412 nm for 1 min interval at 37 ± 0.2°C. For determining the blank value, additionally 50 µL buffer replaced the enzyme solution. *In vitro* the BuChE assay uses the similar method to that described above.

Each concentration was analyzed in triplicate. The 50% inhibitory concentration (IC_{50}) was calculated from a dose–response curve obtained by plotting the percentage of inhibition versus the log concentration with the use of GraFit 4.09 software[22]. The results were expressed as the mean ± standard deviation (SD).

Results and discussion

5-Substituted (1,3,4-thiadiazol-2-yl)benzene-1,3-diols were obtained by the reaction of the commercially available hydrazides or thiosemicarbazides with sulfinylbis[(2,4-dihydroxyphenyl)methanethione] (STB) or with its analogue sulfinylbis[(5-chloro-2,4-dihydroxyphenyl)methanethione] (SClTB) in methanol under reflux (1.5–4 h) in moderate to good yields (60–89%) as outlined in Figure 1. The key intermediates were

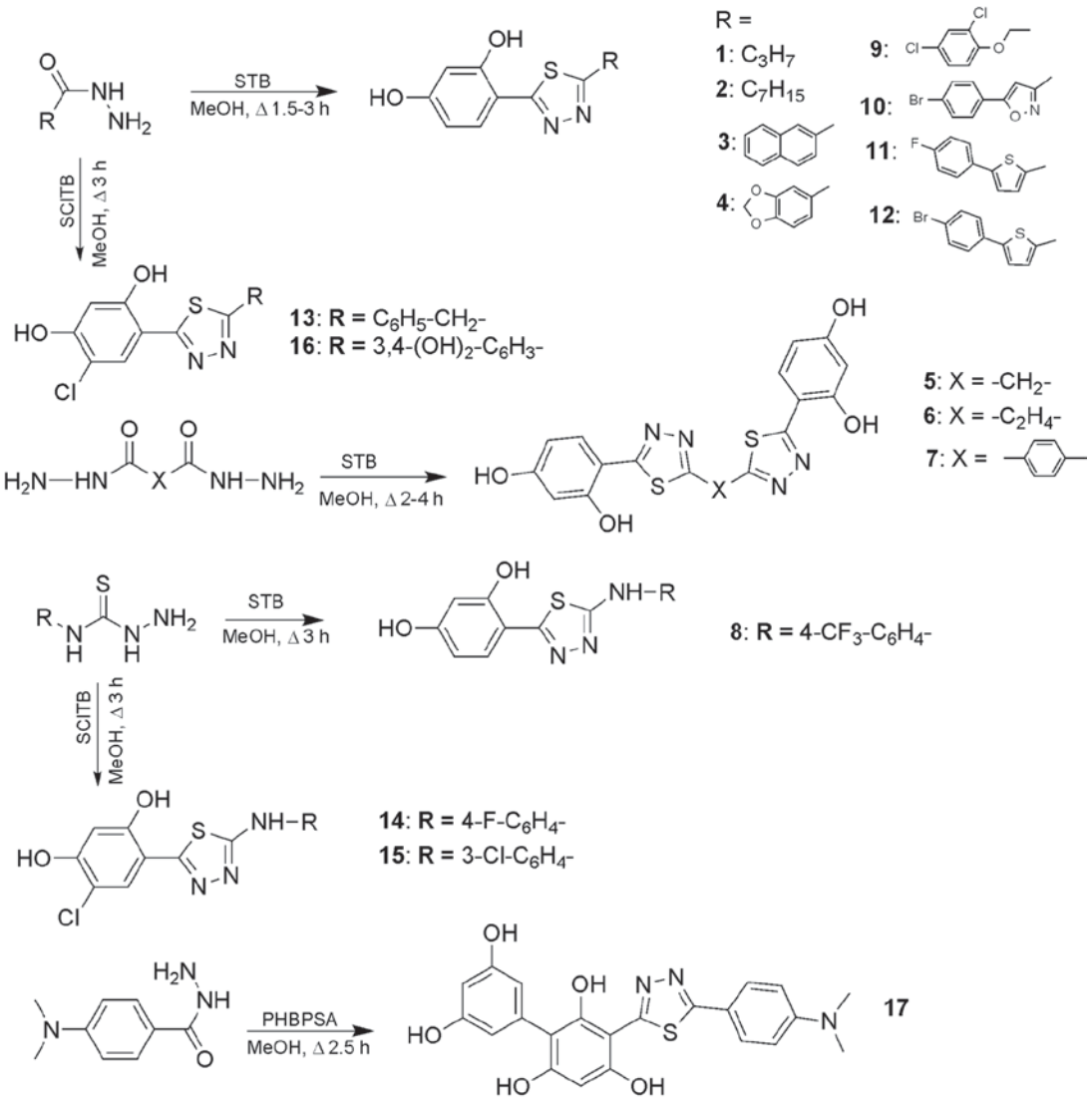

Figure 1. Synthesis scheme of (1,3,4-thiadiazol-2-yl)benzene-1,3-diol derivatives.

obtained from 2,4-dihydroxybenzenecarbodithioic acids and $SOCl_2$ in diethyl ether[23]. The substituents panel of compounds is shown in Figure 1. Purity of compounds was monitored by the reversed-phase (RP-18) HPLC chromatography (methanol–water).

