College of Chemistry and Environmental Science, Anyang Institute of Technology, Anyang, China

Design, synthesis, and biological evaluation of acetophenone derivatives as dual binding acetylcholinesterase inhibitors

YANHONG SHEN, BAOLI LI, HU XU, GUOQIANG ZHANG

Received October 20, 2012, accepted November 23, 2012

Yanhong Shen, College of Chemistry and Environmental Science, Anyang Institute of Technology, Anyang 455000, China
yanhong0809@163.com

Pharmazie 68: 307–310 (2013) *doi: 10.1691/ph.2013.2839*

As part of a project aimed at developing new agents for potential application in Alzheimer's disease, a new series of acetophenone derivatives which possess alkylamine side chains were designed, synthesized and assayed as acetylcholinesterase (AChE) inhibitors that could simultaneously bind to the peripheral and catalytic sites of the enzyme. The compounds were synthesized, and the inhibitory activities toward AChE and butyrylcholinesterase (BuChE) *in vitro* were determined using a modified Ellman method. Of the compounds tested, 6 derivatives were found to inhibit AChE in the micromolar range. The best compound, **2e**, had an IC_{50} of 0.13 μM. A detailed molecular modeling study was performed to explore the interaction of **2e** with AChE.

1. Introduction

Alzheimer's disease (AD) is a chronic, neurodegenerative disorder, characterized by the loss of memory and cognition, severe behavioral abnormalities, and ultimately death. Low levels of acetylcholine (ACh) are thought to play a significant role in the disease, therefore, the use of reversible inhibitors of acetylcholinesterase (AChE) is considered to be a viable and attractive approach to treat the disease (Bachurin 2003; Munoz-Torrero and Camps 2006; Orhan et al. 2006). To date only acetylcholinesterase (AChE) inhibitors, such as tacrine, donepezil, rivastigmine and galantamine and a NMDA receptor antagonist, memantine, are available for AD treatment (Giacobini 2004).

Recent studies suggest that β-amyloid peptide (Aβ) in the brain may be a key factor in the pathogenesis of the disease, as its accumulation may result in a cascade of biochemical events leading to neuronal dysfunction (Hardy and Selkoe 2002; Piazzi et al. 2003). AChE is able to promote the aggregation of Aβ into amyloid fibrils through residues located in the peripheral anionic site (PAS) of the enzyme. AChE inhibitors that interact with both the catalytic site and PAS might exert a dual pharmacological effect by simultaneously inhibiting acetylcholine hydrolysis and AChE-induced Aβ aggregation (Camps et al. 2008; Kwon et al. 2007; Tumiatti et al. 2010).

In our previous study, a series of 2-phenoxy-indane-1-one derivatives (compound **1**) which possess alkylamine side chains have been designed and synthesized. Most of them demonstrated potent AChE inhibitory activity, and a docking study revealed that compound **1** bound with the active and peripheral sites simultaneously (Shen et al. 2008; Sheng et al. 2005). In this paper, 5,6-dimethoxy-indan-1-one in compound **1** was replaced by 3,4-dimethoxyacetophenone to result in compound **2** with a more flexible structure that may lead to a higher affinity or better selectivity for acetylcholinesterase. This provided an opportunity to explore the relationship between structure flexibility and inhibitory activity (Fig. 1).

2. Investigations, results and discussion

2.1. Chemistry

The target compounds **2a–g** were synthesized as illustrated in the Scheme. The readily available material 3,4-dimethoxyacetophenone **3** was brominated with $CuBr_2$ to obtain 2-bromo-1-(3,4-dimethoxyphenyl) ethanone **4** (Belluti et al. 2005). For the synthesis of intermediates **5a–g**, different routes were followed according to previously published methods (Grove et al. 2002; Tramontini 1973). Finally, the target compounds were obtained by refluxing **5a–g** with **4** in acetonitrile in the presence of K_2CO_3.

