Synthesis and biological evaluation of 1,3,4-thiadiazole analogues as novel AChE and BuChE inhibitors

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · JANUARY 2013

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2012.12.060 · Source: PubMed

5 AUTHORS, INCLUDING:

Alicja Skrzypek
University of Life Sciences in Lublin
8 PUBLICATIONS **28** CITATIONS
SEE PROFILE

Joanna Matysiak
University of Life Sciences in Lublin
70 PUBLICATIONS **780** CITATIONS
SEE PROFILE

Paweł Szymański
Medical University of Łódź
29 PUBLICATIONS **143** CITATIONS
SEE PROFILE

European Journal of Medicinal Chemistry 62 (2013) 311–319

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of 1,3,4-thiadiazole analogues as novel AChE and BuChE inhibitors

Alicja Skrzypek [a], Joanna Matysiak [a,*], Andrzej Niewiadomy [a,b], Marek Bajda [c], Paweł Szymański [d]

[a] Department of Chemistry, University of Life Sciences, Akademicka 15, 20-950 Lublin, Poland
[b] Institute of Industrial Organic Chemistry, Annopol 6, 03-236 Warszawa, Poland
[c] Department of Physicochemical Drug Analysis, Chair of Pharmaceutical Chemistry, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland
[d] Department of Pharmaceutical Chemistry and Drug Analyses, Medical University, Muszyńskiego 1, 90-151 Łódź, Poland

ARTICLE INFO

Article history:
Received 8 September 2012
Received in revised form
19 November 2012
Accepted 22 December 2012
Available online 11 January 2013

Keywords:
1,3,4-Thiadiazole
Acetylcholinesterase
Butyrylcholinesterase
Inhibitor
Molecular docking
In silico pharmacokinetics

ABSTRACT

In this paper a series of new 1,3,4-thiadiazole derivatives has been designed, synthesized and evaluated as the acetyl- and butyrylcholinesterase inhibitors. Some analogues showed promising inhibition of both enzymes *in vitro* in the nM range. Generally, inhibitory potency of compounds was stronger against AChE than BuChE, and one of them was 1154-fold more active inhibiting AChE (IC_{50} = 0.17 μM) than BuChE. The kinetic studies showed that one of the most active analogues **8** (IC_{50} = 0.09 μM, AChE) acted as a non-competitive AChE inhibitor and was characterized by the high selectivity index (300). The other derivative (**1**) exhibited a mixed-type of AChE inhibition. Docking simulations enabled the detection of key binding interactions of the compounds with AChE and revealed that they occupied mainly the catalytic active site. The scoring function for the novel compounds was similar or higher than for the reference inhibitor. Additionally, based on Lipinski and other filters, the drug-likeness of compounds was assessed. They revealed that the compounds possess properties which can suggest the favourable pharmacokinetics in the human body after oral admission.

1. Introduction

Alzheimer's disease (AD) is a progressive degeneration of the central nervous system, characterised by a loss of memory and reduced ability to perform basic everyday activities of daily living. Based on the cholinergic hypothesis, the mainstays of current pharmacotherapy of AD are drugs aimed at increasing the levels of acetylcholine (ACh) through the inhibition of cholinesterases (ChEs) [1–3]. The studies have shown that AChE performs secondary noncholinergic functions. It colocalizes with the β-amyloid peptide (Aβ) deposit present in the brain of Alzheimer's patients. It is postulated that AChE binds to Aβ and induces a conformational transition from Aβ into its amyloidogenic form *in vitro* [4,5].

Two types of the ChE enzymes are found in the nervous system – acetylcholinesterase (AChE, E.C. 3.1.1.7) and butyrylcholinesterase (BuChE, E.C. 3.1.1.8). Both enzymes are able to hydrolyse ACh, but AChE has a 10^{13} – fold higher hydrolytic ACh activity than BuChE under the same conditions. In the normal brain AChE predominates over the BuChE activity but it is reported that BuChE has a key role that can partly compensate for the action of AChE.

Cholinesterase inhibitors have been approved as efficacious treatment to reduce the symptoms of early medium stage of AD. Several antiacetylcholinesterase agents such as donepezil [6], tacrine [7], galantamine [8], and ensaculin [9] have shown to induce modest improvement in memory and cognitive functions. Unfortunately, the potential effectiveness offered by these inhibitors is often limited by the appearance of central and peripheral side effects. For example, clinical studies have shown that tacrine has hepatotoxic liability [10,11]. A large diversity of multi-target-directed AChE inhibitors of tacrine and nimodipine hybrids has been also evaluated [12,13].

Recent research aims at new types of compounds as potential AChE and BuChE inhibitors. To synthesize more metabolically stable cholinomimetic ligands, it points out to the possibility of replacing the ester group with five-membered rings like thiadiazoles, triazoles, tetrazoles as well as oxadiazoles. Some scientists have studied widely 1,3,4-thiadiazole derivatives as potential drugs to treat AD [14,15]. The thiadiazole ring can act as the "hydrogen binding domain", "two-electron donor system" as well as it enables to create π–π stocking interactions.

3-(Thiadiazolyl)pyridine 1-oxide derivatives possessing the antioxidant and muscarinic receptor binding properties were

* Corresponding author. Tel.: +48 81 4456816; fax: +48 81 5333549.
E-mail address: joanna.matysiak@up.lublin.pl (J. Matysiak).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.12.060

reported as potential acetylcholinesterase inhibitors [16]. Sarkandi et al. described 1-benzyl-4-[2-(5-phenyl-1,3,4-thiadiazole-2-yl) aminoethyl]piperidines [17]. Recently, the thiadiazolidin-3,5-dione (TDZD) analogues have been reported as the first non-ATP competitive inhibitors of glycogen synthase kinase 3β (GSK-3β). This is one of the most attractive molecular targets for the development of effective inhibitors of AD treatment [18].

Recently 1,3,4-thiadiazole based compounds have been obtained and tested by our team [19]. Some of them acted as strong AChE and BuChE inhibitors with the IC_{50} values in the nM ranges. The other derivatives were highly selective towards AChE, exhibiting selectivity ratios of *ca.* 950. Behavioural studies performed on mice revealed considerable analgesic activity and anxiolytic effect of some 4-(1,3,4-thiadiazol-2-yl)benzene-1,3-diols. At the same time none of the studied derivatives displayed any neurotoxic activity and their LD_{50} values were in the range of 1000–2500 mg/kg of body weight (unpublished results). These studies can suggest that the compounds under consideration can cross the blood–brain-barrier. Additionally, cytotoxic studies performed on normal and cancer cells confirmed relatively low toxicity of (1,3,4-thiadiazol-2-yl)benzene-1,3-diol set. One of them showed even neuoroprotective properties [20]. This encouraged us to explore (1,3,4-thiadiazol-2-yl)benzene-1,3-diols as potential AD drugs.

The synthesis and biological evaluation of a new series of 4-(5-phenyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol derivatives as the AChE and BuChE inhibitors are discussed in the paper. To gain more insights into the molecular determinants responsible for the observed ability to inhibit the AChE activity, a modelling study was undertaken through molecular docking of the most active compounds. Recently the attention has been paid to optimization of the pharmacokinetics of the substance at a very early stage of its research as a potential drug. Therefore, based on the molecule structure, the number of descriptors necessary for its estimation was determined. Lipinski and other drug-likeness filters were used to predict pharmacokinetics of the obtained compounds [21–24].

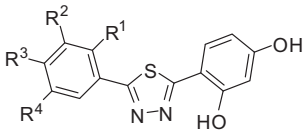
2. Chemistry

5-Phenyl(modified)-(1,3,4-thiadiazol-2-yl)benzene-1,3-diols were formed by the reaction of the commercially available hydrazides with sulfinylbis[(2,4-dihydroxyphenyl)methanethione] (**STB**) in methanol under reflux (3–4 h) as presented in Scheme 1. The yields of processes were in the range of 64–84%. The starting reagent STB was prepared from 2,4-dihydroxybenzenecarbodithioic acid and $SOCl_2$ in ethoxyethane [25]. In the reaction the electrophilic substrate STB also acts as an endogeneous cyclising reagent. Purity of the obtained compounds was checked by the reversed-phase (RP-18) HPLC chromatography using the MeOH–H_2O mixture as a mobile phase. The structures of the obtained compounds are shown in Table 1. Their analytical and spectroscopic data are in accordance with the predicted structures.

In the ^{1}H NMR spectra the resonance signals of hydroxyl groups protons are usually registered as broad *singlets* in the range *ca.* 11.1 and 10.1 ppm. The aromatic protons of the benzenediol substituent are detected as two *doublets* at 8.1 ppm ($J = 8.7$ Hz) and 6.6–6.5 ppm ($J = 2.3$ Hz) corresponding to H–C(5) and H–C(2) respectively. The third proton appears as *doublets* of *doublets* in the range of 6.5 to 6.4 ppm ($J = 8.7$ and 2.3 Hz) of H–C(6). The ^{13}C NMR spectrum shows characteristic signals of aryl-substituted 1,3,4-thiadiazole ring carbon atoms in the range of 166–160 ppm [26].

