Bioorganic Chemistry 41-42 (2012) 28–34

Contents lists available at SciVerse ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Adamantane-substituted guanylhydrazones: Novel inhibitors of butyrylcholinesterase

Marina Šekutor [a], Kata Mlinarić-Majerski [a,*], Tomica Hrenar [b], Srđanka Tomić [b], Ines Primožič [b,*]

[a] Department of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička cesta 54, P.O. Box 180, 10002 Zagreb, Croatia
[b] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102A, 10000 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 26 December 2011
Available online 24 January 2012

Keywords:
Adamantane-substituted guanylhydrazones
Inhibitors
Butyrylcholinesterase
Kinetic study
Molecular docking

ABSTRACT

A series of novel adamantane-substituted guanylhydrazones was synthesized and used in a study of inhibitory potential toward butyrylcholinesterase. The experimental results were further supported by using docking studies to examine the behavior of the inhibitors within the active site regions of the enzyme. The enzyme-inhibitor dissociation constants K_i were determined from Hunter–Downs diagrams using Ellman's method for cholinesterase activity determination. Compounds 2-(*N*-guanidino)iminoadamantane hydrochloride (**1**) and 2,4-bis(*N*,*N*′-guanidino)iminoadamantane dihydrochloride (**2**) were found to be the best BChE inhibitors and their affinities for the enzyme active site were about five times higher compared to the enzyme peripheral site. The strongest interaction observed in complexes obtained by docking studies was the H-bond between the guanidine and the carboxylate of Glu199 and the second guanidine group in bisguanidine compounds was stabilized with additional H-bonds.

1. Introduction

Butyrylcholinesterase (BChE) is a serine hydrolase related to acetylcholinesterase (AChE) [1]. Cholinesterases catalyse the hydrolysis of esters of choline, including neurotransmitter acetylcholine. The two enzymes differ genetically, structurally and in their substrate specificities and sensitivities to a wide range of inhibitors. Butyrylcholine, the best choline substrate for BChE, is not its natural substrate and BChE is not physiologically essential for humans [2]. The function of BChE has not been clarified, although it has been suggested that it scavenges anti-cholinesterase agents and protects synaptic AChE from inhibition. Compared to AChE, BChE has a wider substrate specificity and interacts with a broader range of inhibitors. Consequently, BChE has been explored as a possible stoichiometric bioscavenger in organophosphorus nerve agent poisoning [3]. Recently it has been shown that BChE is widely distributed in the nervous system of mammals, pointing to its possible involvement in the neural functions and in neurodegenerative diseases [4]. It was found that the specific inhibition of BChE may be an adequate strategy for the development of more effective drugs to treat dementias such as Alzheimer's disease. Some selective butyrylcholinesterase inhibitors have already been reported to increase acetylcholine levels and to reduce the formation of abnormal amyloid found in Alzheimer's disease [5].

The guanidine moiety plays an important biological role [6]. It was found that compounds with a guanidinium group have antimicrobial [7], anti-influenza [8] and anticancer [9] activity. In addition, anti-trypanosome potency of heterocyclic guanylhydrazones [10] and alkylguanylhydrazones [11] was reported. Likewise, guanidinium compounds act as thrombin [12] and Na^+/H^+ exchange [13] inhibitors. It has been recently shown that the guanidinium group can also act as an allosteric site in an allosteric hydrolase model by supramolecular combination of adamantyl guanidinium and Cu(II)-complex of tris(2-aminoethyl)amine modified cyclodextrin [14]. Furthermore, alkyl guanylhydrazones are extensively used as nitric oxide synthase (NOS) inhibitors [15].

Similarly, adamantane derivatives possess several attractive pharmacological activities, such as antiviral [16], antibacterial [17], antifungal [18], and anticancer [19] activities, as well as anticonvulsive [20] effects and as a result of these findings many researchers consider them to be highly promising candidates in drug design. The discovery of antiviral properties of 1-aminoadamantane hydrochloride [21] stimulated a large number of investigations in which structurally modified compounds were assayed for biological activity. In many studies that have appeared, the adamantane moiety has been used primarily to modify the potency of substances already recognized as drugs [22]. It was clear that the adamantyl group confers additional potency in drugs in which it was present. The property that has been emphasized was the lipophilicity of adamantane that increases the ease with which the

* Corresponding authors. Fax: +385 1 4680 195 (K. Mlinarić-Majerski), +385 1 4606 401 (I. Primožič).
E-mail addresses: majerski@irb.hr (K. Mlinarić-Majerski), ines.primozic@chem.pmf.hr (I. Primožič).