2.2. Biological activity

The synthetic compounds **2a–g** were then assayed for their AChE (rat cortex homogenate) and BuChE (rat serum) inhibition potency using the modified Ellman method (Cheng and Tang 1998; Ellman et al. 1961), with donepezil as the reference standard. The ChE inhibitory results were summarized in the Table.

Most of the tested compounds demonstrated moderate or high inhibitory activities against AChE. Among them, compounds **2a**, **2e** and **2 g** showed sub-micromolar inhibition of AChE. Compound **2e** possessed the most potent activity with IC_{50} of 0.13 μM. Based on the previously published data on compound **1**, the replacement of the indanone moiety of compound **1** by 3,4-dimethoxyacetophenone resulted in a decrease in the AChE inhibitory activity of compound **2** compared to compound **1** (Shen et al. 2008). This indicates that a more flexible structure may not lead to a higher affinity for the peripheral sites of AChE than the rigid indanone moiety. Comparing series **2a–d** with **2e–g**, it appeared that the compounds with a carbonyl group in the linker between the benzene ring and tertiary amino group generally possessed higher inhibitory potency (i.e. **2e** and

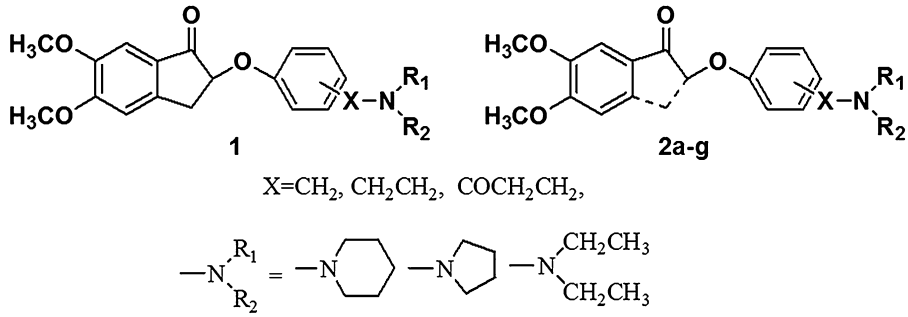
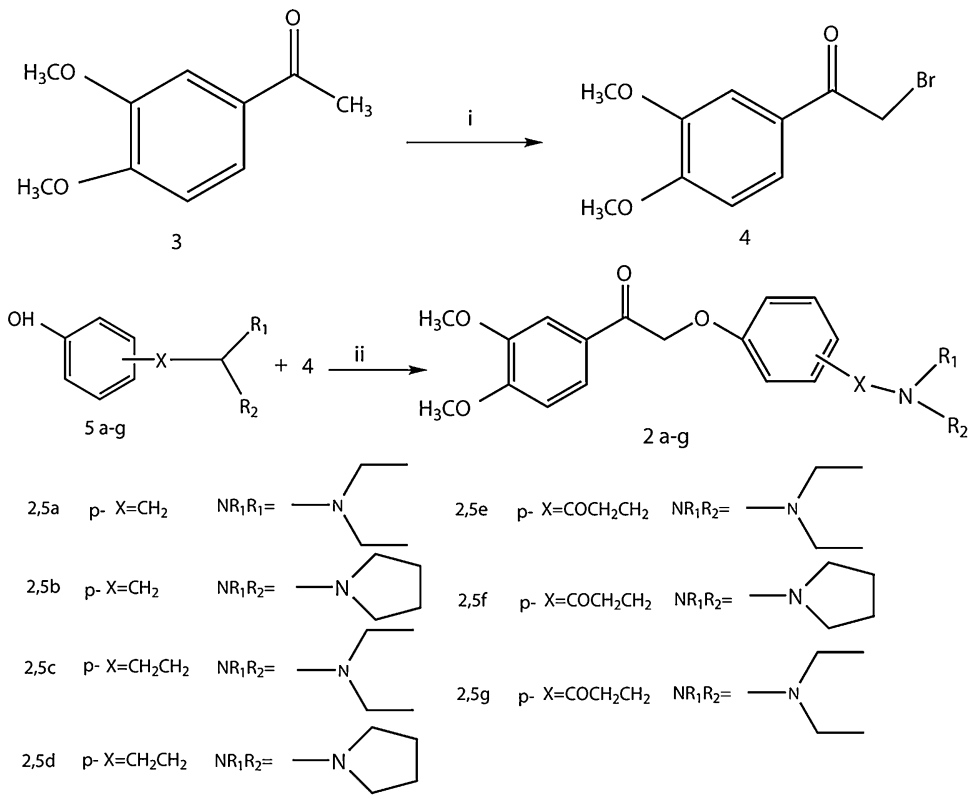