The IR spectra show two strong bands in the region about 3480–3140 and 1632–1620 cm^{-1}, corresponding to ν (O–H) and ν (C=N) respectively [26]. In the mass spectra (EI), the molecular ion peaks M^+ shown that the predicted compound has formed.

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



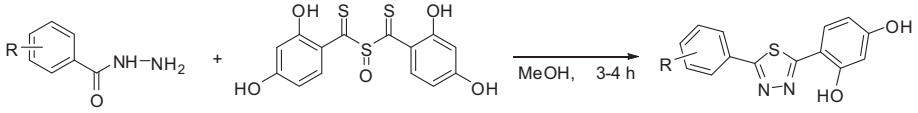
No.	R^1	R^2	R^3	R^4	IC_{50} for AChE[a] [μM]	IC_{50} for BuChE[b] [μM]	Selectivity for AChE[c]
1.	H	Me	H	H	0.16 ± 0.01	17.02 ± 0.40	106.4
2.	F	H	H	H	1.07 ± 0.02	>500	>467
3.	Cl	H	H	H	1.55 ± 0.02	196.24 ± 7.00	126.6
4.	Br	H	H	H	0.06 ± 0.003	0.29 ± 0.04	4.8
5.	Cl	H	Cl	H	128.42 ± 7.13	>500	>3.9
6.	Cl	H	H	Cl	112.01 ± 43.42	19.06 ± 0.92	0.17
7.	H	Cl	H	Cl	45.26 ± 0.50	>500	>11.1
8.	H	H	CF_3	H	0.09 ± 0.004	26.49 ± 1.22	294.3
9.	OMe	H	H	H	26.70 ± 0.21	212.38 ± 6.13	7.9
10.	H	OMe	H	H	1.87 ± 0.11	27.32 ± 0.30	25.5
11.	H	H	OMe	H	2.18 ± 0.20	96.34 ± 0.31	44.2
12.	H	OMe	OMe	H	19.34 ± 0.52	>500	>25.9
13.	OMe	H	H	OMe	1.56 ± 0.08	63.28 ± 0.05	40.5
14.	H	OMe	OMe	OMe	0.17 ± 0.01	196.21 ± 7.22	1154.2
15.	OH	H	OH	H	1.89 ± 0.04	26.74 ± 0.90	14.2
16.	H	OH	OH	H	0.19 ± 0.05	2.16 ± 0.20	11.4
17.	H	OMe	OH	H	19.21 ± 1.00	112.41 ± 2.33	5.9
18.	H	NH_2	H	H	1.73 ± 0.23	84.32 ± 1.00	48.7
19.	NO_2	H	H	H	2.12 ± 0.08	57.32 ± 0.74	27.0
20.	H	NO_2	H	H	0.67 ± 0.04	32.14 ± 0.81	47.9
21.	H	Me	NO_2	H	20.86 ± 0.91	147.62 ± 4.31	7.1
Neostigmine					0.05 ± 0.007	0.07 ± 0.009	1.4
Donepezil					0.02 ± 0.008	7.52 ± 0.20	376

[a] IC_{50}: 50% inhibitory concentration (means ± SD of three independent experiments) of AChE.
[b] IC_{50}: 50% inhibitory concentration (means ± SD of three experiments) of BuChE.
[c] Selectivity for AChE = IC_{50}(BuChE)/IC_{50}(AChE).

3. Biological results and discussion

3.1. Inhibition studies of AChE and BuChE

All considered compounds have been assessed as the AChE and BuChE inhibitors. Their inhibitory potency was described as the half of maximal inhibitory concentration, IC_{50}. The modified Ellman's method has been used in the evaluation [27]. Two drugs: neostigmine and donepezil were used as the standards (Table 1). The results are summarized in Table 1. They clearly show that most of the designed compounds exhibit good to moderate inhibitory activities. The IC_{50} values for AChE are ranged from 128.42 to 0.06 μM and for BuChE from >500 to 0.29 μM. At the same time all



Scheme 1. Synthesis scheme of modified 4-(5-phenyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diols.

synthesized compounds are significantly more active towards AChE than BuChE, with the exception for compound **6**. Some of the derivatives proved to be highly selective for AChE with respect to BuChE (**1**, **3**, **8**, **14**).

To find the influence of the type of substitution of the aryl ring on the potency of compounds the structure–activity analyses have been performed. To obtain better results some previously described compounds were included in the biological screening. The results show that compound **4** with the Br substituent at the *ortho*-position exhibits the most promising activity with the IC_{50} values of 0.06 and 0.29 μM against AChE and BuChE respectively. Compounds **2** and **3** with F or Cl atom in the same position are slightly weaker inhibitors. The activity of these analogues changes in the following order: Br > Cl > F. A similar trend against BuChE is observed, however, activity changes are much larger. The presence of OMe or NO_2 substituents at C(2) of the phenyl ring decreased potency of the compounds against both enzymes (**9**, **19**).

Placement of the Me group in the *meta*-position of the phenyl ring (compound **1**) resulted favourably ($IC_{50} = 0.16$ μM), suggesting the importance of the hydrophobic character for the interaction with enzyme. Compounds **10** and **18** bearing OMe or NH_2 groups ($\pi < 0$) in the same position are weaker inhibitors. High potency of the compound with the strong electron-withdrawing NO_2 substituent for AChE was also observed (**20**). In the *para*-position the presence of the hydrophobic electron-withdrawing CF_3 substituent is strongly preferable to the hydrophilic electron donating OMe substituent.

Analysing the compounds with the OMe substituents (**9**–**14**), it can be found that the analogue with three MeO groups in 3, 4, 5 positions (compound **14**) shows higher potency compared to less substituted ones **9**–**13**. Different electronic effects of the OMe groups depending on their position, hydrophobic character of the flexible group as well as the presence of O atoms as the potential proton acceptors in the hydrogen bond formation could enable access to the enzyme-binding site.

Interposition of 3-Me and 4-NO_2 substituents on the phenyl ring (compound **21**) led to a significant decrease in activity ($IC_{50} = 20.86$ μM) as compared with **1**. Also compounds with two Cl atoms (**5**, **6**, **7**) are weaker inhibitors of both enzymes. In the case of analogue **6** the inversion of the affinity is observed (Table 1). Similarly to tacrine it has a small selectivity for AChE over BuChE [28].

It is interesting that compounds **8** and **14** are much weaker inhibitors against BuChE compared with AChE, indicating that these compounds can serve as selective inhibition agents for AChE. Particularly **14** is 1154-fold more active inhibiting AChE not BuChE, being slightly more selective than donepezil and neostigmine.

3.2. Molecular modelling

Molecular modelling studies were performed to find a possible binding mode of the most active compounds (**4**, **8**, **14**) with AChE. The structure of enzyme was obtained from the Protein Data Bank [29]. The 1ACJ complex was selected for docking according to the validation process. All compounds were bound mainly with the catalytic active site. Interactions with a peripheral anionic site were reduced.

The derivatives were located between the residues Trp84 and Phe330. The central part of each molecule – the thiadiazole ring was parallel to the indole moiety of Trp84, creating π–π stacking interactions (distance 3.1–3.2 Å for each compound). The two hydroxyl substituents in compound **4** created the following hydrogen bonds: the first one in position 4 with the amine group of Gly117 (2.7 Å) and with the hydroxyl substituent of Tyr130 (2.5 Å), the second one in position 2 formed H-bond with Glu199 (2.3 Å) (Fig. 1). The bromine atom was directed to the conserved water

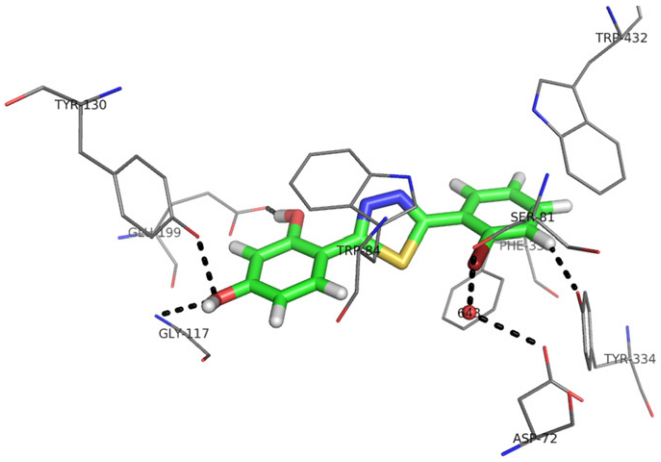


Fig. 1. Binding mode of compound **4** with AChE.

molecule (WAT643), thus phenyl ring could form π–π stacking and π–CH interactions with the aromatic side chains of Phe330 and Trp432. The interaction CH–oxygen atom from the hydroxyl substituent in Tyr334 (2.2 Å) was also observed in the case of this ring.

Compound **8** due to the lack of bromine atom and introduction of CF_3 was slightly shifted (Fig. 2). It caused a few changes in binding. The OH group in position 4 created H-bond with the OH substituent of Tyr130 (3.2 Å) and =C(=O) from Gly117 (2.2 Å), the second hydroxyl substituent still formed the hydrogen bond with Glu199 (2.9 Å). The thiadiazole moiety was rotated and nitrogen atoms were directed towards the conserved water (WAT 643) (3.1 and 3.5 Å). Phenyl substituted by the CF_3 group formed two kinds of interactions: π–π with Phe330 (3.6 Å), CH–oxygen atom from OH Tyr334 (2.8 Å). Trifluoromethyl could create hydrophobic interactions with Trp432.