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doi:10.1016/j.bioorg.2012.01.004

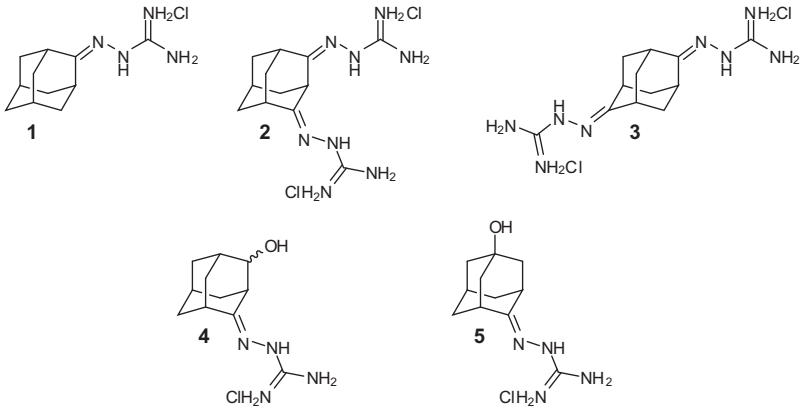


Fig. 1. Structures of compounds **1**–**5**.

compound reaches the site of activity. The second property was the bulkiness, suggesting that a steric factor is also important for the interactions at the site of activity. However, if the biological interaction involves inhibition of an enzyme, both of these factors reflect on the net effect of activity, as was demonstrated for the series of alkyl substituted diaminopyridines [23].

In the present study we explored the possible effect of the bulky adamantane moiety attached to the terminal nitrogen position of aminoguanidine on the inhibitory potential of butyrylcholinesterase. We report the synthesis of a series of adamantane-substituted guanylhydrazones **1**–**5** (Fig. 1) and the evaluation of their inhibitory potential toward BChE. In the docking study, similarities, differences and relations among the obtained orientations of guanylhydrazones within the active site regions of BChE are discussed.

2. Material and methods

2.1. Synthesis of the inhibitors

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-300 or 600 Spectrometer at 300 or 600 MHz, respectively. All NMR spectra were measured in [D$_6$]DMSO using tetramethylsilane as a reference. IR spectra were recorded on a FT-IR ABB Bomem MB 102 spectrophotometer. MALDI-TOF MS spectra in reflectron mode were obtained on an Applied Biosystems Voyager DE STR instrument (Foster City, CA). Melting points were obtained using an Original Kofler Mikroheitztisch apparatus (Reichert, Wien) and are uncorrected. All solvents were purified by distillation and aminoguanidine hydrochloride was purchased from Sigma–Aldrich, Co., and used without further purification.

2.1.1. General procedure for the synthesis of (N-guanidino) iminoadamantane hydrochlorides

The respective ketone (1.0 mmol) was dissolved in absolute ethanol (10 mL) and then aminoguanidine hydrochloride (1.0 mmol) was added in the solution in one portion. The reaction mixture was refluxed for 5 h and after cooling the solvent was evaporated yielding the crude product quantitatively. The obtained solid was purified by washing with ethyl acetate/methanol solvent mixture and the product was isolated as a white solid.

2.1.2. 2-(N-guanidino)iminoadamantane hydrochloride (1)

The title compound was synthesized from commercially available adamantan-2-one (300 mg, 2.0 mmol), yield 481 mg (99%); mp: 291–294 °C; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 1.72 (d, J = 11.6 Hz, 2H), 1.78 (d, J = 11.6 Hz, 2H), 1.83 (s, 2H), 1.92–1.99 (m, 6H), 2.59 (s, 1H), 3.37 (s, 1H), 7.44–7.60 (br s, 4H), 11.19 ppm (s, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 26.9, 31.7, 35.5, 37.0, 38.4, 38.5, 156.2, 166.2 ppm; IR (KBr): $\tilde{\nu}$ = 3301(br), 2924(s), 2852(m), 1667(s), 1663(s), 1597(m), 1449(w), 1085(w), 982(w) cm^{-1}; HRMS-MALDI m/z [M + H]$^+$ Calcd for $C_{11}H_{18}N_4$: 207.1604. Found: 207.1608.

2.1.3. 2,4-Bis(N,N'-guanidino)iminoadamantane dihydrochloride (2)

Compound **2** was obtained from adamanatan-2,4-dione [24] (164 mg, 1.0 mmol) as a mixture of isomers, yield 338 mg (97%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.57–2.17 (m, Ad-CH$_2$), 2.72 (s, Ad-CH), 3.32 (br s, Ad-CH), 3.38 (br s, Ad-CH), 3.48 (br s, Ad-CH), 3.55 (br s, Ad-CH), 4.14 (s, Ad-CH), 7.48–7.84 (m, guan. NH), 11.44 (s, guan. NH), 11.59 (s, guan. NH); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 26.5, 26.6, 31.2, 31.5, 33.6, 35.4, 35.8, 35.9, 36.1, 37.0, 37.1, 37.7, 37.8, 38.2, 39.3, 41.4, 43.3, 48.5, 49.4, 155.8, 156.2, 156.4, 158.7, 159.0, 160.2, 162.3 ppm; IR (KBr): $\tilde{\nu}$ = 3341(br), 3137(br), 2926(m), 1665(s), 1631(s), 1604(s), 1448(w), 1100(m) cm^{-1}; HRMS-MALDI m/z [M + H]$^+$ Calcd for $C_{12}H_{20}N_8$: 277.1883. Found: 277.1880.