Fig. 1: Chemical structure of compound **1** and acetophenone derivatives **2a–g**



Scheme: Synthesis of **2a–g**. Reagents and conditions: (i): $CuBr_2$, $CHCl_3$, AcOEt, reflux (ii) K_2CO_3, CH_3CN, reflux

2 g). Finally, most of the synthetic compounds showed inhibitory selectivity for AChE over BuChE. The most potent compound **2e** was 206-fold more active in inhibiting AChE than BuChE, but less selective than donepezil.

2.3. Molecular modeling

To explore the possible binding conformation and protein–ligand interactions, a molecular modeling study of the optimal compound **2e** was performed using the Tripos FlexiDock program and visualized using PyMOL program. As seen in Fig. 2, compound **2e** extended well into the gorge of the enzyme. Fig. 3 illustrates that compound **2e** makes several interactions with both the catalytic site and the PAS. Near the bottom of the gorge, the protonated nitrogen of the ligand made a cation–π interaction with Trp84. At the midgorge recognition site, the ethereal oxygen atom was hydrogen-bonded with the OH group of Tyr121 with a bond length of 2.41 Å, and the carbonyl group in the $COCH_2CH_2$ linker formed direct hydrogen bonds with the OH group in Ser200. At the peripheral site, the methoxy groups interacted with residues Phe288 and Arg289 through hydrophobic interactions. Therefore, when 5,6-dimethoxy-indan-1-one in compound **1** was replaced by 3,4-dimethoxyacetophenone, compound **2e** assumed a slightly different orientation compared to compound **1**. Especially at the PAS, compound **2e** could not make a close contact with the key residue Trp279, which may have resulted in the decreased AChE inhibitory activity.

Table: AChE and BuChE inhibition data (IC_{50}, μM) of prepared compounds[a]

Compd.	IC_{50} AChE (μM)	IC_{50} BuChE (μM)	Selectivity for AChE[b]
2a	0.28	76.3	272.5
2b	32.8	89.4	2.73
2c	3.40	156.7	46.1
2d	7.46	42.6	29.2
2e	0.13	26.9	206.9
2f	7.2	46.3	6.43
2g	0.73	28.6	39.17
Donepezil	0.011	6.72	610.9

[a] Values are means of three different experiments.
[b] Selectivity for AChE is defined as IC_{50} (BuChE)/IC_{50} (AChE)

2.4. Conclusion

In conclusion, a novel class of acetophenone derivatives were designed, synthesized and tested for their biological activity, and

