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Original Article

Rhodolirium andicola: a new renewable source of alkaloids with acetylcholinesterase inhibitory activity, a study from nature to molecular docking

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ABSTRACT

Acetylcholinesterase is an important target for control of neurodegenerative diseases causing cholinergic signaling deficit. Traditionally, galanthamine has been used as an Amaryllidaceae-derived acetylcholinesterase inhibitor, although new Amaryllidaceae plants could serve as source for better acetylcholinesterase inhibitors. Therefore, the objective of this study was to characterize the alkaloid composition from bulbs of *Rhodolirium andicola* (Poepp.) Traub, a native Chilean Amaryllidaceae specie, and assess their inhibitory activity on acetylcholinesterase by *in vitro* and *in silico* methodologies. Alkaloidal extracts from *R. andicola* exhibited an inhibitory activity with IC_{50} values between 11.25 ± 0.04 and 57.78 ± 1.92 μg/ml that included isolated alkaloid, galanthamine (2.3 ± 0.18 μg/ml), Additionally, 12 alkaloids were detected using gas chromatography–mass spectrometry and identified by comparing their mass fragmentation patterns with literature and database NIST vs.2.0. To better understand the bioactivity of isolated compounds and alkaloidal extracts against acetylcholinesterase, a molecular docking approach was performed. Results suggested that alkaloids such as lycoramine, norpluvine diacetate and 6α-deoxy-tazettine expand the list of potential acetylcholinesterase inhibitors to not only galanthamine. The role of *R. andicola* as a source for acetylcholinesterase inhibitors is further discussed in this study.

Introduction

The enzyme acetylcholinesterase (AChE) is known by its rapid hydrolysis of neurotransmitter acetylcholine (ACh) in the cholinergic synapses (Barnard, 1974; Stryer, 1995). Inhibition of AChE is an important strategy for the treatment of diseases that involve cholinergic transmission deficit such as myasthenia gravis and Alzheimer's disease (AD) (Rahman and Choudhary, 2001; Mehndiratta et al., 2011). AD is the most common form of dementia in our society (World Alzheimer Report, 2015). Worldwide, it is currently estimated that 46 million people have AD or a related dementia, and considering that life expectancy will increase, it is estimated that people with AD will reach to 131.5 million by 2050 (World Alzheimer Report, 2015). These facts make AD one of the most investigated diseases throughout the world (Perry, 1986; Greig et al., 2001). Although AChE inhibition is an established therapeutic strategy to ameliorate cognitive dysfunction and memory loss associated with AD (Rahman and Choudhary, 2001), only a few compounds, such as tacrine, donepezil, physostigmine and galanthamine (Zarotsky et al., 2003) have been approved by the Food and Drug Administration (FDA) in the United States. However, several side-effect such as hepatotoxicity and problems associated with gastrointestinal symptoms, have been reported for the synthetic drugs tacrine and donepezil, respectively (Schulz, 2003; Mehta et al., 2012). In contrast, physostigmine and galanthamine, both from natural origin, have fewer side effects in patients with mild to moderate AD (Mehta et al., 2012). Consequently, many research groups have focused their studies on finding new renewable sources of compounds with acetylcholine esterase inhibitory activity (Mukherjee et al., 2007). In this regard, after the

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isolation of natural compound galanthamine, a long-acting, selective, reversible and competitive AChE inhibitor, approved in 2001 by FDA (Razadyne®), for clinical treatment of mild and moderate AD, several Amaryllidaceae species have been evaluated as new sources of galanthamine or other alkaloids with potential AChE inhibitory activity (López et al., 2002; Rhee et al., 2004; Ortiz et al., 2012). Although the chemical synthesis of galanthamine is available (Marco and Carreiras, 2006; Bulger et al., 2008), current pharmaceutical production of this compound is mainly limited to the extraction of natural populations of the Amaryllidaceae *Leucojum aestivum* and *Narcissus* spp. (Heinrich and Teoh, 2004).

In Chile, around 35 species of the Amaryllidaceae family are present covering a wide variety of eleven genera (Ravena, 2003). Particularly, *Rhodolirium andicola* (Poepp.) Traub, part of endemic Amaryllidaceae species growing in Chile, represents a potential source of alkaloids with AChE inhibitory activity. In this study, we describe the alkaloidal composition of *R. andicola* for the first time. We isolated three well-known alkaloids and evaluated their AChE inhibitory by the Ellman method (Ellman et al., 1961) as a first approach to probe *R. andicola* as a source of alkaloids. Additionally, we tested twelve other alkaloids identified by gas chromatography–mass spectrometry (GC–MS) by molecular docking using a crystal structure of AChE to propose new alkaloids as potential AChE inhibitors that could be used in further assays and likely treatments of neurodegenerative diseases.