2.1.4. 2,6-Bis(N,N'-guanidino)iminoadamantane dihydrochloride (3)

Similar to the synthesis of compound **2**, adamantane-2,6-dione [25] (82 mg, 0.5 mmol) was used in the synthesis of hydrazone **3**, yield 171 mg (98%); mp: >300 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.96–2.07 (m, 8H), 2.70 (s, 2H), 3.46 (s, 2H), 7.47–7.70 (br s, 8H), 11.33 ppm (s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 30.9, 36.8, 37.8, 38.1, 39.4, 156.2, 163.1 ppm; IR (KBr): ν~ = 3316(br), 3165(br), 2939(m), 1661(s), 1635(s), 1598(s), 1449(w), 1107(w) cm^{-1}; HRMS-MALDI m/z [M + H]$^+$ Calcd for $C_{12}H_{20}N_8$: 277.1883. Found: 277.1882.

2.1.5. 4-Hydroxy-2-(N-guanidino)iminoadamantane hydrochloride (4)

Hydrazone **4** was synthesized from 2-hydroxyadamantane-4-one [24] (166 mg, 1.0 mmol) in a 94% yield and was obtained as a diastereomer mixture (244 mg); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.37–2.81 (m, Ad-CH$_2$ and Ad-CH), 3.13–3.69 (m, Ad-CH), 3.90 (br s, Ad-CH), 3.96 (br s, Ad-CH), 4.15 (br s, Ad-CH), 7.23–7.87 (m, guan. NH), 10.98 (s, guan. NH), 11.15 (s, guan. NH), 11.45 (s, guan. NH), 11.60 (s, guan. NH); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 26.0, 26.4, 26.5, 26.6, 29.3, 30.8, 31.2, 31.4, 31.5, 32.6, 33.6, 33.8, 34.6, 35.5, 35.9, 37.1, 37.3, 37.6, 37.8, 38.2, 38.3, 43.4, 45.4, 45.8, 74.6, 74.7, 156.0, 156.1, 156.2, 156.4, 160.3, 162.4, 164.9, 165.4 ppm; IR (KBr): $\tilde{\nu}$ = 3401(br), 3156(br), 2922(m), 2858(m), 1670(s), 1639(s), 1602(s), 1091(w) cm^{-1}; HRMS-MALDI m/z [M + H]$^+$ Calcd for $C_{11}H_{18}N_4O$: 223.1553. Found: 223.1558.

2.1.6. 5-Hidroxy-2-(N-guanidino)iminoadamantane hydrochloride (5)

Compound **5** was obtained from 1-hydroxyadamantan-4-one [25] (166 mg, 1.0 mmol) in a 99% yield (256 mg); mp: 269–271 °C;

^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.57–1.83 (m, 8H), 2.17 (s, 1H), 2.67 (s, 1H), 3.35 (s, 2H), 3.42 (s, 1H), 4.69 (br s, 1H), 7.54 (br s, 4H), 11.16 (s, 1H) ppm; ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 29.5, 32.8, 36.1, 37.5, 39.9, 43.8, 44.0, 45.3, 65.6, 156.2, 164.8 ppm; IR (KBr): $\tilde{\nu}$ = 3302(br), 3165(br), 2929(m), 2911(m), 2858(m), 1670(s), 1647(s), 1602(s), 1455(w), 1353(w), 1112(m), 1093(m), 926(w) cm^{-1}; HRMS-MALDI m/z $[M+H]^+$ Calcd for $C_{11}H_{18}N_4O$: 223.1553. Found: 223.1553.

2.2. Kinetic study

BChE (EC 3.1.1.8), type IV-S lyophilized powder from horse serum, acetylthiocholine (ATCh), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co. and were used without further purification. Enzyme activities were determined by the method of Ellman et al. [26] The enzyme activities were measured under the following conditions: 0.1 M phosphate buffer pH = 7.4, 0.33 mM DTNB and 0.1–2.0 mM acetylthiocholine as substrate, at 25 °C. The assay volume was 3.0 mL. The increase in absorbance is recorded at 412 nm up to several minutes (depending on the enzyme activity). Enzyme activity was corrected for the spontaneous, nonenzymic substrate hydrolysis. All spectrophotometric measurements were performed on Cary III, Varian Inc., Australia. 3–5 inhibitor concentrations were used. The activities of the enzyme were measured at different substrate concentrations (s) in the absence (v_o) and presence (v_i) of the given inhibitor concentration (i). For each substrate concentration, the apparent dissociation constant (K_{app}) was calculated, $K_{app} = (v_i \cdot i)/(v_o - v_i)$. The enzyme-inhibitor dissociation constants K_i were evaluated from the Hunter–Downs equation [27] using linear regression analysis.