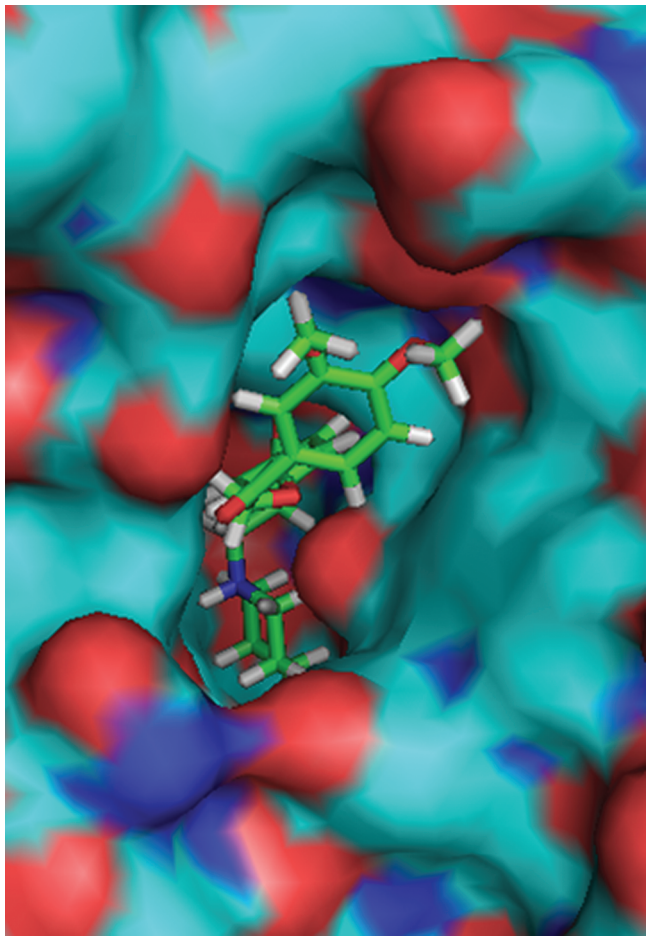


Fig. 2: Compound **2e** in the gorge of AChE

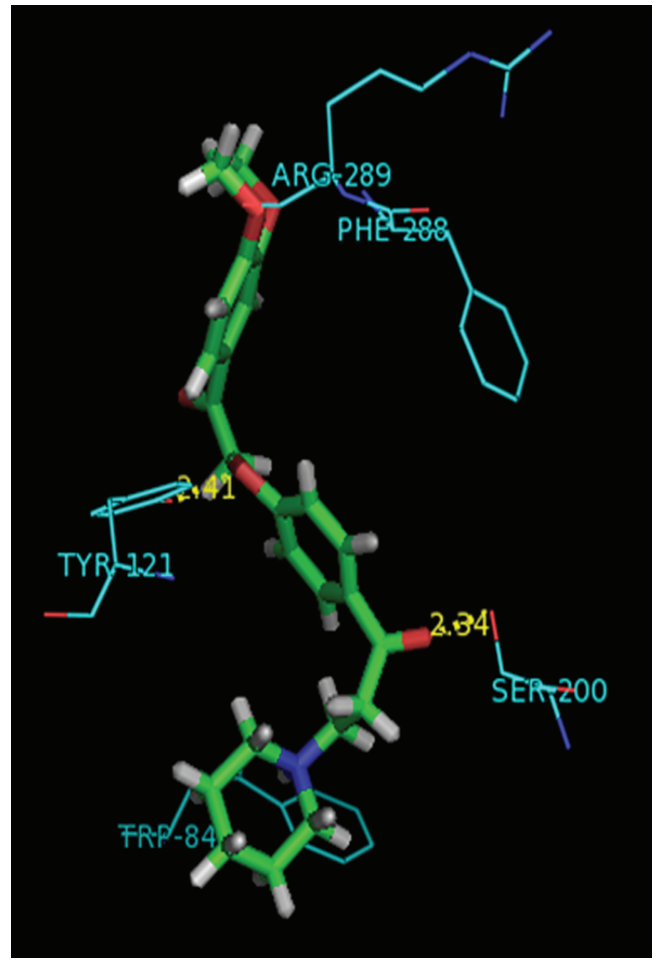


Fig. 3: Interaction between compound **2e** and AChE

most of the tested compounds demonstrated moderate or high inhibitory activities against AChE. The optimum inhibitor **2e** showed potent inhibitory activity with an IC_{50} value of 0.13 μM. The molecular modeling results showed that it likely interacts with both the catalytic site and the PAS of the enzyme, but without close contact with the key residue Trp279, which may result in decreased of AChE inhibitory activity.