Materials and methods

Chemicals

Silica gel 60 (Merck, 70–230 mesh) was used for column chromatography (CC) and silica gel 60 F_{254} for thin layer chromatography (TLC) analytical and preparative. MeOH and water (HPLC grade), $CHCl_3$, H_2SO_4, Et_2O, NH_4OH, hexane, BuOH, NH_3, EtOAc (analytical grade) were purchased from J.T. Baker (México). Acethyltiocholine iodide (ATCI), acetylcholinesterase (AChE) from *Electhrophorus electricus* (type VI-S lyophilized powder), 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) and hydrocarbon mixture (C_6–C_{26}) (chemical purity >99%) were obtained from Sigma–Aldrich (St. Louis, MO, USA) whereas, Reminyl® (galanthamine hydrobromide salt) were purchased from Janssen-Cilag (Spain).

Plant material

Rhodolirium andicola (Poepp.) Traub, Amaryllidaceae, bulbs were collected during the flowering season in December 2016 from National park Conguillio, Araucanía Region, Chile (S 38°44,426′ WO 72°38,887 height: 1389 m.a.s.l.). The plant was identified by Dr. Marcelo Baeza and deposited at the herbarium of Universidad de Concepción, Concepción, Chile (voucher no. CONC 182466).

Alkaloid extraction and fractionation

Dried bulbs (2.5 kg) were extracted three times with MeOH (1 g of dry sample by 10 ml of solvent) at room temperature for one week (Rhee et al., 2004). The solution was filtered and the solvent was evaporated under reduced pressure on a rotary evaporator (40 °C). The residue (150 g) was dissolved in water (250 ml) and acidified to pH 2 with H_2SO_4 (2%, v/v). The acid solution was defatted with Et_2O (5 × 100 ml) and $CHCl_3$ (5 × 100 ml). Then, the acid solution was basified with 25% ammonia solution up to pH 9–10 and the alkaloids were extracted with hexane (5 × 100 ml), $CHCl_3$ (5 × 100 ml) and BuOH (5 × 80 ml), to obtain the hexanic (0.27 g), chloroformic (1.3 g), and buthanolic alkaloidal extracts (2.5 g) respectively (Ortiz et al., 2012; Sheng-Dian et al., 2013; de Andrade et al., 2016). The hexanic alkaloidal extract was roughly separated by column chromatography on 10 g of silica gel 60 (Merck, 70–230 mesh) using a gradient with hexane (100%), gradually enriching with $CHCl_3$ (0 → 100%) and subsequently increasing the polarity with EtOAc, and finally increasing it with MeOH (0 → 50%) (de Andrade et al., 2014; Ortiz et al., 2016) to give five fractions (I–V). Column fractions were monitored by TLC, and similar ones were combined and evaporated to dryness. Fractions I and II were combined and subjected to preparative TLC, (silica gel 60 F_{254} with $CHCl_3$/hexane/MeOH, 5:2:3, in NH_3 atmosphere) to give the compound-A (10 mg). Column chromatographic on Sephadex LH-20 of fractions III–V in MeOH gave three subfractions. The second subfraction, positive to Dragendorff reagent, was subjected to preparative TLC, (silica gel 60 F_{254} with $CHCl_3$/MeOH, 9:1, in NH_3 atmosphere) to give the compound-B (15 mg). Whereas, the separation of compounds from chloroformic alkaloidal extract (1.3 g) was performed by preparative column chromatography on 50 g of silica gel 60 (Merck, 70–230 mesh), as stationary phase (Ortiz et al., 2012). The elution started with chloroform increasing the polarity with methanol, enriched gradually with 10% methanol up to 100% methanol (Elisha et al., 2013) to give one hundred fractions of 10 ml. Fractions with similar profiles based on visualized under ultraviolet light (254 nm), and analysis by Dragendorff reagent were combined and evaporated to dryness. Column chromatographic on Sephadex LH-20 of fractions 60–100 in MeOH gave four subfractions. The third subfraction, positive to Dragendorff reagent, was subjected to preparative TLC, (silica gel 60 F_{254} with $CHCl_3$/MeOH, 9:1, in NH_3 atmosphere) to give the compound-C (20 mg) and compound-D (40 mg) respectively.

GC/MS analysis

The extracts were analyzed by coupled gas chromatography–mass spectrometry (GC–MS) with electron impact ionization (70 eV) using an Agilent, model 7890A chromatograph equipped with a HP-5ms capillary column (30 m × 0.25 mm × 0.25 μm; J&W Scientific) with helium carrier gas. The GC oven was programmed to ramp from 100 °C (for 3 min) to 280 °C at 10 °C/min and held for 19 min. The injector and transfer line temperatures were 250 °C and 285 °C respectively. The alkaloid compounds were identified by comparing their GC mass spectra with data from the NIST MS Search 2.0 library, Kovats indices (RI) and mass spectra reported in the literature (Mukherjee et al., 2007; Ortiz et al., 2016). The Kovats retention indexes of the compounds were recorded with standard of an *n*-hydrocarbon mixture (C_9–C_{26}). The proportion of each alkaloid in the basic extracts is expressed as a percentage of ion current (TIC).

