Talanta 116 (2013) 647–652

Contents lists available at ScienceDirect

Talanta

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

Acetylcholinesterase immobilized capillary reactors coupled to protein coated magnetic beads: A new tool for plant extract ligand screening

Kenia Lourenço Vanzolini [a,b], Zhengjin Jiang [c], Xiaoqi Zhang [c], Lucas Campos Curcino Vieira [a], Arlene Gonçalvez Corrêa [a], Carmen Lucia Cardoso [d], Quezia Bezerra Cass [a], Ruin Moaddel [b,c,*]

[a] Departamento de Química, Universidade Federal de São Carlos, caixa postal 676, São Carlos 13565-905, Brazil
[b] Biomedical Research Center, National Institute on Aging, National Institutes of Health, 251 Bayview Boulevard, Baltimore, MD 21224, USA
[c] Department of Pharmacy and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of Traditional Chinese Medicine & New Drug Research, Jinan University, Guangzhou 510632, China
[d] Departamento de Química, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14040-901 SP, Brazil

ARTICLE INFO

Article history:
Received 21 May 2013
Received in revised form
19 July 2013
Accepted 22 July 2013
Available online 31 July 2013

Keywords:
Binding affinities
Acetylcholinesterase inhibitor
Non-linear chromatography

ABSTRACT

The use of immobilized capillary enzyme reactors (ICERs) and enzymes coated to magnetic beads ((NT or CT)-MB) for ligand screening has been adopted as a new technique of high throughput screening (HTS). In this work the selected target was the enzyme acetylcholinesterase (AChE), which acts on the central nervous system and is a validated target for the treatment of Alzheimer's disease, as well as for new insecticides. A new approach for the screening of plant extracts was developed based on the ligand fishing experiments and zonal chromatography. For that, the magnetic beads were used for the ligand fishing experiments and capillary bioreactors for the activity assays. The latter was employed also under non-linear conditions to determine the affinity constants of known ligands, for the first time, as well as for the active fished ligand.

Published by Elsevier B.V.

1. Introduction

The enzyme acetylcholinesterase (AChE) acts in the central nervous system and rapidly hydrolyzes the active neurotransmitter acetylcholine into the inactive compounds choline and acetic acid [1]. Low levels of acetylcholine in the synaptic cleft are associated with a decrease in cholinergic function characterizing Alzheimer's disease, which is the most common cause of dementia among the elderly. Because AChE inhibitors are currently one of the few therapies approved for the treatment of Alzheimer's disease, the identification of novel ligands that could modulate AChE activity is of great therapeutic importance.

Newmann and Cragg [2] determined that more than 70% of all drugs approved from 1981 and 2006, were either derived from or structurally similar to nature based compounds. Considering the structural diversity of known AChE inhibitors, a logical extension is to screen plant extracts to identify potentially unknown modulators of AChE. In fact, a number of known inhibitors of AChE have been derived from plant extracts, including galanthamine, a drug accepted for Alzheimer's disease treatment by the FDA since 2001 that is extracted from *Galantus nivalis* species [3]; Huperzine A (HupA), an alkaloid from the extract of *Lycopodium* genus [4]; and indole alkaloids from *Ervatamia hainanensis* [5]. To date, plant extracts from *Melodinus* genus, Apocynaceae family, have not been investigated for AChE activity. Pharmacological assays using the crude extract or purified compounds of *Melodinus* species, i.e. *Melodinus tenuicaudatus*, demonstrated potent cytotoxicity against human cancer and bacterial cell lines. Although *Melodinus* genus has not been used for short term memory loss, it is known to be a good source of alkaloids, [4,6], and therefore could be a good source for an AChE inhibitor.

Currently, bioguided fractionation is most commonly used for traditional drug screens. However, this method is costly and time consuming. Dereplication is the most common approach for screening complex matrixes (plant extracts) to identify known compounds and has been approached using a variety of methods including HPLC-SPE coupled to NMR-MS for structural identification [7]. Another method that is commonly used to screen plant extracts is on-line screening with bioactive detection, which has also been previously used to identify inhibitors of AChE [8–10]. In this case, the enzymatic hydrolysis of acetylcholine is monitored and the inhibition of this activity is used to identify active

* Corresponding author at: Bioanalytical and Drug Discovery Unit, Laboratory of Clinical Investigation, National Institute on Aging, National Institutes of Health Suite 100, 8C232 Biomedical Research Center, 251 Bayview Boulevard, Baltimore, MD 21224-6825, USA. Tel.: +1 410 558 8294; fax: +1 410 558 8409.
E-mail address: moaddelru@grc.nia.nih.gov (R. Moaddel).