All new compounds showed analytical and spectroscopic data in good accordance with the proposed structure. In the ^{1}H NMR spectra the OH protons are usually detected as broad bands in the range *ca.* 11 and 10 ppm. The resonance signals of aromatic protons of the β-resorcinol moiety appear as two characteristic *dublets* at *ca.* 8 ppm (J = 8.7 Hz) and 6.5 ppm (J = 2.3 Hz) corresponding to H-C(5) and H-C(2), respectively and as *doublet* of *doublets* at *ca.* 6.45 ppm (J = 8.7 and 2.3 Hz) of H-C(6). In the case of additional substitution in position 5 by chlorine atom protons of that ring appear as two characteristic *singlets* at *ca.* 6.7 and 8 ppm corresponding to H-C(2) and H-C(5), respectively[13–16]. *Singlet* is registered for H-C(isoxazole) at 7.88 ppm in the case of compound **10**. Protons of 2,5-disubstituted thiophene appeared as two *doublet*s at *ca.* 7.77 and 7.63 ppm with J = 3.9 Hz[11,12]. In the ^{13}C NMR spectrum characteristic signals of substituted 1,3,4-thiadiazole ring carbon atoms appear in the range 169-162 ppm.

In the IR spectrum there are strong bands in the region about 3400–3100 corresponding to ν (O-H) and additionally ν (N-H) in the case of compounds **8**, **14**, **15**. Band of ν (C=N) appears in the region 1635–1610 cm^{-1}.

The mass spectra (EI) of the compounds show molecular ion peaks, however, with various intensities. The major fragmentation pathway of 4-(1,3,4-thiadiazol-2-yl) benzene-1,3-diols involves the cleavage of the C(2)-N(3) and S-C(5) bonds 1,3,4-thiadiazole ring with the formation of $(OH)_2C_6H_3CS^+$ (m/z 153) ion. The cleavage of S-C(2) and N-N bonds which directs to $(OH)_2C_6H_3CN^+$ (m/z 135) fragmentation is also observed. Characteristic

relatively strong band corresponding to tropylic cation $C_7H_7^+$ (m/z 91) is observed for benzyl derivative[13]. It is the effect of α and β atoms disconnection in relation to the phenyl ring.

All compounds have been evaluated as AChE and BuChE inhibitors. Their inhibitory potency was expressed as the half of maximal inhibitory concentration, IC_{50}. It was determined by the modified Ellman's method[21]. Donepezil and neostigmine were used as the reference drugs. Table 1 shows that most of the studied compounds were able to inhibit *in vitro* both enzymes – AChE and BuChE but in a very broad range of concentrations. The IC_{50} values for AChE are ranged from > 500 to 0.053 μM and for BuChE from > 500 to 0.105 μM. At the same it proves that all synthesized particles are significantly more active towards AChE than BuChE, with the exception of compounds **5** and **14**. Compound **9** of the highest activity against AChE shows inhibition effect similar to that of neostigmine. Simultaneously it is 947-fold more active against AChE compared to BuChE. That compound can serve as a selective inhibition agent for AChE over BuChE.

To find the influence of the type of 1,3,4-thiadiazole ring substitution on potency of compounds the structure-activity analyses SAR have been performed. To obtain better results some previously described compounds were included in the biological screening. SAR shows that alkyl derivatives (compounds **1** and **2**) are good inhibitors of both enzymes. Compound **2** with the heptyl substituent is one of the most active derivatives. From the group of compounds with two 1,3,4-thiadiazole rings[5,6,7] compound **7** with benzene as a link shows the highest affinity for AChE. Derivative **9** with the 2,4-dichlorophenoxymethyl substituent is the most active against AChE of all compounds of 1,3,4-thiadiazole series. The presence of the additional heterocyclic ring (oxazolyl, thiophenyl) does not improve activities of compounds. Placement of chlorine atom in the benzenodiole moiety does not change substantially affinity of compounds for both enzymes[13–16]. The analogs possessing naphthalenyl[3] or biphenylyl[12] substituent are inactive.

The linear Lineweaver-Burk equation which is a double reciprocal form of the Michaelis-Menten equation was used to evaluate the type of inhibition. The graphical analysis of steady-state inhibition data for representative compounds **2** and **7** is shown in Figure 2A and 2B, respectively. In Figure 2A the graph shows that the mechanism of AChE inhibition of compound **2** is the mixed-type. In Figure 2B the lines crossing the *x* axis in the same point indicate unchanged K_M and decreased V_{max} with the increasing inhibitor concentrations. This is a typical trend of non-competitive inhibition which is similar to that of donepezil[4].