Acetylcholinesterase inhibitory activity

Inhibition of AChE by alkaloidal extracts and isolated compounds was determined using the spectrophotometric method according to Ellman et al. (1961) and modified by Ortiz et al. (2012). Fifty microliters of AChE (0.25 U/ml) in phosphate buffer saline (8 mM KH_2PO_4, 2.3 mM Na_2HPO_4, 0.15 M NaCl, pH 7.5) and 50 μl of the samples dissolved in the same buffer were added to the wells. The plates were incubated for 30 min at 25 °C before the addition of 100 μl of the substrate solution (0.04 M Na_2HPO_4, 0.2 mM, 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), 0.24 nM acethyltiocholine iodide (ATCI) in HPLC grade water). The absorbance was read in a microplate reader (Varioskan™ Flash) at 405 nm after 5 min. Inhibition of enzyme was calculated as a percentage compared with an assay using a buffer without any inhibitor. The IC_{50} values were the means ± SD of three determinations. Reminyl® was used as positive control.

Table 1
Alkaloid composition of *Rhodolirium andicola*.

Alkaloid	Area % in alkaloidal extract		[M⁺] and characteristic ions (%)	RI[a]	RI reference	MS reference
	Hexanic	Chloroformic				
Galanthamine	4.96	1.64	287 (90), 286 (99); 244 (27)	2433	[1]	[2] [3] [4]
Lycoramine	1.05	1.53	289 (62), 288 (99), 274 (10)	2449	[1]	[1]
Galanthaminon	0.70	–	284 (99), 285 (93), 216 (42)	2508	[1]	[1]
6α-Deoxy-tazettine	0.45	0.43	315 (31), 300 (36), 231 (71)	2516	[NIST]	[4]
Norpluviine diacetate	6.49	–	357 (46), 296 (13), 270 (14)	2554		[NIST]
3-*O*-Acetyl-1,2-dihidro-galanthamine	40.77	–	330 (77), 270 (21), 213 (14)	2577		[NIST]
Heamanthamine	17.60	3.58	301 (77), 272 (99), 257 (53)	2595	[6]	[2] [4]
Undulatine diol	19.25	–	349 (38), 247 (99), 318 (78)	2594		NIST
Tazettine	–	90.21	331 (19), 247 (71), 240 (20)	2593	[7] [NIST]	[2] [4]
Acetylnatalensine	0.51	–	343 (76), 250 (28), 225 (25)	2592		[NIST]
Undulatine	0.27	–	331 (99), 258 (35), 205 (69)	2587		[5]
3-*epi*-Macronine	7.92	–	314 (19), 245 (65), 201 (83)	2583		[2]
Crinan-3-one	–	2.23	271 (99), 270 (48), 238 (16)	2580		[4]

(–) Not found in extract.
[a] Experimental Kovats index; [NIST]; NIST reference; [1] Berkov et al. (2012); [2] de Andrade et al. (2016); [3] Ortiz et al. (2016); [4] Berkov et al. (2004); [5] Tram et al. (2014); [6] de Andrade et al. (2014); [7] Gotti et al. (2006).

AChE refinement and molecular docking

Binding affinities of alkaloids were evaluated through molecular docking against a refined 3D structure of AChE 1C2B.pdb and the human AChE 4PQE.pdb. For 1C2B, 5 ns of molecular dynamics were run to refine the crystal structure of AChE. The AChE structure was refined using molecular dynamics with NAMD v2.9 and CHARMM36 force field. The protein was solvated with water (TIP3P model) in a cubic box with a minimum distance of 15 Å between the protein and the edge of the box. Likewise, the system was neutralized by adding Na^+ or Cl^- randomly placed in the box. All protein preparation was carried out using Visual Molecular Dynamics software (VMD). Configuration files were prepared in order that the system was simulated under periodic boundary conditions with a cutoff radius of 12 Å for non-bonded interactions and a time step of 2 fs. Extensive energy minimizations (50,000 steps) were performed followed by heating through short simulations of 1 ps at 50, 100, 150, 200, 250 and 300 K. Long simulations were kept at 298 K and 1 bar pressure in the NTP (referred to a constant number of particles, temperature and pressure) during 5 ns. Root-mean-square deviation (RMSD) trajectory tool in VMD was used to calculate the RMSD with reference to the starting structure. When the plotted RMSD did not showed any big changes, coordinates were analyzed every fifty frames to obtain the best representative structure (lowest energy, kcal mol^{-1}). When the representative structure of the enzyme was selected, molecular docking was performed using Autodock 4.2 (Morris et al., 1998). Thus, ligands were prepared as PDBQT files including torsional bonds when corresponding using Autodock Tools (ADT). Two hundred runs of Lamarckian genetic algorithm (GA) as the best method to find the lowest energy structures were used (Morris et al., 1998). Likewise, a grid box with dimension $50 \times 50 \times 50$ and orientations 27.05 (*x*-center), 77.14 (*y*-center) and 20.109 (*z*-center) with default space of 0.375 Å using Autogrid, was set for 1C2B. Moreover, dimensions of $50 \times 50 \times 50$ and orientations −25.3 (*x*-center), 24.623 (*y*-center) and −6.754 (*z*-center) were set for the human AChE (4PQE). Ligands were optimized using SPARTAN Software with Hartree–Fock with basis set 6−31G* and water environment, considered flexible while the protein was rigid. Every docked conformation and clusters were analyzed by ADT and the best binding modes were selected according to the lowest binding energy.