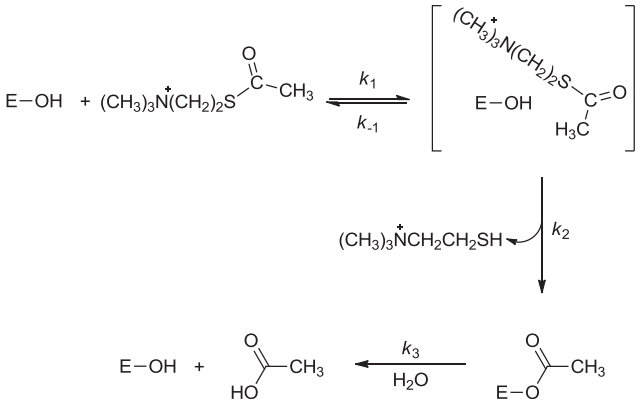
2.3. Docking studies: Setting up of receptor and ligands

A theoretical 3D structure of human BChE was derived by homology modeling on the basis of the known X-ray structure of *Torpedo* acetylcholinesterase [28]. The polar hydrogen atoms on amino acid residues were added and atom types together with Gasteiger [29] atomic partial charges were assigned using the AutoDockTools 1.5.4.

Geometries of all ligands (Fig. 2) were optimized using the B3LYP functional and the 6-31G(d) basis set. Non-polar hydrogens on ligands were removed and Gasteiger charges were added to all atoms using the AutoDockTools. Geometries of enantiomeric pairs were prepared by mirroring the coordinates of one enantiomer. All quantum chemical calculations were performed by using the Gaussian 09 package [30].

2.3.1. Flexible ligand docking

Docking studies were performed by using AutoDock 4.2.2 suite of programs [31] and included flexible ligand docking to the rigid receptor. To speed up the docking calculation, AutoDock requires a precalculated grid map for each atom type present in the ligand molecule. Those maps were generated by the auxiliary program AutoGrid and their consistencies were ascertained by checking



Scheme 1. Catalytic process of BChE.

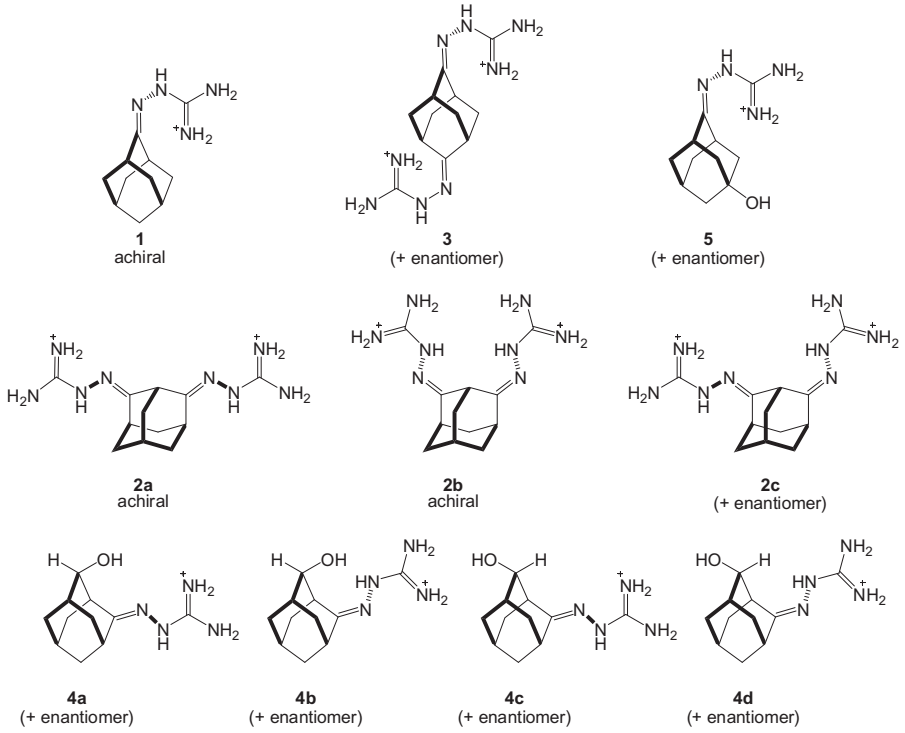


Fig. 2. Seventeen stereoisomers of compounds **1**–**5** chosen for docking studies.

maximum and minimum values of van der Waals' energies and electrostatic potentials.