For compound **14** (Fig. 3) the thiadiazole moiety was placed in the same way as in the case of compound **8** but the outermost phenyl rings were replaced with each other. Trimethoxyphenyl was orientated towards Ser122 and Gly123. The oxygen atom from the 3-CH_3O group created the hydrogen bond with OH Ser122 (3.5 Å) and oxygen from 4-CH_3O with NH of Gly123 (2.6 Å). The HO–C(2) substituent from the second phenyl ring formed H-bond with the carbonyl group of His440 (2.6 Å), 4-hydroxy group interacted with π electrons of Trp432 (3.5 Å). The phenyl ring was engaged in π–π stacking with Phe330 (3.6 Å) and CH–OH interactions with Tyr334

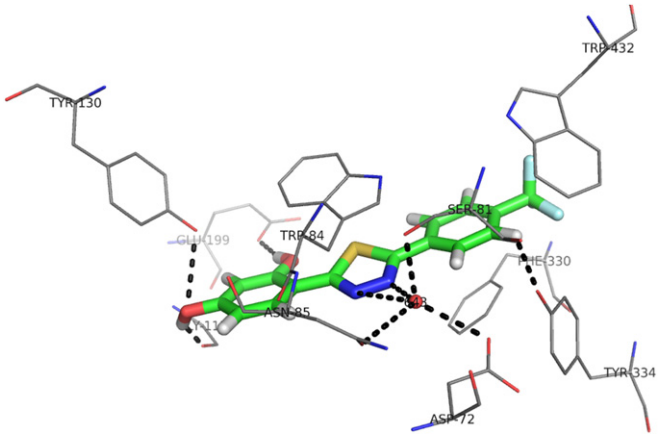


Fig. 2. Binding mode of compound **8** with AChE.

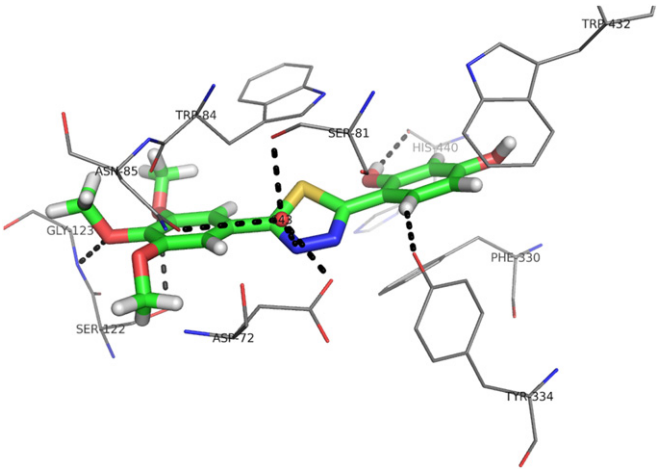


Fig. 3. Binding mode of compound **14** with AChE.

(2.5 Å). These differences in binding of compounds **4**, **8** and **14** could be the reason for differences in activity.

The scoring function for the novel compounds is similar or higher than for the reference inhibitor – tacrine (GoldScore: 67.95; IC_{50} 180 nM) [30]. Two most active compounds **4** and **8** obtained 76.48 and 69.17 (GoldScore) and are more potent than the reference (IC_{50} 60 nM and 90 nM, respectively).

3.3. Kinetic studies

The linear Lineweaver–Burk equation which is a double reciprocal form of the Michaelis–Menten one was converted to evaluate the type of inhibition. The graphical analysis of steady-state inhibition data for representative compounds **1** and **8** is shown in Fig. 4A and B, respectively. In Fig. 4A the graph shows that the mechanism of AChE inhibition of compound **1** is of the mixed-type.

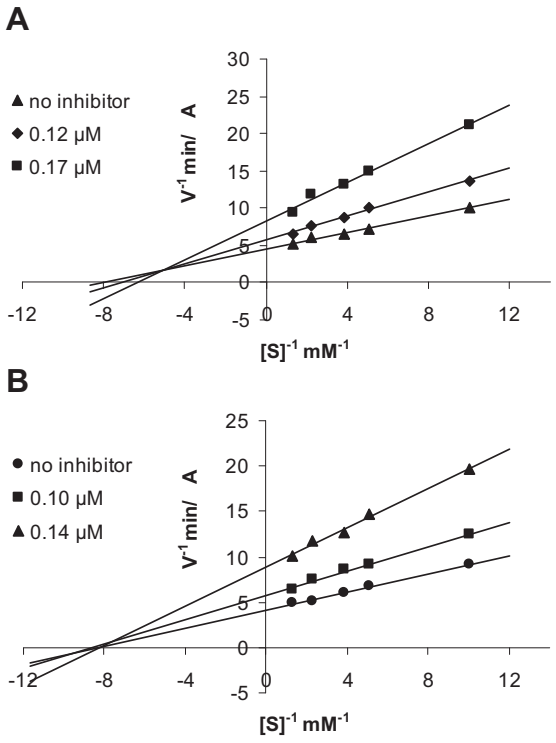


Fig. 4. Steady state inhibition of **1** (A) and **8** (B) against AChE. The plot A shows mixed-type inhibition and the plot B non-competitive inhibition.

In Fig. 4B the lines crossing the *x* axis in the same point indicate unchanged K_M and decrease V_{max} with the increasing inhibitor concentrations. This is a typical trend of non-competitive inhibition which is similar to that of donepezil [31].

Docking simulation showed that compounds bound mainly with the catalytic active site of AChE creating with them a lot of interactions significant for activity. The studies revealed that the compounds interact in a different way depending on the type of aryl ring modification. Only 1,3,4-thiadiazole ring occupies the same space but its orientation and type of interactions are also different. This can explain significant differences in the activity, selectivity as well as different types of inhibitions of the studied analogues.

3.4. Virtual screening of pharmacokinetic properties

To predict some aspects of the pharmacokinetics of the obtained compounds, their physicochemical and topological properties were calculated. Table 2 presents the octanol–water partition coefficients expressed as Clog *P* and Mlog *P* (Moriguchi log *P*), a number of H-bond donors (HBD), a number of H-bond acceptors (HBA), a number of rotatable bonds (RBC), and the polar surface area (tPSA).

The descriptors obtained *in silico* were compared with the filters for prediction of solubility and permeability of drug candidates after the oral admission. The results show that the compounds under consideration obey the Lipinski rule of 5: their MW $\leq$ 500, HBD $\leq$ 5, HBA $\leq$ 10 and Clog *P* $\leq$ 5 (Table 2) [21,22]. The molecular weight MW of compounds is found between 284 and 360 Da, their Clog *P* is in the range of 1.49–3.97, they possess 2-4 H-bond donors and 4-7 H-bond acceptors. All properties are of medium value. According to Lipinski a compound that fails the alert will likely to be poorly bioavailable because of poor absorption or permeation [21].

The derivatives also meet the Oprea's criterions, which additionally includes a number of ring $\leq$5 and MLog *P* in the range −2.0 to 4.5. The compounds possess 2 rings and their Mlog *P* values are in the range of 0.64–2,71 [23]. A filter of two properties is proposed by Veber: a number of HBA and HBD $\leq$ 12 (tPSA $\leq$ 140 Å^2), RBC $\leq$ 10. It is postulated that limited molecular flexibility, expressed as the number of rotatable bonds (RBC), and low polar

Table 2
Molecular descriptors[a] *in silico* of (1,3,4-thiadiazol-2-yl)benzene-1,3-diols.

No.	HBD	HBA	Clog *P*	Mlog *P*	tPSA [Å^2]	RBC
1.	2	4	3.04	2.46	65.18	2
2.	2	4	2.65	2.33	65.18	2
3.	2	4	3.01	2.46	65.18	2
4.	2	4	3.11	2.59	65.18	2
5.	2	4	3.72	2.71	65.18	2
6.	2	4	3.72	2.71	65.18	2
7.	2	4	3.97	2.71	65.18	2
8.	2	4	3.43	2.83	65.18	3
9.	2	5	2.04	1.67	74.41	3
10.	2	5	2.60	1.67	74.41	3
11.	2	5	2.60	1.67	74.41	3
12.	2	6	2.32	1.15	83.64	4
13.	2	6	2.11	1.15	83.64	4
14.	2	7	2.04	0.65	92.87	5
15.	4	6	1.49	0.64	105.64	2
16.	4	6	1.86	0.64	105.64	2
17.	3	6	1.99	0.90	96.64	3
18.	4	5	1.59	1.42	91.20	2
19.	2	6	2.29	1.98	116.99	3
20.	2	6	2.29	1.98	116.99	3
21.	2	6	2.71	2.24	116.99	3

[a] Clog *P* – the octanol–water partition coefficient, Mlog *P* – Moriguchi log *P*, HBD – a number of H-bond donors, HBA – a number of H-bond acceptors, tPSA – the polar surface area, RBC – a number of rotatable bonds.

surface are important predictors of oral bioavailability, independent of molecular weight [24].

The results show that the synthesized compounds would have favourable pharmacokinetics: solubility and permeability after the oral admission as drug candidates. They are drug likeliness independent of the criterion used.