3. Experimental

3.1. Chemistry

Melting points were obtained on a B-540 Buchi melting-point apparatus and uncorrected. ESI-MS were recorded on an Esquire-LC-00075 mass spectrometer (Bruker, USA). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker Advance DMX 400 MHz spectrometer (chemical shifts are expressed as δ values relative to TMS as internal standard). Infrared spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. Elemental analyses were performed on a Flash EA 1112 elemental analyzer.
2-Bromo-1-(3,4-dimethoxyphenyl)ethanone **4** and key intermediates **5a–g** were synthesized according to published methods (Belluti et al. 2005; Grove et al. 2002; Tramontini 1973).

3.2. General procedure for the synthesis of 2a–g

A solution of 2-bromo-1-(3,4-dimethoxyphenyl) ethanone (0.124 g, 0.48 mmol) in CH_3CN (3 mL) was added dropwise to a mixture of compound **5** (0.4 mmol), K_2CO_3 (0.066 g, 0.048 mmol) in CH_3CN (3 mL) and the mixture was refluxed for 1 h. After cooling to room temperature, the reaction mixture was filtered and the solvent was removed *in vacuo* to give syrups that were dissolved in dichloromethane (50 mL) and washed with water and brine. The organic phase was dried and evaporated under reduced pressure and the residue was purified by a silica-gel column chromatography (CH_2Cl_2/MeOH/TEA = 100: 5:1)

*3.2.1. 2-(4-((Diethylamino)methyl)phenoxy)-1-(3,4-dimethoxyphenyl)ethanone **(2a)***

Yield 51%, pale yellow syrup. MS(ESI): 358(M + 1). ^{1}H NMR ($CDCl_3$): δ 7.64(d, 1H, J = 8.4 Hz, H-6), 7.57(s, 1H, H-2), 7.27(d, 2H, J = 8 Hz, H-3', H-5'), 6.90(m, 3H, H-5, H-2', H-6'), 5.21(s, 2H, $COCH_2$), 3.96 (s, 3H, OCH_3), 3.93(s, 3H, OCH_3),3.62(s, 2H, benzylic CH_2), 2.60 (q, 4H, NCH_2CH_3, J = 7.6 Hz), 1.08 (t, 6H, NCH_2CH_3, J = 7.6 Hz); IR (KBr), ν (cm^{-1}): 2967, 1689, 1593, 1510, 1266, 1162, 760. Anal. calcd for $C_{21}H_{27}NO_4$: C, 70.56; H, 7.61; N, 3.92; found: C, 70.40; H, 7.53; N, 3.98.

*3.2.2. 1-(3,4-Dimethoxyphenyl)-2-(4-(pyrrolidin-1-ylmethyl)phenoxy)ethanone **(2b)***

Yield 46%, pale yellow syrup. MS(ESI): 356(M + 1). ^{1}H NMR ($CDCl_3$): δ 7.65 (d, 1H, J = 8.4 Hz, H-6), 7.59 (s, 1H, H-2), 7.26 (d, 2H, J = 8 Hz, H-3', H-5'), 6.88-6.94 (m, 3H, H-5, H-2', H-6'), 5.22 (s, 2H, $COCH_2$), 3.96 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.63(s, 2H, benzylic-CH_2), 2.55 (m, 4H, pyrrolidine-CH_2, H-2", H-5"), 1.81 (m, 4H, H-3", H-4"); IR (KBr), ν (cm^{-1}): 2876, 1683, 1592, 1511, 1467, 1163, 773. Anal. calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94; found: C, 71.15; H, 7.02; N, 3.90.