Dimensions of the grid box were set to 100 × 100 × 100 Å (with spacing of 0.2 Å) ensuring the appropriate size of the ligand-accessible space. The grid was centered at the γ-oxygen atom of catalytic Ser200.

Flexible ligand docking was performed for 17 new compounds (7 enantiomeric pairs and 3 non-chiral molecules). Docking calculations were carried out using the Lamarckian genetic algorithm (LGA) and all parameters were the same for each docking run. The initial population of random individuals was 5000, the maximum number of energy evaluations and generations were 2×10^7 and 1×10^6, respectively. An elitism value of 1, mutation rate of 0.02 and crossover rate of 0.08 were used. For the local search, the pseudo-Solis and Wets method was used with a maximum of 10,000 iterations per local search. The probability of performing a local search on an individual in the same population was 0.06 and the maximum number of consecutive successes or failures before changing the rho was 4 in both cases. The size of local search space to sample was 1.0 and the lower bound on rho was 0.01. At the end of each docking procedure (100 independent docking runs), the resulting positions were clustered according to a r.m.s. criterion of 0.5 Å and obtained structures were analyzed visually.

Table 1
Inhibition constants for the inhibition of BChE by compounds **1**–**5**.

Compound	K_i[a] (mM)	
	K_a[b]	K_{ss}[c]
1	0.55 ± 0.05	2.9 ± 0.6
2	0.67 ± 0.03	3.1 ± 0.6
3	2.5 ± 0.4	–
4	1.1 ± 0.2	–
5	4.0 ± 0.5	–

[a] Enzyme-inhibitor dissociation constant K_i was determined from Hunter–Downs plots, standard deviation was obtained from four experiments on average.
[b] Dissociation constant for the catalytic site K_a.
[c] Dissociation constant for the peripheral site K_{ss}.