Results and discussion

Spectroscopic analysis by GC–MS is a valuable tool for the detection, identification and quantification of alkaloids in Amaryllidaceae plants (Cortes et al., 2015). For this study, the technique was used to detect thirteen alkaloids from extracts of bulbs from *R. andicola*, six of them identified from their mass spectra and retention index (Table 1). Galanthamine was found in hexanic and chloroformic alkaloidal extracts and ranged from 1.64% to 4.96% of total ion current (TIC), respectively. Furthermore, galanthamine-type alkaloids such as lycoramine, galanthaminon and 3-*O*-acetyl-1,2-dihidro-galanthamine were detected in hexanic alkaloidal extract. The isolated and purified compounds were identified as galanthamine (A), haemanthamine (B, C) and tazettine (D) from their mass spectra and retention index.

Table 2
Enzymatic inhibition activity of alkaloidal extracts and isolated compounds from bulbs of *Rhodolirium andicola* expressed as IC_{50} values.

Sample	IC_{50} (μg/ml)[a]
Hexanic alkaloidal extract	11.25 ± 0.04
Chloroformic alkaloidal extract	17.34 ± 1.13
Buthanolic alkaloidal extract	57.78 ± 1.92
Tazettine	441.04 ± 1.67
Hemanthamine	287.32 ± 1.82
Galanthamine	2.3 ± 0.18
Galanthamine (Reminyl®)[b]	0.17 ± 0.15

[a] Expressed as mean ± standard error mean (SEM).
[b] Reference compound.

The alkaloidal extracts and purified compounds were tested to evaluate their AChE inhibitory activity, using Reminyl® as positive control. The results expressed as half-maximal inhibitory concentration (IC_{50}) values, are showed in Table 2. The hexanic and chloroformic alkaloidal extracts showed a notable AChE inhibitory effect, considering that they are fractions and not pure compounds, with IC_{50} values of 11.25 ± 0.04 and 17.34 ± 1.13 μg/ml respectively, compared with IC_{50} value of 0.17 ± 0.15 μg/ml for Reminyl® (positive control). On the other hand, buthanolic alkaloidal extract showed a low activity against AChE with an IC_{50} value of 57.78 ± 1.92 μg/ml which was 300-fold less than that of Reminyl®. The results expressed as IC_{50} values can be compared with values obtained by Cortes et al. (2015), where AChE inhibitory activity of alkaloidal extracts from five Amaryllidaceae plants was evaluated obtaining IC_{50} values between 5.97 ± 0.24 and 70.22 ± 0.24 μg/ml, compared with IC_{50} value of 1.55 μg/ml for galanthamine (reference compound). Cortes and his colleagues suggested that the notable activity showed by alkaloidal extract from *Zephyranthes carinata* (5.97 ± 0.24 μg/ml) could be specifically related to the presence of lycoramine, galanthamine and lycorine. In this regard, Ortiz et al. (2012) evaluated six alkaloidal extracts

from different Amaryllidaceae species that grow in Argentina against AChE-activity by spectrophotometric Ellman assay (Ellman et al., 1961), obtaining a high AChE inhibitory activity with IC_{50} values between 1.0 ± 0.01 to 2.0 ± 0.20 μg/ml, compared with the IC_{50} value 0.29 ± 0.07 μg/ml reported for galanthamine. The authors describing that the highest AChE inhibitory activity showed by alkaloid extract from *Habranthus jamesonii*, could be related to the high content of galanthamine and galanthamine-type alkaloids showed by GC–MS analysis. On the other hand, Elisha et al. (2013) showed both a high and low AChE inhibitory activity for buthanolic and ethyl acetate alkaloidal extracts from bulbs of *Ammocharis coranica* with IC_{50} values of 0.05 ± 0.02 and 43.1 ± 1.22 μg/ml respectively, compared with IC_{50} value of reference compound physostigmine (1.51 μg/ml). In our study, the notable AChE inhibitory activity showed by hexanic and chloroformic alkaloidal extracts could be associated with the content of galanthamine-type alkaloids showed by GC–MS analysis. For the isolated compounds, galanthamine showed an IC_{50} value (2.3 ± 0.18 μg/ml) lower than all alkaloidal extracts and other isolated compounds (Table 2), which corroborates its selective, competitive and reversible affinity for AChE (López et al., 2002). In contrast, haemanthamine showed a weak AChE inhibitory activity with a IC_{50} value of 287.32 ± 1.82 μg/ml, similar to reported in literature (López et al., 2002; Houghton et al., 2006). The other major isolated alkaloid, tazettine, did not show AChE inhibitory activity (López et al., 2002).