Table 1. *In vitro* inhibition (IC_{50}, μM) and selectivity of the studied compounds on AChE and BuChE.

No.	IC_{50} for AChE* [μM]	IC_{50} for BuChE** [μM]	Selectivity for AChE***
1.	0.109 ± 0.02	18.2 ± 0.1	166.9
2.	0.060 ± 0.003	0.24 ± 0.02	4.0
3.	>500	>500	—
4.	0.22 ± 0.01	25.1 ± 0.02	114.1
5.	0.26 ± 0.02	0.184 ± 0.01	0.71
6.	3.11 ± 0.06	14.3 ± 0.20	4.59
7.	0.092 ± 0.004	15.21 ± 0.42	165.3
8.	3.24 ± 0.01	1.32 ± 0.01	0.41
9.	0.053 ± 0.003	50.20 ± 0.20	947.2
10.	45.2 ± 0.52	193.4 ± 1.13	4.3
11.	37.2 ± 0.31	> 500	>13.44
12.	0.125 ± 0.01	18.6 ± 0.23	148.8
13.	18.0 ± 0.14	44.2 ± 0.72	2.45
14.	0.143 ± 0.02	0.105 ± 0.01	0.73
15.	2.68 ± 0.02	>500	186.5
16.	1.27 ± 0.01	35.20 ± 0.24	27.72
17.	>500	>500	—
neostigmine	0.05 ± 0.007	0.07 ± 0.009	1.4
donepezil	0.02 ± 0.008	7.52 ± 0.20	376

*IC_{50}: 50% inhibitory concentration (means ± SD of three experiments) of AChE.
**IC_{50}: 50% inhibitory concentration (means ± SD of three experiments) of BuChE.
***Selectivity for AChE = IC_{50} (BuChE)/IC_{50} (AChE).

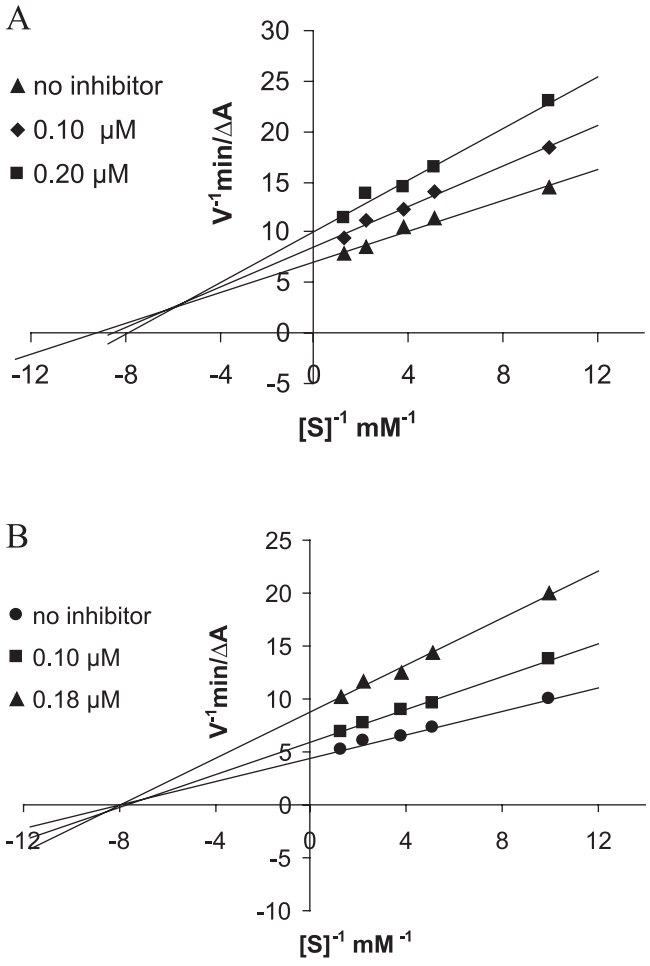

Figure 2. Steady state inhibition of **2** (A) and **7** (B) against AChE. Plot A shows the mixed-type inhibition and plot B the non-competitive inhibition.

Conclusion

In conclusion, we synthesized new (1,3,4-thiadiazol-2-yl) benzene-1,3-diol derivatives and evaluated their anticholinesterase activities. The results indicate that compound **9** is the most potent inhibitor of AChE being 947-fold more active against AChE than BuChE. That compound can serve as a selective inhibition agent. Whereas the heptyl derivative shows comparable high affinity for both enzymes in the low nM range. The kinetic studies suggest that in a series of the investigated compounds, inhibition mechanisms can be various. The obtained results could be useful for designing of new AChE and BuChE inhibitors, resulting in greater selectivity as well as the increasing inhibitory potency.

Declaration of interest

The authors declared no conflict of interest.

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