4. Conclusions

In summary, a series of new 1,3,4-thiadiazole analogues has been synthesized and evaluated as the AChE and BuChE inhibitors. Compounds **4** and **8** (IC_{50} = 0.06 and 0.09 μM respectively) are the most potent ones in the series. At the same time they show strong affinity for BuChE but compound **14** is 1154-fold more active inhibiting AChE (IC_{50} = 0.17 μM) than BuChE. It is slightly more selective than donepezil and neostigmine. That compound can serve as a selective inhibition agent for AChE over BuChE. The kinetic studies suggest that in a series of the investigated compounds, the inhibition mechanisms can be various.

Docking simulation showed that the compounds are bound mainly with the catalytic active site of AChE. The scoring function for the novel compounds is similar or higher than for the reference inhibitor. Modelled derivatives **4**, **8** and **14** create a lot of interactions with the catalytic active site of AChE which confirms their high inhibitory potency. However, the compounds locate in different ways depending on the type of aryl ring modification. Particularly a molecule of compound **14** docs otherwise, probably due to a large volume of trimetoxyphenyl substituent. This can cause a poor fit and week interactions with the BuChE residues and its low inhibition. It can explain very high selectivity of **14** towards AChE.

The analysis of adequate descriptors revealed that the compounds possess properties which can suggest favourable pharmacokinetics in the human body after the oral admission. They are drug likeliness independent of the criterion used.

5. Experimental section

5.1. Chemistry

The IR spectra were measured with a Perkin–Elmer FT-IR 1725X spectrophotometer (in KBr) or a Varian 670-IR 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 a Varian Mercury 400 or a Bruker DRX 500 instrument. Chemical shifts (δ, ppm) were described in relation to tetramethylsilane (TMS). The spectra MS (EI, 70 eV) were recorded using the apparatus AMD-604. Elemental analyses (C, H, N) were performed using a Perkin–Elmer 2400 instrument 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 HPLC 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 MeOH 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_o) was determined by the injection of a small amount of acetone dissolved in water. The log k values for 70% of methanol (v/v) in the mobile phase are presented. The log k values were calculated as $\log k = \log(t_R - t_o)/t_o$, where: t_R = the retention time of a solute, t_o = the retention time of an unretained solute.

5.2. Synthesis of compounds

5.2.1. A general procedure for the synthesis of compounds

A mixture of the corresponding hydrazide (0.01 mol) and STB (0.0075 mol) in MeOH (50 mL) was refluxed for 3 h. The hot mixture was filtered. Water (50 mL) was added to the filtrate and the filtrate was left at room temperature (48 h) (compounds **1**, **9**–**11**, **14** and 18) or the filtrate was concentrated (compounds **3**, **5**–**8**, **12**, **13**, **16**, **19**, **21**). The obtained solid was recrystallized from MeOH/H_2O.

5.2.2. 4-[5-(3-Methylphenyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**1**)

Yield: 74%; pale yellow needles; HPLC: (C-18): log k = 0.308; m.p.: 234–236 °C; anal. calc. for $C_{15}H_{12}N_2O_2S$ (284.06): C, 63.36; H, 4.25; N, 9.85; found: C, 63.42; H, 4.23; N, 9.89; ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 11.14 (s, 1H, HO–C(3)), 10.10 (s, 1H, HO–C(3)), 8.08 (d, J = 8.73 Hz, 1H, HO–C(5)), 7.83 (m, 2H, HO–C(2′,4′)), 7.45 (t, J = 7.55 Hz, 1H, H–C(5′)), 7.36 (m, 1H, H–C(6′)), 6.53 (d, J = 2.18 Hz, 1H, HO–C(2)), 6.48 (dd, J = 8.74 and 2.36 Hz, 1H, HO–C(6)), 2.41 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 166.2, 162.6, 161.3, 156.3, 138.8, 131.3, 130.1, 129.2, 128.9, 127.7, 124.4, 108.4, 108.3, 102.3, 20.8 (CH_3); IR (KBr, cm^{-1}): 3138 (OH), 1632 (C=N), 1598, 1528, 1471, 1443, 1318 (C=C), 1281, 1262, 1214, 1175 (C–O), 1141, 1095 (H–Ar), 1041 (N=C–S–C=N), 986, 966, 877, 846, 778 (H–Ar), 684, 665 (C–S–C), 596, 522; EI-MS (m/z, %): 284 (M^+, 100), 168 (4), 167 (38), 153 (6), 150 (3), 149 (15), 142 (4), 135 (13), 119 (5), 118 (4), 117 (5), 116 (4), 107 (5), 91 (9), 80 (3), 65 (4).

5.2.3. 4-[5-(2-Chlorophenyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**3**)

Yield: 68%; yellow needles; HPLC (C-18): log k = 0.175; m.p.: 268–269 °C; anal. calc. for $C_{14}H_9ClN_2O_2S$ (304.75): C, 55.18; H, 2.98; N, 9.19; found: C, 55.06; H, 2.99; N, 9.21; ^1H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.17 (s, 1H, HO–C(3)), 10.14 (br.s., 1H, HO–C(1)), 8.17 (m, 1H, H–C(3′)), 8.14 (d, J = 8.70 Hz, 1H, H–C(5)), 7.69 (m, 1H, H–C(6′)), 7.55 (m, 2H, H–C(4′,5′)), 6.56 (d, J = 2.32 Hz, 1H, H–C(2)), 6.51 (dd, J = 8.70 and 2.33 Hz, 1H, H–C(6)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.2, 161.8, 161.4, 156.4, 131.8, 131.3, 130.9, 130.6, 129.0, 128.9, 127.8, 108.4, 108.1, 102.4; IR (KBr, cm^{-1}): 3186 (OH), 1629 (C=N), 1598 (C=C), 1522 (C=C), 1473 (C=C), 1417, 1314, 1230, 1183 (C–O), 1068 (N=C–S–C=N), 1042, 995, 982, 965, 845 (H–Ar), 798, 757, 733, 714, 681 (C–S–C), 648, 632; EI-MS (m/z, %): 304 (M^+, 100), 171 (6), 169 (19), 168 (7), 167 (78), 157 (8), 156 (5), 155 (24), 153 (12), 140 (4), 139 (10), 138 (8), 137 (14), 136 (3), 135 (18), 134 (10), 125 (5), 111 (15), 108 (10), 107 (19), 106 (8), 102 (13), 97 (8), 95 (5), 90 (4), 84 (5), 80 (13), 79 (7), 76 (6), 75 (15), 69 (17), 66 (5), 65 (7), 63 (11), 62 (6), 53 (6), 52 (24), 51 (18), 50 (10), 45 (7), 39 (22), 38 (5).

5.2.4. 4-[5-(2,4-Dichlorophenyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**5**)

Yield: 67%; orange needles; HPLC (C-18): log k = 0.533; m.p.: 272–273 °C; anal. calc. for $C_{14}H_8Cl_2N_2O_2S$ (339.20): C, 49.57; H, 2.38; N, 8.26; found: C, 49.66; H, 2.36; N, 8.23; ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 11.21 (s, 1H, HO–C(3)), 10.15 (s, 1H, HO–C(1)), 8.22 (dd, J = 8.56 and 0.28 Hz, 1H, H–C(5′)), 8.14 (d, J = 8.71 Hz, 1H, H–C(5)), 7.89 (dd, J = 2.11 and 0.27 Hz, 1H, H–C(3′)), 7.65 (dd, J = 8.57 and 2.11 Hz, 1H, H–C(6′)), 6.54 (d, J = 2.20 Hz, 1H, H–C(2)), 6.41 (dd, J = 8.71 and 2.29 Hz, 1H, H–C(6)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.3, 161.5, 160.0, 156.4, 135.6, 132.2, 132.0, 130.0, 128.9, 128.4, 128.1, 108.5, 108.1, 102.3; IR (KBr, cm^{-1}): 3350, 3196 (OH), 1630 (C=N), 1595 (C=N), 1524 (C=N), 1478 (C=N), 1411, 1341, 1315, 1246, 1246, 1177 (C–O), 1142, 1110 (C–Cl), 1067 (N=C–S–C=N), 994, 966, 947, 815 (H–Ar), 745, 684, 668 (C–S–C), 648, 614; EI-MS (m/z, %): 338 (M^+, 100), 205 (7), 203 (10), 191 (5),

189 (7), 171 (5), 169 (6), 168 (7), 167 (65), 153 (8), 135 (8), 119 (6), 107 (7), 80 (4), 69 (5), 52 (6), 39 (4).