In general it is difficult to compare the results obtained from different studies in relation to AChE inhibitory activities for alkaloids isolated from Amaryllidaceae plants. The possibility of false-positive results in the AChE inhibitory activity values due to chemical inhibition (Rhee et al., 2003) should not be ruled out; however, different authors have showed that members of the galanthamine and lycorine group have notable AChE inhibitory activities (López et al., 2002; Elisha et al., 2013). Recently, a series of lycorine derivatives were synthesized and evaluated for anti-cholinesterase activity; in fact the lycorine derivate compound, 2-*O*-*tert*-butyldimethylsilyl-1-*O*-(methylthio)methyl lycorine, showed dual cholinesterase inhibitory activities of human AChE and butyrylcholinesterase with IC_{50} values of 11.40 ± 0.66 μM and 4.17 ± 0.29 μM, respectively (Wang et al., 2012). On the other hand, galanthamine-type alkaloid such as sanguinine and *N*-(14-methylallyl) norgalanthamine have showed an IC_{50} value lower than galanthamine (0.10 against 1.07 μM and 0.16 vs 1.82 μM respectively) (López et al., 2002; Berkov et al., 2008).

The present study demonstrates that *R. andicola* serves as source of alkaloids with AChE inhibitory activity, containing thirteen alkaloid compounds, including galanthamine and other galanthamine-type alkaloids. Our efforts that led to the isolation of three alkaloids (galanthamine, haemanthamine and tazettine) allowed us to corroborate *R. andicola* as a rich source of AChE inhibitors, mainly based on successful inhibition assays with galanthamine. However, in an attempt to explain the bioactivity of hexanic and chloroformic alkaloidal extracts and considering the difficulties to obtain enough plant material for the isolation of the other 10 compounds, molecular docking was conducted to simulate the interactions of the entire profile of alkaloidal compounds in the catalytic site of a crystal structure of *E. electricus* AChE (PDB: 1C2B), same enzyme as the one used in inhibitory assays. Additionally, we performed molecular docking studies on the human AChE 4PQE.pdb and compared the resulting binding energies between the top scoring alkaloids.

Molecular docking is a useful tool to predict the binding mode of small molecules to target proteins as well as giving an approximation of the binding affinity of small molecules. Table 3 shows the results of binding interactions with the active site of the crystal structure of AChE from *E. electricus* (1C2B) with alkaloids identified in extracts from *R. andicola* bulbs as ligands. In order to extrapolate potential human activity, the top four scoring ligands at 1C2B were carried forward for docking calculations at the human AChE with scores shown in Table 4. The 1C2B AChE active-site gorge is shown as a schematic in Fig. 1. The bottom of the gorge is characterized by several subsites: the 'anionic' site (Trp86, Phe338 and Glu202), in which the choline moiety of ACh interacts by π-cation interactions; the 'catalytic' site, which contains the three residues of the catalytic triad (Ser203, Glu334 and His447); the 'oxyanion' hole (Gly121, Gly122, Ala204), and the "acyl pocket" (Phe295, Phe297), which confers substrate specificity (Houghton et al., 2006). The molecular docking calculations from 1C2B predict that the binding energy of galanthamine is −8.0 kcal/mol (1.38 μM of predicted K_i), whereas the binding energies for compounds lycoramine, norpluvine diacetate and 6α-deoxy-tazettine are −9.07 (0.23 μM of predicted K_i), −8.94 (0.28 μM of predicted K_i) and −8.12 kcal/mol (1.13 μM of predicted K_i), respectively. In human AChE (4PQE), alkaloids galanthamine, lycoramine, norpluvine diacetate and 6α-deoxy-tazettine had comparable binding affinities as shown in Table 4. For the complexes lycoramine, norpluvine diacetate and 6α-deoxy-tazettine with 1C2B, the amino acids Glu202 (anionic site), Ser203 and His447 (catalytic site) have a structural arrangement similar to that observed in the AChE-galatanthamine complex (Bartolucci et al., 2001). The similar binding affinities observed for all ligands and side chain conformations of amino acids are unsurprising considering the 88.52% global sequence identity and near complete conservation of residues at the level of the active site between both models (Edgar, 2004; Waterhouse et al., 2009) (Fig. 3). In AChE-lycoramine complex, the inhibitor binds at the base of the active site gorge. The hydroxyl oxygen of lycoramine forms a closed hydrogen bond with Ser203 (2.0 Å), similar to that observed in AChE-galanthamine complex (Ortiz et al., 2016), where the hydroxyl oxygen of galanthamine forms a closed hydrogen bond with the charged Glu202 (2.1 Å) and methoxy group with Ser203 (3.2 Å). There may also be hydrogen bonding interactions between the N-methyl group of the inhibitor lycoramine and amino acid residues Tyr449 and Tyr337. It must be assumed that the binding energy for lycoramine and galanthamine comes from a number of smaller enthalpic contributions, coupled to an unusually small entropic penalty (Lee et al., 2007; Cortes et al., 2015). This latter point arises from the rigidity of the molecule, which allows the numerous interactions to occur with minimal loss of entropy (Greenblatt et al., 1999). The theoretical representation presented in Fig. 2 provides information about how the galanthamine inhibitor is stabilized by the AChE enzyme. These same interactions occurred with lycoramine, norpluvine diacetate and 6α-deoxy-tazettine. The binding poses for these molecules suggest that the hydroxyl functional group could play a key role to stabilizing these alkaloids through hydrogen bonds with Ser203 and Glu202 (Fig. 2A and B). Likewise, epoxides present in 6α-deoxy tazzetine could form hydrogen bonds with close residues (Fig. 2C). For norpluvine diacetate, residues such as Ser125 and Ser203 are candidates to establish hydrogen bonds due to their proximity to alcohol groups. Moreover, hydrophobic interactions seem to predominate in the stabilization of norpluvine diacetate with the participation of Tyr and Phe residues (Fig. 2D). The most stable conformation of this complex shows that the Ser203 and His447 residue are close to the inhibitor. Besides, the amino acid residues Tyr86 (involved in π-cation interactions with the protonated head of ACh) and Glu202 are in a close conformation to the inhibitor molecule, similar to the AChE-galanthamine complex (Atanasova et al., 2005). Therefore, the amino acid Glu202 (anionic site) is implicated in AChE inhibitory mechanism.