*3.2.3. 2-(4-(2-(Diethylamino)ethyl)phenoxy)-1-(3,4-dimethoxyphenyl)ethanone **(2c)***

Yield 37%, white solid, mp 82–84 °C. MS(ESI): 372(M + 1). ^{1}H NMR ($CDCl_3$): δ7.67(d, 1H, J = 8.4 Hz, H-6), 7.57 (s, 1H, H-2), 7.16 (d, 2H, J = 8 Hz, H-3', H-5'), 6.88-6.94 (m, 3H, H-5, H-2', H-6'), 5.24 (s, 2H, $COCH_2$), 3.98 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.03 (m, 6H, $PhCH_2CH_2N$, NCH_2CH_3), 2.19 (m, 2H, $PhCH_2CH_2N$), 1.32 (m, 6H, CH_2CH_3). IR (KBr), ν (cm^{-1}): 2969, 1689, 1591, 1512, 1267, 1162, 1018, 758. Anal. calcd for $C_{22}H_{29}NO_4$: C, 71.13; H, 7.87; N, 3.77; found: C, 71.36; H, 7.79; N, 3.81.

3.2.4. 1-(3,4-Dimethoxyphenyl)-2-(4-(2-(pyrrolidin-1-yl)ethyl)phenoxy)ethanone ***(2d)***

Yield 41%, white solid, mp 116–118 °C. MS(ESI): 370(M + 1). ^{1}H NMR ($CDCl_3$): δ 7.63 (d, 1H, J = 8.4 Hz, H-6), 7.52 (s, 1H, H-2), 7.14 (d, 2H, J = 8 Hz, H-3', H-5'), 6.84-6.91 (m, 3H, H-5, H-2', H-6'), 5.22 (s, 2H, $COCH_2$), 3.94 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.22 (m, 6H, pyrrolidine-CH_2, H-2", H-5", CH_2CH_2N), 2.09 (m, 2H), 1.39 (m, 4H). IR (KBr), ν (cm^{-1}): 2940, 1685, 1595, 1514, 1465, 1264, 1162, 771. Anal. calcd for $C_{22}H_{27}NO_4$: C, 71.52; H, 7.37; N, 3.79; found: C, 71.66; H, 7.39; N, 3.63.

3.2.5. 1-(4-(2-(3,4-Dimethoxyphenyl)-2-oxoethoxy)phenyl)-3-(piperidin-1-yl)propan-1-one ***(2e)***

Yield 45%, white solid, mp 62–64 °C. MS(ESI): 412(M + 1). ^{1}H NMR ($CDCl_3$): 7.96 (d, 2H, H-2', H-6'), 7.65 (d, 1H, J = 8.4 Hz, H-6), 7.56 (s, 1H, H-2), 6.92-6.98 (m, 3H, H-5, H-3', H-5'), 5.34 (s, 2H, $COCH_2$), 3.98 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.27 (t, 2H, J = 7.6 Hz, $COCH_2CH_2$), 2.93 (t, 2H, J = 7.6 Hz, CH_2CH_2N), 2.58 (m, 4H, piperidine-CH_2, H-2", H-6"), 1.70 (m, 4H, H-3", H-5"), 1.49 (m, 2H, H-4"). IR (KBr), ν (cm^{-1}): 2932, 1682, 1595, 1512, 1264, 1162, 1014, 763. Anal. calcd for $C_{24}H_{29}NO_5$: C, 70.05; H, 7.10; N, 3.40; found: C, 70.18; H, 7.10; N, 3.27.