5.2.5. 4-[5-(2,5-Dichlorophenyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**6**)

Yield: 69%; orange needles; HPLC (C-18): log k = 0.530; m.p.: 250–252 °C; anal. calc. for $C_{14}H_8Cl_2N_2O_2S$ (339.20): C, 49.57; H, 2.38; N, 8.26; found: C, 49.62; H, 2.39; N, 8.30; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.18 (s, 1H, HO–C(3)), 10.11 (s, 1H, HO–C(1)), 8.20 (d, J = 2.61 Hz, 1H, H–C(6′)), 8.13 (d, J = 8.67 Hz, 1H, H–C(5)), 7.74 (d, J = 8.65 Hz, 1H, H–C(3′)), 7.64 (dd, J = 8.64 and 2.63 Hz, 1H, H–C(4′)), 6.52 (d, J = 2.29 Hz, 1H, H–C(2)), 6.47 (dd, J = 8.69 and 2.31 Hz, 1H, H–C(6)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.5, 161.5, 160.58, 156.5, 132.8, 132.3, 131.3, 130.7, 130.0, 129,9, 128.9, 108.5, 108.1, 102.3; IR (KBr, cm^{-1}): 3372 (OH), 3065 (C_{Ar}–H), 1621 (C=N), 1590 (C=C), 1519 (C=C), 1473 (C=C), 1402, 1339, 1314, 1291, 1259, 1218, 1184 (C–O), 1141, 1105 (C–Cl), 1062 (N=C–S–C=N), 1015, 986, 970, 881, 844 (H–Ar), 825, 763, 742, 721, 683 (C–S–C), 658, 638; EI-MS (m/z, %): 338 (M^+, 100), 205 (8), 203 (12), 191 (7), 189 (11), 173 (8), 171 (11), 169 (7), 168 (10), 167 (77), 154 (4), 153 (12), 136 (5), 135 (13), 133 (5), 124 (4), 119 (10), 112 (5), 109 (4), 108 (6), 107 (11), 100 (4), 97 (4), 80 (8), 69 (9), 52 (10), 51 (6), 39 (7).

5.2.6. 4-[5-(3,5-Dichlorophenyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**7**)

Yield: 66%; orange needles; HPLC (C-18): log k = 0.781; m.p.: 273–275 °C; anal. calc. for $C_{14}H_8Cl_2N_2O_2S$ (339.20): C, 49.57; H, 2.38; N, 8.26; found: C, 49.65; H, 2.40; N, 8.31; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.21 (s, 1H, HO–C(1)), 10.12 (s, 1H, HO–C(3)), 8.11 (d, J = 8.71 Hz, 1H, H–C(5)), 8.02 (d, J = 1.93 Hz, 2H, H–C(2′, 6′)), 7.78 (t, J = 1.89 Hz, 1H, H–C(4′)), 6.53 (d, J = 2.31 Hz, 1H, H–C(2)), 6.48 (dd, J = 8.70 and 2.31 Hz, 1H, H–C(6)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 163.7, 163.3, 161.6, 156.5, 135.0 (2C), 133.4, 129.7, 128.9, 135.5 (2C), 108.5, 108.1, 102.3; IR (ATR, cm^{-1}): 3200 (OH), 3077 (H–Ar), 2917 (C–H), 2850 (C–H), 1626 (C=N), 1606 (C=C), 1590 (C=C), 1567 (C=C), 1523 (C=C), 1486, 1417, 1330, 1278, 1261, 1193 (C–O), 1124, 1110 (C–Cl), 1060 (N=C–S–C=N), 988, 872, 855, 816 (H–Ar), 765, 673 (C–S–C); EI-MS (m/z, %): 338 (M^+, 100), 205 (13), 203 (19), 191 (7), 189 (9), 173 (7), 171 (10), 170 (4), 169 (7), 168 (10), 167 (74), 153 (13), 145 (5), 135 (18), 133 (4), 119 (11), 112 (5), 109 (5), 108 (7), 107 (13), 106 (5), 97 (5), 80 (9), 79 (4), 69 (9), 52 (12), 51 (6), 39 (17), 38 (6).

5.2.7. 4-[5-(4-Trifluoromethylphenyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**8**)

Yield: 64%; orange needles; HPLC (C-18): log k = 0.494; m.p.: 252–254 °C; anal. calc. for $C_{15}H_9F_3N_2O_2S$ (338.30): C, 53.25; H, 2.68; N, 8.28; found: C, 53.30; H, 2.65; N, 8.30; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.20 (s, 1H, HO–C(1)), 10.11 (s, 1H, HO–C(3)), 9.23 (d, J = 8.11 Hz, 2H, H–C(3′,5′)), 8.10 (d, J = 8.69 Hz, 1H, H–C(5)), 7.90 (d, J = 8.32 Hz, 2H, H–C(2′,6′)), 6.54 (d, J = 2.30 Hz, 1H, H–C(2)), 6.46 (dd, J = 8.70 and 2.31 Hz, 1H, H–C(6)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.1, 163.4, 161.6, 156.5, 134.0, 129.0, 128.9, 128.0, 125.1 (CF_3), 126.3, 126.2, 125.0, 108.4, 108.2, 102.3; IR (KBr, cm^{-1}): 3452, 3202 (OH), 1631 (C=N), 1600, 1523, 1473 (C=C), 1413, 1320 (CF_3), 1235, 1170 (C–O), 1141 (C_{AR}–CF_3), 1115, 1067 (N=C–S–C=N), 1012, 996, 985, 964, 843, 829, 799, 671 (C–S–C); EI-MS (m/z, %): 338 (M^+, 100), 319 (9), 309 (4), 203 (20), 189 (14), 171 (4), 169 (4), 167 (47), 153 (13), 152 (6), 145 (12), 139 (8), 135 (15), 121 (7), 119 (9), 112 (5), 108 (7), 107 (14), 106 (5), 95 (7), 80 (90), 69 (11), 51 (8), 39 (11).

5.2.8. 4-(5-(2-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**9**)

Yield: 70%; yellow needles; HPLC (C-18): log k = −0.301; m.p.: 218–220 °C; anal. calc. for $C_{15}H_{12}N_2O_3S$ (300.33): C, 59.99; H, 4.03; N, 9.33; found: C, 59.91; H, 4.01; N, 9.36; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 10.22 (s, 1H, HO–C(3)), 9.20 (s, 1H, HO–C(1)), 7.52 (dd, J = 7.53 and 1.71 Hz, 1H, H–C(6′)), 7.24 (d, J = 8.64 Hz, 1H, H–C(5)), 6.75 (m, 1H, H–C(4′)), 6.49 (m, 1H, H–C(5′)), 6.37 (m, 1H, H–C(3′)), 5.69 (d, J = 2.32 Hz, 1H, H–C(2)), 5.66 (dd, J = 8.64 and 2.34 Hz, 1H, H–C(6)), 3.21 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.4, 161.0, 160.2, 156.4, 155.5, 131.9, 129.1, 127.6, 121.1, 118.9, 112.4, 108.5, 108.3, 102.5, 56.1 (OCH_3); IR (KBr, cm^{-1}): 3410 (OH), 2935 (C–H), 2846 (Ar–OCH_3), 1628 (C=N), 1601 (C=C), 1519 (C=C), 1468 (C=C), 1428, 1320, 1257 (C–O–C), 1170 (C–O), 1136, 1059 (N=C–S–C=N), 1019, 990, 967, 848, 799, 754 (H–Ar), 693 (C–S–C), 658, 632, 601, 527; EI-MS (m/z, %): 300 (M^+, 24), 284 (7), 256 (73), 167 (7), 166 (6), 165 (9), 153 (12), 149 (15), 148 (7), 142 (5), 137 (45), 136 (21), 135 (100), 132 (6), 78 (5), 77 (29), 71 (5), 69 (9), 64 (7), 63 (9), 57 (5), 53 (5), 52 (7), 51 (11), 50 (6), 43 (5), 41 (5), 39 (11), 36 (5).

5.2.9. 4-(5-(3-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**10**)

Yield: 78%; orange needles; HPLC (C-18): log k = 0.242; m.p.: 145–147 °C; anal. calc. for $C_{15}H_{12}N_2O_3S$ (300.33): C, 59.99; H, 4.03; N, 9.33; found: C, 59.94; H, 4.01; N, 9.38; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.14 (s, 1H, HO–C(3)), 10.10 (s, 1H, HO–C(1)), 8.09 (d, J = 8.67 Hz, 1H, H–C(5)), 7.58 (m, 1H, H–C(4′)), 7.54 (m, 1H, H–C(2′)), 7.45 (t, J = 8.04 Hz, 1H, H–C(5′)), 7.13 (m, 1H, H–C(6′)), 6.55 (d, J = 2.37 Hz, 1H, H–C(2)), 6.50 (dd, J = 8.68 and 2.33 Hz, 1H, H–C(6)), 3.87 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 165.0, 163.0, 161.4, 159.7, 156.5, 131.5, 130.6, 129.0, 119.9, 116.6, 111.9, 108.4, 108.3, 102.5, 55.4 (OCH_3); IR (KBr, cm^{-1}): 3626, 3128 (OH), 2834 (Ar–OCH_3), 1604 (C=N), 1580 (C=C), 1455 (C=C), 1408, 1382, 1295, 1270 (C–O–C), 1218, 1198, 1173 (C–O), 1107 (Ar–H), 1016 (N=C–S–C=N), 976, 885, 873, 807, 792, 774 (Ar–H), 682 (C–S–C), 644; EI-MS (m/z, %): 300 (M^+, 100), 299 (4), 271 (4), 168 (4), 167 (40), 165 (11), 153 (6), 151 (10), 150 (5), 136 (6), 135 (13), 134 (5), 133 (11), 122 (6), 119 (6), 112 (6), 108 (8), 107 (8), 103 (5), 90 (4), 80 (5), 77 (8), 69 (5), 64 (4), 63 (6), 52 (8), 51 (5), 39 (7).