Considering the results from molecular docking and the presence of lycoramine, norpluvine diacetate, galanthamine and 6α-deoxy-tazettine in hexanic and chloroformic alkaloidal

Table 3
Estimated binding energies of inhibition for alkaloid compounds from *Rhodolirium andicola* in the active site of AChE (PDB: 1C2B).

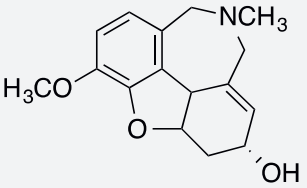
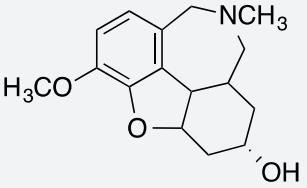
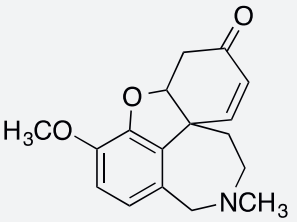
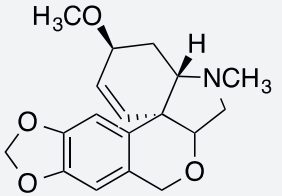
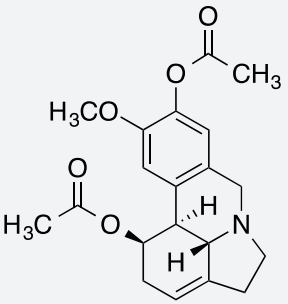
Alkaloid	Type	Chemical structure	Free binding energy (kcal mol^{-1})	Closest residues	Hydrogen bonds
Galanthamine	Galanthamine		−8.00	Trp86, Gly120, Gly121, Gly122, Tyr124, Glu202, Ser203, Ala204, Phe297, Tyr337, Phe338, Tyr341	Glu202
Lycoramine	Galanthamine		−9.07	Trp86, Gly121, Gly122, Tyr124, Glu202, Ser203, Phe297, Tyr337, His447, Tyr449	Ser203, Tyr337
Galanthaminon	Galanthamine		−8.09	Trp86, Gly120, Gly121, Gly122, Gly126, Tyr133, Glu202, Tyr337	Tyr133, Glu202
6α-Deoxy-tazettine	Tazettine		−8.12	Trp86, Gly121, Gly122, Tyr124, Ser125, Glu202, Ser203, Phe297, Tyr337, Phe338, Tyr341, His447	Tyr341
Norpluvine diacetate	Lycorine		−8.94	Gly121, Gly122, Tyr124, Ser125, Glu202, Ser203, Ala204, Trp236, Phe295, Phe297, Tyr337, Phe338, Tyr341, His447	

Table 3
(*Continued*)

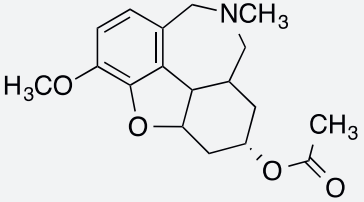
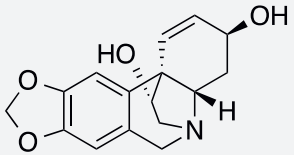
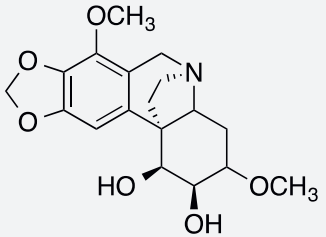
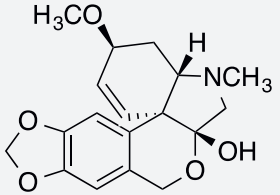
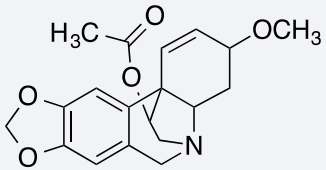
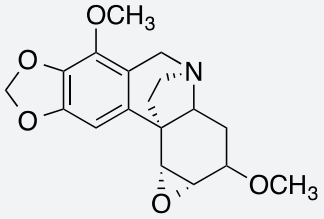
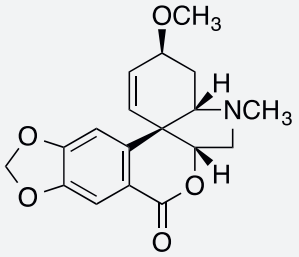
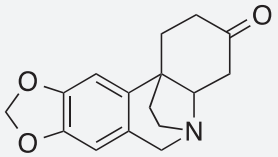
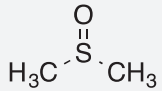
Alkaloid	Type	Chemical structure	Free binding energy (kcal mol^{-1})	Closest residues	Hydrogen bonds
3-*O*-Acetyl-1,2-dihidro-galanthamine	Galanthamine		−−9	Trp86, Gly121, Tyr124, Ser125, Glu202, Phe295, Phe297, Phe338, Tyr341, Tyr337	Glu202
Haemanthamine	Haemanthamine		−7 .22	Gly121, Gly122, Tyr124, Ser125, Glu202, Ser203, Ala204, Trp236, Phe297, Tyr337, Phe338, His447	Gly122, Tyr337
Undulatiane diol	Crinine		−6 .61	Trp86, Gly121, Gly122, Tyr124, Ser125, Glu202, Ser203, Ala204, Tyr337, Phe338, His447	Gly122, Glu202
Tazettine	Tazettine		−8 .08	Trp86, Gly121, Gly122, Ser125, Ser203 Ala204, Phe297, Tyr337, Phe338, Tyr341, His447	
Acetylnatalensine	Crinine		−8 .08	Gly121, Gly122, Tyr124, Ser125, Glu202, Ser203, Trp236, Tyr337, Phe338, Phe297, His447	Gly122