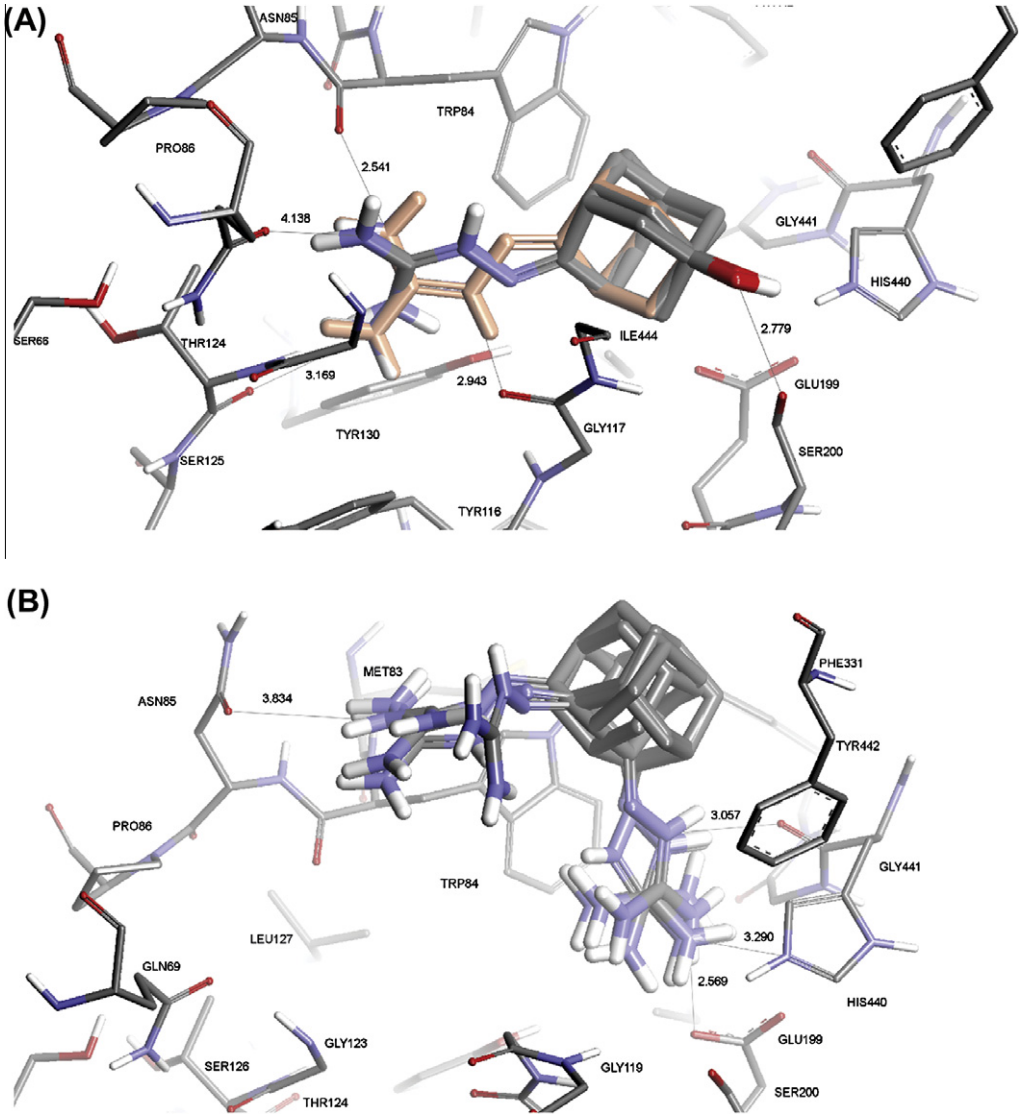


Fig. 3. Inhibitor-BChE complexes derived from docking studies. BChE active site is represented by some structurally important amino acids. Only polar hydrogen atoms are presented. Interatomic distances are given in Å. (A) compound **1** (light orange) and two enantiomers of the compound **5** (color by element); (B) four stereoisomers of compound **2**; (C) two enantiomers of compound **3**; (D) eight stereoisomers of compound **4**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

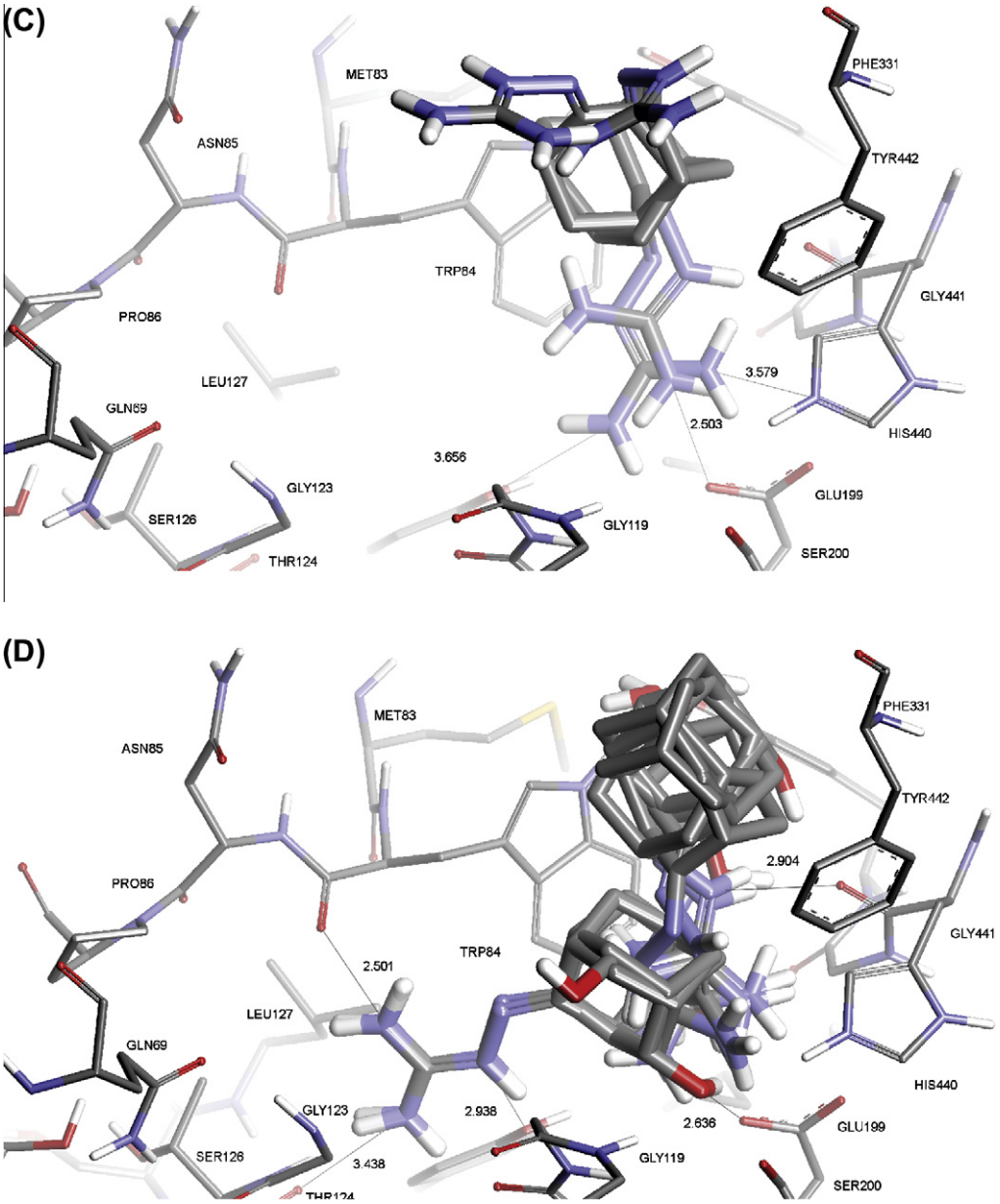


Fig. 3 (*continued*)