5.2.10. 4-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**11**)

Yield: 72%; pale orange needles; HPLC (C-18): log k = 0.193; m.p.: 218–220 °C; anal. calc. for $C_{15}H_{12}N_2O_3S$ (300.33): C, 59.99; H, 4.03; N, 9.33; found: C, 60.06; H, 4.01; N, 9.29; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.07 (s, 1H, HO–C(3)), 10.06 (s, 1H, HO–C(1)), 8.05 (d, J = 8.69 Hz, 1H, H–C(5)), 7.95 (m, 2H, H–C(2′, 6′)), 7.11 (m, 2H, H–C(3′, 5′)), 6.53 (d, J = 2.34 Hz, 1H, H–C(2)), 6.48 (dd, J = 8.66 and 2.34 Hz, 1H, H–C(6)), 3.84 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 165.8, 162.1, 161.2, 161.1, 156.2, 128.9, 128.8 (2C), 122.7, 114.7 (2C), 108.4, 108.3, 102.4, 55.4 (OCH_3); IR (KBr, cm^{-1}): 3848, 3110 (OH), 2837 (C–OCH_3), 2360, 1606 (C=N), 1521 (C=C), 1455 (C=C), 1417, 1309, 1257 (C–O–C), 1178 (C_{Ar}–O), 1137, 1173 (C–O), 1029 (N=C–S–C=N), 981, 963, 885, 834, 801 (Ar–H), 744, 683, 669 (C–S–C), 641; EI-MS (m/z, %): 300 (M^+, 100), 284 (10), 168 (4), 167 (42), 165 (13), 160 (5), 153 (10), 151 (18), 150 (23), 137 (7), 136 (5), 135 (21), 134 (12), 133 (14), 122 (8), 120 (5), 119 (7), 108 (6), 107 (8), 90 (5), 80 (5), 77 (5), 69 (5), 63 (6).

5.2.11. 4-(5-(3,4-Dimethoxyphenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**12**)

Yield: 73%; yellow plaques; HPLC (C-18): log k = −0.012; m.p.: 214–216 °C; anal. calc. for $C_{16}H_{14}N_2O_4S$ (330.36): C, 58.17; H, 4.27; N, 8.48; found: C, 58.15; H, 4.29; N, 8.50; ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 11.07 (s, 1H, HO–C(1)), 10.07 (s, 1H, HO–C(3)), 8.04 (d, J = 8.56 Hz, 1H, H–C(5)), 7.56 (d, J = 2.02 Hz, 1H, H–C(2′)), 7.53 (dd, J = 8.23 and 2.02 Hz, 1H, H–C(6′)), 7.11 (d, J = 8.56 Hz, 1H, H–C(5′)), 6.51 (d, J = 2.35 Hz, 1H, H–C(2)), 6.47 (dd, J = 8.73 and 2.35 Hz, 1H, H–C(6)), 3.88 (s, 3H, CH_3), 3.84 (s,

3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 166.0, 162.3, 161.1, 156.3, 150.9, 149.1, 128.9, 122.8, 120.8, 112.0, 109.7, 108.4, 108.3, 102.4, 55.7 (OCH_3), 55.6 (OCH_3); IR (KBr, cm^{-1}): 3478, 3151 (OH), 2943 (Ar–H), 2840 (C_{Ar}–OCH_3), 1627 (C=N), 1597 (C=C), 1522 (C=C), 1454 (C=C), 1432 (C=C), 1269 (C–O–C), 1246, 1178 (C–O), 1128, 1095 (N=C–S–C=N), 1020, 985, 967, 859, 805, 756 (Ar–H), 679, 643 (C–S–C); EI-MS (*m*/*z*, %): 330 (M^+, 100), 329 (5), 315 (12), 301 (4), 287 (9), 181 (7), 180 (5), 167 (14), 165 (7), 164 (5), 163 (14), 153 (8), 152 (4), 148 (6), 137 (4), 136 (4), 135 (6), 120 (7), 107 (4), 94 (5), 65 (4), 39 (4).

5.2.12. 4-(5-(2,5-Dimethoxyphenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**13**)

Yield: 79%; pale yellow plaques; HPLC (C-18): log k = 0.102; m.p.: 248–249 °C; anal. calc. for $C_{16}H_{14}N_2O_4S$ (330.36): C, 58.17; H, 4.27; N, 8.48; found: C, 58.23; H, 4.29; N, 11.41; ^1H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.03 (s, 1H, HO–C(3)), 10.03 (s, 1H, HO–C(1)), 8.05 (d, J = 8.64 Hz, 1H, H–C(5)), 7.87 (d, J = 3.16 Hz, 1H, H–C(6′)), 7.25 (d, J = 9.12, 1H, H–C(3′)), 7.11 (dd, J = 9.07 and 3.19 Hz, 1H, H–C(4′)), 6.50 (d, J = 2.29 Hz, 1H, H–C(2)), 6.47 (dd, J = 8.64 and 2.32 Hz, 1H, H–C(6)), 3.97 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.5, 161.0, 160.0, 156.4, 153.3, 149.9, 129.1, 119.4, 117.9, 114.0, 111.3, 108.5, 108.3, 102.4, 56.6, 55.6; IR (KBr, cm^{-1}): 3434 (OH), 3001 (Ar–H), 2938 (Ar–H), 2836 (CH_3), 1629 (C=N), 1598 (C=C), 1503 (C=C), 1465 (C=C), 1427 (C=C), 1402, 1338, 1289 (C–O–C), 1224, 1182 (C–O), 1129, 1047 (N=C–S–C=N), 1018(Ar–H), 970, 876, 846, 805, 728, 691, 669 (C–S–C), 644, 616; EI-MS (*m*/*z*, %): 330 (M^+, 100), 329 (16), 301 (15), 287 (9), 271 (7), 196 (10), 195 (49), 194 (18), 180 (13), 179 (7), 178 (11), 168 (7), 167 (46), 166 (17), 165 (14), 163 (13), 162 (23), 162 (23), 153 (28), 152 (9), 150 (9), 149 (16), 148 (27), 137 (8), 136 (17), 135 (22), 134 (21), 120 (11), 107 (9), 79 (8), 69 (7).

5.2.13. 4-(5-(3,4,5-Trimethoxyphenyl)-1,3,4-thiadiazol-2-yl) benzene-1,3-diol (**14**)

Yield: 71%; yellow needles; HPLC (C-18): log k = −0.022; m.p.: 202–204 °C; anal. calc. for $C_{17}H_{16}N_2O_5S$ (360.38): C, 56.66; H, 4.47; N, 7.77; found: C, 56.74; H, 4.45; N, 7.80; ^1H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.05 (s, 1H, HO–C(1)), 10.02 (s, 1H, HO–C(3)), 8.08 (d, J = 8.60 Hz, 1H, H–C(5)), 7.85 (d, J = 3.19 Hz, 2H, H–C(2′, 6′)), 6.50 (d, J = 2.29 Hz, 1H, H–C(2)), 6.48 (dd, J = 8.63 and 2.30 Hz, 1H, H–C(6)), 3.90 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 166.0, 162.7, 161.2, 156.3, 156.3, 153.4, 139.5, 128.9, 125.7, 108.4, 108.3, 104.6 (2C), 102.3, 60.2 (OCH_3), 56.1 (OCH_3, 2C); IR (KBr, cm^{-1}): 3408 (OH), 2934 (Ar–H), 2836 (CH_3), 1628 (C=N), 1592 (C=C), 1517 (C=C), 1467 (C=C), 1414, 1332, 1248 (C–O–C), 1172 (C–O), 1130, 1045 (N=C–S–C=N), 1002, 968, 925, 845, 804, 767, 737, 681, 650 (C–S–C), 600; EI-MS (*m*/*z*, %): 360 (M^+, 100), 347 (4), 346 (11), 345 (6), 331 (4), 317 (10), 314 (5), 302 (7), 193 (4), 180 (4), 178 (10), 167 (7), 153 (14), 152 (4), 150 (12), 135 (9), 120 (4), 118 (4).

5.2.14. 4-(5-(3,4-Dihydroxyphenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**16**)

Yield: 82%; yellow plaques; HPLC (C-18): log k = −0.293; m.p.: 296–298 °C; anal. calc. for $C_{14}H_{10}N_2O_4S$ (302.31): C, 55.62; H, 3.33; N, 9.27; found: C, 55.70; H, 3.32; N, 9.31; ^1H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.03 (s, 1H, HO–C(3)), 10.02 (s, 1H, HO–C(1)), 9.55 (br.s., 2H, HO–C(3′, 4′)), 8.00 (d, J = 8.68 Hz, 1H, H–C(5)), 7.44 (d, J = 2.14 Hz, 1H, H–C(2′)), 7.28 (dd, J = 8.2 and 2.21 Hz, 1H, H–C(6′)), 6.88 (d, J = 8.19 Hz, 1H, H–C(5′)), 6.51 (d, J = 2.29 Hz, 1H, H–C(2)), 6.47 (dd, J = 8.66 and 2.3 Hz, 1H, H–C(6)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 166.4, 161.8, 161.0, 156.2, 148.2, 145.8, 128.9, 121.4, 119.5, 116.2, 113.9, 108.4, 108.3, 102.4; IR (KBr, cm^{-1}): 3392 (OH), 1631 (C=N), 1530 (C=C), 1440 (C=C), 1335, 1292, 1228, 1185 (C–O), 1141, 1020 (N=C–S–C=N), 987, 969, 886, 884 (Ar–H), 805, 772, 745, 728, 686 (C–S–C), 644; EI-MS (*m*/*z*, %): 302 (M^+, 100), 169 (6), 168 (9), 167 (67), 153 (39), 138 (5), 137 (5), 136 (10), 135 (34), 121 (11), 119 (8), 112 (5), 108 (7), 107 (13), 106 (10), 97 (7), 89 (6), 81 (7), 80 (10), 79 (9), 75 (7), 69 (13), 65 (7), 63 (17), 62 (7), 55 (6), 53 (7), 52 (21), 51 (18), 50 (7), 39 (17), 38 (6).