Table 3
(*Continued*)

Alkaloid	Type	Chemical structure	Free binding energy (kcal mol^{-1})	Closest residues	Hydrogen bonds
Undulatine	Crinine		−8.09	Trp86, Gly121, Gly122, Tyr124, Ser125, Ser203, Ala204, Trp236, Phe297, Tyr337, Phe338, His447	Gly122
3-*epi*-Macronine	Tazettine		−7.97	Trp86, Gly121, Gly122, Try124, Ser125, Phe297, Tyr337, Phe338, Try341, His447	
Crinan-3-one	Crinine		−7.55	Trp86, Gly121, Tyr124, Gly126, Glu202, Typ337, Phe338	
Dimethyl sulfoxide[a]	–		−3.32	Gly120, Gly121, Gly122, Ser203, Ala204, Phe297, His447	Gly122, Ala204

[a] Ligand considered as negative control for molecular docking.

Table 4
Estimated binding energies between four best alkaloids and the human acetylcholinesterase (PDB: 4PQE).

Alkaloid	Free binding energy (kcal mol^{-1})	Closest residues	Hydrogen bonds
Galanthamine	−8.58	Tyr72, Trp86, Thr83, Asn87, Gly121, Tyr124, Ser125, Tyr337,	Tyr72
Lycoramine	−8.83	Gln71, Val73, Asp74, Thr83, Trp86, Asn87, Gly121, Tyr124, Ser125, Tyr337, Tyr341	
6α-Deoxy-tazettine	−8.57	Arg18, Gly19, Ile20, Val59, Thr63	
Norpluvine diacetate	−8.92	Ser203, Ser125, Phe295	Ser125, Phe295

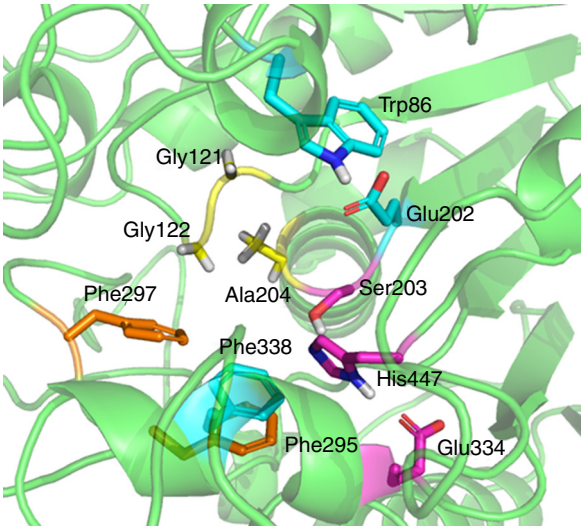


Fig. 1. Schematic view of the active-site gorge of AChE from *Electrophorus electricus* (1C2B) 'anionic' site (Trp86, Phe338 and Glu202), 'catalytic' (Ser203, Glu334 and His447) 'oxyanion' hole (Gly121, Gly122, Ala204) "acyl pocket" (Phe295, Phe297).