3. Results and discussion

3.1. Design and synthesis

Guanylhydrazones used in this study were synthesized according to the general procedure (see Experimental Section) starting from the corresponding adamantanone. After condensation with aminoguanidine hydrochloride, all the obtained compounds have been purified, identified and afterwards used in the enzymatic experiments. It should be noted that compounds **2** and **4** have been isolated as a mixture of diastereoisomers, while for guanylhydrazones **1**, **3** and **5** only one diastereomeric form was obtained due to the high symmetry present in the structure.

3.2. Enzyme-inhibitor dissociation constants

Butyrylcholinesterase has similar enzymatic properties as AChE [32]. The overall catalytic process of BChE proceeds in three steps: initial formation of an enzyme-substrate complex (e.g. BChE–ATCh), an acylation step, and deacylation by hydrolysis (Scheme 1).

The enzyme-inhibitor dissociation constants K_i were evaluated from the kinetics of competition between ATCh and inhibitors **1**–**5** towards BChE. Two stages of the competition were considered: one at lower ATCh concentrations (up to 1 mM) and the other at higher concentrations (Table 1). The enzyme-inhibitor dissociation constant was calculated from the equation $K_{app} = K_i + (K_i/K_M)\cdot s$, where K_{app} is the apparent enzyme-inhibitor dissociation constant at a given substrate concentration (s) and K_M is the Michaelis constant for the substrate (ATCh). When the relationship between K_{app} vs. s is linear, the intercept on the ordinate K_i is either the enzyme-inhibitor dissociation constant for the catalytic site (K_a) or the dissociation constant for the peripheral site (K_{ss}). K_a and K_{ss} were determined based on the numerical values of the calculated K_M (K_M corresponds to $(k_{-1}/k_1 + k_2/k_1)[k_3/(k_2 + k_3)]$). The K_M constant in the Hunter–Downs equation [27] corresponds to either the dissociation constant of enzyme-substrate Michaelis complex that

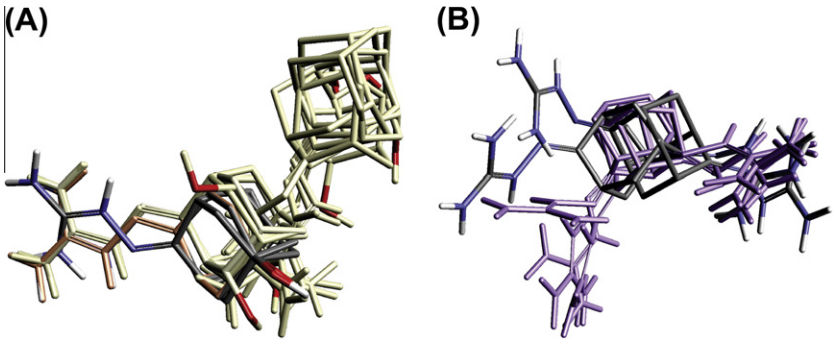


Fig. 4. Superimposed geometries obtained by docking studies of all stereoisomers of (A) adamantane monoguanidine inhibitors **1** (light orange), **4** (light yellow) and **5** (color by element); (B) adamantane bisguanidine inhibitors **2** (light purple) and **3** (color by element). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

could be approximated to the Michaelis constant, or the dissociation constant of the enzyme-substrate complex when the binding to the peripheral site occurs as well (K_{ss}). Typically, K_a can be calculated from K_{app} values determined at low substrate concentrations and K_{ss} from K_{app} values determined at higher substrate concentrations. At low substrate concentrations, it is expected that inhibitors compete for the catalytic site (K_a) with the substrate and at higher substrate concentrations for the peripheral site (K_{ss}). The peripheral anionic site of cholinesterases is located at the rim of the active site gorge and is sometimes critical for dictating specificity of ligands. Occupation at this site may affect the conformation of the active center.[1]

All tested compounds were reversible inhibitors of BChE, Table 1. Reversible inhibition of BChE by compounds **1** and **2** was not linear which implied the competition between inhibitors and ATCh at two sites of the enzyme. A linear relationship between the apparent dissociation constants and the substrates was obtained for compounds **3**–**5**. The best inhibitor was the monoguanidine **1** closely followed by bisguanidine **2** while compounds **3**, **4** and **5** inhibited the enzyme two, four and eight times weaker, respectively. The affinities of the compounds **1** and **2** were approximately five times lower towards the enzyme peripheral site.