5.2.15. 4-(5-(3-Aminophenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**18**)

Yield: 69%; yellow needles; HPLC (C-18): log k = 0.107; m.p.: 160–162 °C; anal. calc. for $C_{14}H_{11}N_3O_2S$ (285.32): C, 58.93; H, 3.89; N, 14.73; found: C, 58.98; H, 3.91; N, 14.68; ^1H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.14 (s, 1H, HO–C(3)), 10.07 (s, 1H, HO–C(1)), 8.08 (d, J = 8.68 Hz, 1H, C(5)), 7.88 (m, 2H, H–Ar), 7.61 (m, 1H, H–Ar), 6.53 (d, J = 2.34 Hz, 1H, C(2)), 6.47 (dd, J = 8.70 and 2.33 Hz, 1H, C(6)), 6.38 (s, 2H, NH_2), 6.36 (d, 1H, H–Ar); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 165.5, 161.4, 156.2, 147.5, 133.9, 130.2, 128.9, 120.8, 118.8, 112.0, 109.7, 108.5, 107.9, 102.3; IR (KBr, cm^{-1}): 3433 (OH, NH), 1622 (C=N), 1506 (C=C), 1464 (C=C), 1341, 1234 (C–O–C), 1171 (C–O), 978, 892, 843, 801, 744, 682 (C–S–C), 649; EI-MS (*m*/*z*, %): 28 (5), 258 (4), 256 (10), 192 (7), 160 (11), 149 (4), 128 (9), 110 (7), 96 (6), 91 (6), 81 (4), 80 (7), 77 (5), 76 (5), 73 (4), 69 (4), 66 (10), 64 (100), 60 (6), 57 (7), 55 (8), 51 (7), 48 (12), 45 (8), 44 (80), 43 (14), 41 (13), 40 (46), 39 (12), 38 (6), 36 (15), 34 (4).

5.2.16. 4-(5-(2-Nitrophenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**19**)

Yield: 64%; orange needles; HPLC (C-18): log k = −0.360; m.p.: 282–284 °C; anal. calc. for $C_{14}H_9N_3O_4S$ (315.30): C, 53.33; H, 2.88; N, 13.33; found: C, 53.41; H, 2.90; N, 13.29; ^1H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.30 (s, 1H, HO–C(3)), 10.18 (s, 1H, HO–C(1)), 8.14 (d, J = 8.80 Hz, 1H, H–C(5)), 8.09 (dd, J = 7.80 and 1.28 Hz, 1H, H–C(3′)), 7.97 (dd, J = 7.52 and 1.46 Hz, 1H, H–C(6′)), 7.85 (td, J = 7.52 and 1.46 Hz, 1H, H–C(5′)), 7.79 (td, J = 7.70 and 1.47 Hz, 1H, H–C(4′)), 6.55 (d, J = 2.20 Hz, 1H, H–C(2)), 6.48 (dd, J = 8.70 and 2.33 Hz, 1H, H–C(6)); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.1, 161.6, 161.1, 156.5, 148.5, 133.1, 132.0, 131.6, 128.9, 124.6, 123.4, 108.5, 108.1, 102.3; IR (KBr, cm^{-1}): 3443, 3143 (OH), 3039 (Ar–H), 1606 (C=N), 1529, 1466 (C=C), 1411, 1368, 1350, 1317, 1291 (NO_2), 1216 (C–O), 1109, 1042 (N=C–S–C=N), 984, 852, 812, 771, 745, 719, 684 (C–S–C), 648; EI-MS (*m*/*z*, %): 315 (M^+, 49), 155 (5), 154 (9), 153 (100), 150 (3), 135 (7), 134 (12), 108 (4), 107 (4), 104 (9), 97 (4), 90 (4), 76 (4), 69 (3).

5.2.17. 4-(5-(3-Methyl-4-nitrophenyl)-1,3,4-thiadiazol-2-yl) benzene-1,3-diol (**21**)

Yield: 84%; yellow needles; HPLC (C-18): log k = 0.193; m.p.: 295–296 °C; anal. calc. for $C_{15}H_{11}N_3O_4S$ (329.33): C, 54.71; H, 3.37; N, 12.76; found: C, 54.62; H, 3.39; N, 12.81; ^1H NMR (500 MHz, DMSO-d_6, ppm) δ: 11.22 (s, 1H, HO–C(1)), 10.13 (s, 1H, HO–C(3)), 8.15 (m, 2H, H–Ar), 8.12 (d, J = 8.71 Hz, 1H, H–C(5)), 8.10 (m, 1H, H–Ar), 6.54 (d, J = 2.3 Hz, 1H, C(2)), 6.48 (dd, J = 8.72 and 2.34 Hz, 1H, C(6)), 2.63 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.0, 163.7, 161.6, 156.6, 149.5, 134.3, 134.2, 131.2, 128.9, 125.6, 125.5, 108.5, 108.2, 102.3, 19.5 (CH_3); IR (ATR, cm^{-1}): 3358 (OH), 2947 (CH), 2835 (CH), 1633 (C=N), 1558 (C=C), 1507 (C=C), 1449, 1424, 1346, 1323, 1284, 1214, 1185 (C–O), 1112, 1023, 865; EI-MS (*m*/*z*, %): 329 (M^+, 100), 299 (9), 284 (5), 283 (8), 167 (37), 153 (20), 148 (10), 136 (5), 135 (14), 134 (7), 132 (9), 122 (7), 121 (9), 119 (7), 116 (10), 108 (7), 107 (10), 106 (5), 104 (10), 97 (6), 90 (8), 89 (13), 80 (7), 78 (5), 77 (9), 69 (9), 65 (5), 63 (11), 52 (13), 51 (10), 45 (6), 39 (13).

Compounds: 4-[5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**2**), 4-[5-(2-bromophenyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**4**), 4-[5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**15**), 4-[5-(4-hydroxy-3-methoxyphenyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**17**) and 4-[5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (**20**) were prepared according to the procedure already described [26,32,33].

5.3. Biological assay

5.3.1. In vitro AChE and BuChE inhibition assay

Acetylcholinesterase (AChE, E.C. 3.1.1.7, from the electric eel), butyrylcholinesterase (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. [27], using donepezil and neostigmine as the reference compounds. 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^{-1} 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 in the 1 min intervals at 37 °C. For determining the blank value, additionally 50 μL buffer replaced the enzyme solution. *In vitro* the BuChE assay uses a similar method to that described above.

Each concentration was analysed 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 [34]. The results were expressed as the mean ± standard deviation (SD).

5.3.2. Molecular modelling studies

The inhibitor structures were created and prepared by Corina online (Molecular Networks) and Sybyl 8.0 (Tripos). Types of atoms were checked, hydrogen atoms were added and then Gasteiger-Marsili charges were assigned. Ligands were docked to acetylcholinesterase from the 1ACJ crystal complex. Before docking with GoldSuite (CCDC) the protein was prepared. All histidine residues were protonated at Nε, the hydrogen atoms were added, a few water molecules (616, 634, 643) were retained, the others and ligands were removed and the binding site was defined as all amino acid residues within 12 Å from tacrine. A standard set of genetic algorithm with the population size 100, the number of operations 100,000 and clustering with a tolerance of 1Å were applied. As a result, 10 ligand poses, sorted by the GoldScore function value were obtained. The results were visualized by PyMOL 0.99 (DeLano Scientific LLC).

5.3.3. Kinetic studies of AChE inhibition

Kinetic characterization of AChE was performed using a reported method [35]. Different concentrations of AChE and inhibitors (**1** and **8**) were mixed in the assay buffer (pH 8.0), containing 350 μM of DTNB, 0.035 unit mL^{-1} AChE and 550 μM ATCh. The test compounds were added to the assay solution and pre-incubated with the enzyme at 37 °C for 15 min, followed by the addition of substrate. Determination of kinetic characterization of the AChE-catalysed hydrolysis of ATCh was made spectrometrically at 412 nm. A parallel control experiment was carried out without the test compound in the mixture.

5.4. Computational methods

The log *P* values and tPSA were calculated using the ChemDraw Ultra 10.0 [36] and Virtual Computational Chemistry Laboratory services [37]. The polar surface area (tPSA) was calculated by the atom-based method [38].