3.2.6. 1-(4-(2-(3,4-Dimethoxyphenyl)-2-oxoethoxy)phenyl)-3-(pyrrolidin-1-yl)propan-1-one ***(2f)***

Yield 42%, white solid, mp 55–57 °C. MS(ESI): 398(M + 1). ^{1}H NMR ($CDCl_3$): 7.97 (d, 2H, H-2', H-6'), 7.66 (d, 1H, J = 8.4 Hz, H-6), 7.57 (s, 1H, H-2), 6.93-6.96 (m, 3H, H-5, H-3', H-5'), 5.34 (s, 2H, $COCH_2$), 3.98 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.23 (t, 2H, J = 7.6 Hz, $COCH_2CH_2$), 2.97 (t, 2H, J = 7.6 Hz, $COCH_2CH_2$), 2.65 (m, 4H, pyrrolidine-CH_2, H-2", H-5"), 1.84(m, 4H, H-3", H-4"). ^{13}C NMR (δ, $CDCl_3$): 197.2, 192.1, 161.9, 154.1, 149.3, 130.5, 130.4, 127.4, 122.7, 114.5, 110.1, 70.2, 56.1, 56.0, 54.2, 50.9, 37.1, 23.4. IR (KBr), ν (cm^{-1}): 2960, 1680, 1596, 1512, 1419, 1266, 1163, 1017, 763. Anal. calcd for $C_{23}H_{27}NO_5$: C, 69.50; H, 6.85; N, 3.52; found: C, 69.47; H, 6.80; N, 3.41.

3.2.7. 3-(Diethylamino)-1-(4-(2-(3,4-dimethoxyphenyl)-2-oxoethoxy)phenyl)propan-1-one ***(2g)***

Yield 35%, pale yellow syrup. MS(ESI): 400(M + 1). ^{1}H NMR ($CDCl_3$): 7.91 (d, 2H, H-2', H-6'), 7.61 (d, 1H, J = 8.4 Hz, H-6), 7.51 (s, 1H, H-2), 6.88-6.94 (m, 3H, H-5, H-3', H-5'), 5.32 (s, 2H, $COCH_2$), 3.93 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.26 (t, 2H, J = 7.6 Hz, $COCH_2CH_2$), 3.09 (t, 2H, J = 7.6 Hz, CH_2CH_2N), 2.78 (m, 4H, NCH_2CH_3), 2.14 (m, 6H, NCH_2CH_3). IR (KBr), ν (cm^{-1}): 2967, 1681, 1596, 1512, 1266, 1163, 1016, 764. Anal. calcd for $C_{23}H_{29}NO_5$: C, 69.15; H, 7.32; N, 3.51; found: C, 69.32; H, 7.18; N, 3.53.

3.3. Enzyme inhibition assays

AChE and BuChE inhibition activities were measured by the spectrophotometric Ellman's method with slight modifications, using rat cortex homogenate and rat serum as the source of AChE and BuChE, respectively. The brain homogenate was preincubated for 5 min with tetraisopropyl pyrophosphoramido (isoOMPA) (0.04 mmol/L), a selective inhibitor of BuChE. To assess AChE or BuChE activity, a reaction mixture containing acetylthiocholine iodide or butyrylthiocholine iodide, sodium phosphate buffer (pH 7.4), homogenate or serum, and different concentrations of the tested compounds was incubated at 37 °C for 15 min. The reaction was terminated by adding 3% sodium lauryl sulphate, then 0.2% 5,5′-dithio-bis (2-nitrobenzoic acid) to produce the yellow anion of 5-thio-2-nitro-benzoic acid. The values of IC_{50} were calculated by UV spectroscopy from the absorbance changes at 450 nm.

3.4. Molecular modeling of compound 2e

Molecular modeling studies were performed using SYBYL 6.9 software implemented in a Silicon Graphics workstation. The X-ray crystal structure of the enzyme AChE complexed with Donepezil (PDB file identificator 1EVE) was retrieved from the Protein Data Bank (PDB). All bound water molecules and ligands were removed from the complexes, and hydrogen atoms were subsequently added. The structures of the ligands were prepared in MOL2 format using the sketcher module, and Gasteiger-Huckel charges were assigned to the ligand atoms. The minimization was run until it converged to a maximum derivative of 0.001 kcal mol^{-1} $Å^{-1}$. The docking and subsequent scoring were performed using the default parameters of the FlexX program in the SYBYL.