3.3. Docking studies

Amino acids of the active site of BChE make specific contributions to the substrate specificity and catalytic power of the enzyme. They form four major domains: (a) the esteratic site containing the active serine as a part of the catalytic triad (Ser200, His440, Glu327), (b) the acyl pocket (Leu285, Trp233, Phe400, Phe331) – a hydrophobic region which accommodates the acyl group of an ester, (c) the choline subsite (Trp84, Tyr130, Glu199), the recognition of the substrate's quaternary ammonium group and (d) the oxyanion hole formed by the main chain N–H dipoles (Gly118, Gly119, Ala201) interacting with the negatively charged carbonyl oxygen of the substrate in a tetrahedral intermediate [1]. In order to shed light on experimental data concerning the inhibitory power of the studied compounds, we applied flexible ligand docking protocol implemented in AutoDock 4.2.2 [31]. All stereoisomers of compounds **1**–**5** (Fig. 2) were docked into the active site of BChE by utilizing the AutoDock program.

According to the Gibbs energies of binding and substrate orientations in the BChE-ester complexes, AutoDock generated several clusters of the binding orientations for all ligands in the active site. Analyses of the obtained complexes revealed that there are numerous close contacts of ligands with the enzyme residues of the active site. Estimated lowest Gibbs energy of binding differs significantly among stereoisomers and valuable information about the important interactions of adamantane-substituted guanylhydrazones were obtained (Fig. 3, Table S1).

Analyses of the docking results revealed that the adamantane and guanidine moieties in most cases occupied only the choline binding subsite (Fig. 3A–D). The strongest interaction observed in all complexes, with the exception of those for compound **5**, was the strong H-bond between the guanidine and the carboxylate of the Glu199. The similarity in the position of adamantane and guanidine moieties is presented in Fig. 4, where the geometries of all stereoisomers of tested monoguanidines (A) and bisguanidines (B) were superimposed.

The position of the adamantane moiety is determined with the accomplishment of the maximum number of H-bonds between the positively charged guanidine and the amino acids in its surroundings. The second guanidine group in compounds **2** and **3** is pointed towards the rim of the active site gorge and can be more or less successfully stabilized with H bonds (the strongest interaction with Asp72, Fig. 3B).

Smaller monoguanidinium inhibitors **1**, **4** and **5**, apart from making H-bonds with the Glu199 in the same manner like bisguanidinium derivatives, can enter deeper into the choline subsite and the guanidinium group is stabilized with 4 H-bonds (Fig. 3A). Derivatives **4** and **5** with 1-hydroxy and 2-hydroxy as substituents, respectively, can make additional H-bonds with donors and acceptors in the surrounding which causes the small change in the positions of guanidine and adamantane moiety compared to compound **1**. Both geometries of hydroxy derivative **5** can be positioned to realize the H-bond between its hydroxy group and the hydroxy group of the Ser200, which is part of the catalytic triad (Ser200, His440, Glu327) (Fig. 3A).

As a result of the rigidity of the tested inhibitors, maximum interactions of hydrophobic adamantane group were not accomplished due to the prevalence of the guanidinium H-bonds stabilization. Hence, further studies on more flexible adamantane-substituted guanylhydrazones will give additional information regarding adamantane and guanidine interactions with BChE.

4. Conclusion

In this paper we have presented the results of the synthesis of a series of adamantane-substituted guanylhydrazones as well as their inhibitory potential toward BChE. Compounds **1** and **2** were found to be the best inhibitors. Their reversible inhibition of BChE was not linear which points towards the existence of a competition between them and ACh at two binding sites. On the other hand, for compounds **3**, **4** and **5** a linear relationship between the dissociation constants and the substrate concentration was found indicating that only binding in the active site of the enzyme occurred.

To support these experimental results stereoisomers of inhibitors **1**–**5** were docked into the active site of BChE and the analysis of the obtained complexes showed that both adamantane and guanidine moiety mostly occupy the choline binding subsite. It was also noted that the strongest interaction (except for inhibitor **5**) was the H-bond between the guanidine and the carboxylate of Glu199. In bisguanidine compounds **2** and **3,** the second guanidine group was stabilized with additional H-bonds (Asp72). When compared to the best inhibitor **1**, hydroxy-derivatives **4** and **5** possess a slight change of position in the active site which is most probably caused by additional H-bonds.

The lipophilic potential of the adamantyl moiety in this series of inhibitors was not entirely utilized, mainly because of the structure rigidity. More flexible derivatives might possess improved inhibition ability due to the multiplied beneficial close contacts. Further studies in this direction are in progress.

Acknowledgments

This research was supported by the Ministry of Science, Education and Sports of the Republic of Croatia (Grants Nos. 098-0982933-2911 and 119-1191344-3121).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioorg.2012.01.004.

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