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.12.060. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] F. Leonetti, M. Catto, O. Nicolotti, L. Pisani, A. Cappa, A. Stefanachi, A. Carotti, Homo- and hetero-bivalent edrophonium-like ammonium salts as highly potent, dual binding site AChE inhibitors, Bioorg. Med. Chem. 16 (2008) 7450–7456.
[2] S. Akasofu, M. Kimura, I. Kosasa, K. Sawada, H. Ogura, Study of neuroprotection of donepezil, a therapy for Alzheimer's disease, Chem. Biol. Interact. 175 (2008) 222–226.
[3] R. Sheng, X. Lin, J.Y. Li, Y.K. Jiang, Z.C. Shang, Y.Z. Hu, Design, synthesis, and evaluation of 2-phenoxy-indan-1-one derivatives as acetylcholinesterase inhibitors, Bioorg. Med. Chem. Lett. 15 (2005) 3834–3837.
[4] X.-C. He, S. Feng, Z.-F. Wang, Y. Shi, S. Zheng, Y. Xia, H. Jiang, X.-C. Tang, D. Bai, Study on dual-site inhibitors of acetylcholinesterase: highly potent derivatives of bis- and bifunctional huperzine B, Bioorg. Med. Chem. 15 (2007) 1394–1408.
[5] M.C. Dinamarca, D. Weinstein, O. Monasterio, N.C. Inestrosa, The synaptic protein neuroligin-1 interacts with the amyloid β-peptide. Is there a role in Alzheimer's disease? Biochemistry 50 (2011) 8127–8137.
[6] H. Sugimoto, Y. Yamanishi, Y. Iimura, Y. Kawakami, Donepezil hydrochloride (E2020) and other acetylcholinesterase inhibitors, Curr. Med. Chem. 7 (2000) 303–339.
[7] P. Camps, X. Formosa, C. Galdeano, T. Gomez, D. Munoz-Torrero, L. Ramirez, E. Viayna, E. Gomez, N. Isambert, R. Lavilla, A. Badia, M. Victoria Clos, M. Bartolini, F. Mancini, V. Andrisano, A. Bidon-Chanal, O. Huertas, T. Dafni, F. Javier Luque, Tacrine-based dual binding site acetylcholinesterase inhibitors as potential disease-modifying anti-Alzheimer drug candidates, Chem. Biol. Interact. 187 (2010) 411–415.
[8] S. Kavanagh, M. Gaudig, B. Van Baelen, M. Adami, A. Delgado, C. Guzman, E. Jedenius, B. Schaeuble, Galantamine and behavior in Alzheimer disease: analysis of four trials, Acta Neurol. Scand. 124 (2011) 302–308.
[9] R. Hoerr, M. Noeldner, Ensaculin (KA-672 center dot HCl): a multitransmitter approach to dementia treatment, CNS Drug Rev. 8 (2002) 143–158.
[10] H. Sugimoto, The new approach in development of anti-Alzheimer's disease drugs *via* the cholinergic hypothesis, Chem. Biol. Interact. 175 (2008) 204–208.
[11] C.P. Smith, G.M. Bores, W. Petko, M. Li, D.E. Selk, D.K. Rush, F. Camacho, J.T. Winslow, R. Fishkin, D.M. Cunningham, K.M. Brooks, J. Roehr, H.B. Hartman, L. Davis, H.M. Vargas, Pharmacological activity and safety profile of P10358, a novel, orally active acetylcholinesterase inhibitor for Alzheimer's disease, J. Pharmacol. Exp. Ther. 280 (1997) 710–720.
[12] J. Marco-Contelles, R. Leon, C. de los Rios, A.G. Garcia, M.G. Lopez, M. Villarroya, New multipotent tetracyclic tacrines with neuroprotective activity, Bioorg. Med. Chem. 14 (2006) 8176–8185.
[13] C. de los Rios, J.L. Marco, M.D.C. Carreiras, P.M. Chinchon, A.G. Garcia, M. Villarroya, Novel tacrine derivatives that block neuronal calcium channels, Bioorg. Med. Chem. 10 (2002) 2077–2088.
[14] A.K.S. Gupta, H.K. Misra, Synthesis and evaluation of substituted quinazolone derivatives for antibacterial, antifungal and antiacetylcholinesterase activities, J. Pharm. Sci. 69 (1980) 1313–1317.
[15] M.D. Altintop, Z.A. Kaplancikli, A. Ozdemir, G. Turan-Zitouni, H.E. Temel, G. Akalin, Synthesis and anticholinesterase activity and cytotoxicity of novel amide derivatives, Arch. Pharm. 345 (2012) 112–116.
[16] A. Martinez, D. Alonso, A. Castro, V.J. Aran, I. Cardelus, J.E. Banos, A. Badia, Synthesis and potential muscarinic receptor binding and antioxidant properties of 3-(thiadiazolyl)pyridine 1-oxide compounds, Arch. Pharm. 332 (1999) 191–194.
[17] D.N. Sarkandi, L. Firoozpour, A. Asadipour, V. Sheibani, M.A.M. Asli, A. Davood, A. Shafiee, A. Foroumadi, Synthesis of 1-benzyl-4-2-(5-phenyl-1,3,4-thiadiazole-2-yl)aminoethyl piperidine as potential Alzheimer's disease modifying agent, Asian J. Chem. 23 (2011) 2503–2505.
[18] M. Alonso, A. Martinez, GSK-3 inhibitors: discoveries and developments, Curr. Med. Chem. 11 (2004) 755–763.
[19] A. Skrzypek, J. Matysiak, M.M. Karpińska, A. Niewiadomy, Synthesis and anticholinesterase activities of novel 1,3,4-thiadiazole based compounds, J. Enzyme Inhib. Med. Chem. (2012). http://dx.doi.org/10.3109/14756366.2012.688041.
[20] W. Rzeski, J. Matysiak, M. Kandefer-Szerszen, Anticancer, neuroprotective activities and computational studies of 2-amino-1,3,4-thiadiazole based compound, Bioorg. Med. Chem. 15 (2007) 3201–3207.
[21] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 23 (1997) 3–25.

[22] C.A. Lipinski, Drug-like properties and the causes of poor solubility and poor permeability, J. Pharmacol. Toxicol. Methods 44 (2000) 235–249.
[23] T.I. Oprea, J. Gottfries, V. Sherbukhin, P. Svensson, T.C. Kuhler, Chemical information management in drug discovery: optimizing the computational and combinatorial chemistry interfaces, J. Mol. Graph. Model. 18 (2000) 512–524.
[24] D.F. Veber, S.R. Johnson, H.Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, Molecular properties that influence the oral bioavailability of drug candidates, J. Med. Chem. 45 (2002) 2615–2623.
[25] J. Matysiak, A. Niewiadomy, Application of sulfinyl bis(2,4-dihydroxythiobenzoyl) in the synthesis of N-substituted 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles, Synth. Commun. 36 (2006) 1621–1630.
[26] J. Matysiak, A. Nasulewicz, M. Pełczynska, M. Switalska, I. Jaroszewicz, A. Opolski, Synthesis and antiproliferative activity of some 5-substituted 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles, Eur. J. Med. Chem. 41 (2006) 475–482.
[27] G.L. Ellman, K.D. Courtney, V. Andres, R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (1961) 88–95.
[28] M. Bartolini, C. Bertucci, V. Cavrini, V. Andrisano, Beta-amyloid aggregation induced by human acetylcholinesterase: inhibition studies, Biochem. Pharmacol. 65 (2003) 407–416.
[29] http://www.rcsb.org/pdb/home/home.do.
[30] J. Marco-Contelles, R. Leon, C. de los Rios, A. Samadi, M. Bartolini, V. Andrisano, O. Huertas, X. Barril, F. Javier Luque, M.I. Rodriguez-Franco, B. Lopez, M.G. Lopez, A.G. Garcia, M. do Carmo Carreiras, M. Villarroya, Tacripyrines, the first tacrine-dihydropyridine hybrids, as multitarget-directed ligands for the treatment of Alzheimer's disease, J. Med. Chem. 52 (2009) 2724–2732.
[31] N. Tsuno, Donepezil in the treatment of patients with Alzheimer's disease, Expert Rev. Neurother. 9 (2009) 591–598.
[32] J. Matysiak, A. Skrzypek, A. Niewiadomy, Synthesis and antifungal activity of novel 5-substituted 4-(1,3,4-thiadiazol-2-yl)benzene-1,3-diols, Heteroat. Chem. 21 (2010) 533–540.
[33] J. Matysiak, Z. Malinski, 2-(2,4-Dihydroxyphenyl)-1,3,4-thiadiazole analogues: antifungal activity *in vitro* against *Candida* species, Russ. J. Bioorg. Chem. 33 (2007) 594–601.
[34] R.J. Leatherbarrow, Stains, Erithacus Software Ltd, UK, 1999.
[35] S.R. Feaster, D.M. Quinn, Mechanism-based inhibitors of mammalian cholesterol esterase, Methods Enzymol. 286 (1997) 231–252.
[36] ChemOffice, CambridgeSoftCorporation, Cambridge, USA, 2006.
[37] Virtual Computational Chemistry Laboratory, http://www.vcclab.org.
[38] P. Ertl, B. Rohde, P. Selzer, Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties, J. Med. Chem. 43 (2000) 3714–3717.