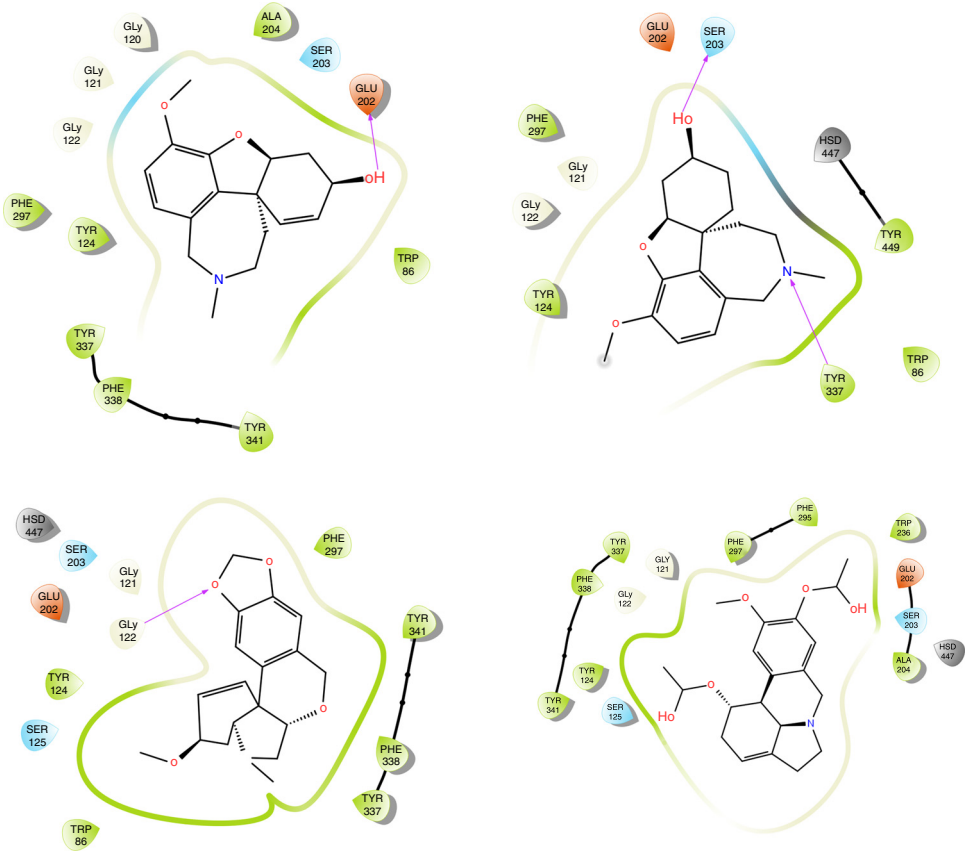


Fig. 2. Schematic representation of main interactions of galanthamine (A), lycoramine (B), tazettine, 6α-deoxy (C) and norpluvine diacetate (D) with AChE- catalytic site (Ser203, Glu334 and His447) and 'anionic' site (Trp86, Phe338 and Glu202). The schematic representations of protein-ligand interactions were created with Maestro software. Green color represents hydrophobic interactions, light blue represents polar interactions, blue represents positively charged residues, red represents negatively charged residues, arrows represent the presence of hydrogen bonds and connected residues by lines represent pi–pi interactions.

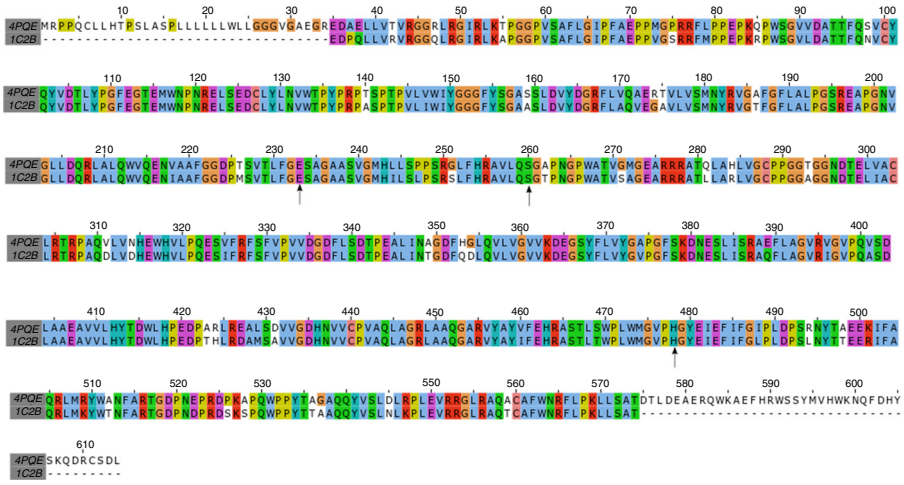


Fig. 3. Sequence alignment of 1C2B and 4PQE generated in MUSCLE (Edgar, 2004) and displayed in Jalview (Waterhouse et al., 2009). Active site residues glutamate, serine and histidine are highlighted by arrows in positions 233, 260 and 478 respectively.

extracts, we propose that these compounds should be considered for further AChE inhibitory activity assays.

Conclusions

The findings of the present study integrated *in vitro* and *in silico* methodologies, which demonstrated the potential of a wild Chilean Amaryllidaceae plant, *R. andicola*, as a new renewable source of galanthamine and other alkaloids with potential use as AChE inhibitors. Thus, molecular docking approaches suggested that lycoramine, 6α-deoxy-tazettine and norpluvine diacetate are interesting AChE-inhibitory alkaloids based on their presence in active hexanic and chloroformic extracts. Although galanthamine is known for its use in treating neurodegenerative diseases, the tazettine-type alkaloids should be evaluated in the search for more selective compounds with potential AChE inhibitory activity, though more experimental evidence is required.

Conflicts of interest

The authors declare no conflicts of interest.

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