References

Bachurin SO (2003) Medicinal chemistry approaches for the treatment and prevention of Alzheimer's disease. Med Res Rev 23: 48–88.

Belluti F, Rampa A, Piazzi L, Bisi A, Gobbi S, Bartolini M, Andrisano V, Cavalli A, Recanatini M, Valenti P (2005) Cholinesterase inhibitors: xanthostigmine derivatives blocking the acetylcholinesterase-induced beta-amyloid aggregation. J Med Chem 48: 4444–4456.

Camps P, Formosa X, Galdeano C, Gomez T, Munoz-Torrero D, Scarpellini M, Viayna E, Badia A, Clos MV, Camins A, Pallas M, Bartolini M, Mancini F, Andrisano V, Estelrich J, Lizondo M, Bidon-Chanal A, Luque FJ (2008) Novel donepezil-based inhibitors of acetyl- and butyrylcholinesterase and acetylcholinesterase-induced beta-amyloid aggregation. J Med Chem 51: 3588–3598.

Cheng DH, Tang XC (1998) Comparative studies of huperzine A, E2020, and tacrine on behavior and cholinesterase activities. Pharmacol Biochem Behav 60: 377–386.

Ellman GL, Courtney KD, Andres V, Feather-Stone RM jr. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7: 88–95.

Giacobini E (2004) Cholinesterase inhibitors: new roles and therapeutic alternatives. Pharmacol Res 50: 433–440.

Grove SJ, Kaur J, Muir AW, Pow E, Tarver GJ, Zhang MQ (2002) Oxyaniliniums as acetylcholinesterase inhibitors for the reversal of neuromuscular block. Bioorg Med Chem Lett 12: 193–196.

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297: 353–356.

Kwon YE, Park JY, No KT, Shin JH, Lee SK, Eun JS, Yang JH, Shin TY, Kim DK, Chae BS, Leem JY, Kim KH (2007) Synthesis, *in vitro* assay, and molecular modeling of new piperidine derivatives having dual inhibitory potency against acetylcholinesterase and Abeta1–42 aggregation for Alzheimer's disease therapeutics. Bioorg Med Chem 15: 6596–6607.

Munoz-Torrero D, Camps P (2006) Dimeric and hybrid anti-Alzheimer drug candidates. Curr Med Chem 13: 399–422.

Orhan G, Orhan I, Sener B (2006) Recent developments in natural and synthetic drug research for Alzheimers disease. Lett Drug Design Discov 3: 268–274.

Piazzi L, Rampa A, Bisi A, Gobbi S, Belluti F, Cavalli A, Bartolini M, Andrisano V, Valenti P, Recanatini M (2003) 3-(4-[[Benzyl(methyl) amino]methyl]phenyl) -6,7-dimethoxy-2H-2-chromenone (AP2238) inhibits both acetylcholinesterase and acetylcholinesterase-induced beta-amyloid aggregation: a dual function lead for Alzheimer's disease therapy. J Med Chem 46: 2279–2282.

Shen Y, Sheng R, Zhang J, He Q, Yang B, Hu Y (2008) 2-Phenoxy-indan-1-one derivatives as acetylcholinesterase inhibitors: a study on the importance of modifications at the side chain on the activity. Bioorg Med Chem 16: 7646–7653.

Sheng R, Lin X, Li J, Jiang Y, Shang Z, Hu Y (2005) Design, synthesis, and evaluation of 2-phenoxy-indan-1-one derivatives as acetylcholinesterase inhibitors. Bioorg Med Chem Lett 15: 3834–3837.

Tramontini M (1973) Advances in the chemistry of Mannich bases. Synthesis: 703–775.

Tumiatti V, Minarini A, Bolognesi ML, Milelli A, Rosini M, Melchiorre C (2010) Tacrine derivatives and Alzheimer's disease. Curr Med Chem 17: 1825–1838.