0039-9140/$ - see front matter Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.talanta.2013.07.046

components. These AChE bioreactors have also been used to determine the affinity, equilibrium and kinetic constants [9,11–13]. This approach uses frontal or zonal (linear and non-linear) chromatography techniques to characterize the desired protein [14–16].

While there are several advantages of bioaffinity chromatography, the process involved for screening plant extracts is nevertheless still challenging. If active compounds are not retained for a significant amount of time by the immobilized protein, binders and non-binders will be co-eluted. In order to circumvent this problem, another plausible approach is the use of protein-coated magnetic beads [17–21]. In this technique, the protein-coated beads are immersed directly into the extract. Any compound with an affinity for the immobilized protein will therefore be retained while non-binders will remain in the extract. The bound compounds can then be eluted to identify active compounds [17,18–20–22]. Previously, Moaddel and collaborators [17], have demonstrated that HSA-MB successfully 'fished' out 3 known binders from a mixture of 6 compounds. In addition, they demonstrated that the HSP-90α-MB could be used to fish out client proteins from a cellular matrix [18]. More recently, Forsberg et al. [9], reported the use of AChE reactors to determine the effect of a complex matrix on the inhibition of AChE activity, while other groups have used AChE coated magnetic beads to determine the inhibitory activity of a known compound [21,22]. However, none of these groups used these bioreactors to 'fish' out the active compound, but rather selected them by their effect on the enzymatic activity. Herein, we report for the first time the use of AChE-coated magnetic beads for the extraction and isolation of an active compound from a complex matrix. In addition, non-linear zonal bioaffinity chromatography using AChE immobilized onto silica fused capillary (AChE-ICER) was used to assess the inhibitors affinity [13] and to assess whether the bound material could inhibit AChE activity.

2. Materials and methods

2.1. Chemicals

Ammonium acetate, tris(hydroxymethyl)aminomethane, acetylcholinesterase from *Electrophorus electricus* (*eel*AChE) type VI-S, choline iodide (Ch), acetylcholine iodide (ACh), galanthamine bromide (GAL), 9-amino-1,2,3,4-tetrahydroacridine hydrochloride hydrate (tacrine –TAC) and dimethyl sulfoxide 99.9% were supplied by Sigma-Aldrich. All chemical materials used during the immobilization procedure were of analytical grade and were purchased from Sigma-Aldrich. BcMag™ amine-terminated magnetic beads were purchased from Bioclone. Methanol and acetonitrile were HPLC grade and the water used for all experiments was deionized in a Millipore Milli-Q system. The mobile phases were prepared daily. The coumarin derivative was prepared as previously described [23].

The LC–MS analyses were performed on an Agilent HP 1100 system, equipped with a vacuum degasser, autosampler, DAD detector and two binary pumps. Data collection was performed using Agilent ChemStation software.

2.2.1. AChE-ICER

The *eel*AChE enzyme was immobilized onto the internal surface of an open tubular silica capillary (100 μm i.d., 30 cm) as previously described [13]. Briefly, the silica-fused capillary was cleaned and coated using a 10% solution (v/v) of APTS in water and left overnight. Then phosphate buffer [50 mM, pH 7.0] containing 4% gluteraldehyde was run through the column followed by phosphate buffer [50 mM, pH 7.0]. An *eel*AChE solution (0.125 mg mL^{-1}) was prepared in phosphate buffer [50 mM, pH 7.0] and run through the column twice. Subsequently, the *eel*AChE was washed with Tris–HCl [100 mM, pH 8.0] and stored at 4.0 °C.

2.2.2. eelAChE-MB

The *eel*AChE was immobilized through the N-terminus onto the surface of magnetic beads resulting in *eel*AChE-MB, following a previously described protocol [17]. Briefly, 25 mg of BcMag was washed three times with 1.0 mL of pyridine [10 mM, pH 6.0] using the manual magnetic separator Dynal MPC-S. The supernatant was discarded and the BcMags were suspended in 1.0 mL of pyridine [10 mM, pH 6.0] containing 5% glutaraldehyde and shaken, at 4.0 °C, for 3.0 h. After magnetic separation, BcMags were washed three more times with 1.0 mL of pyridine [10 mM, pH 6.0], followed by the addition of 0.40 mg of *eel*AChE in 500 μL of pyridine [10 mM, pH 6.0]. The reaction was left for 16 h at 4.0 °C with gentle rotation. The supernatant was discarded and the beads were washed three times with 0.50 mL of Tris–HCl [100 mM, pH 8.0] at 4.0 °C.

2.3. Ligand fishing assay using eelAChE-MB

The prepared *eel*AChE-MB was suspended in 500 μL of an ammonium acetate buffer [15 mM, pH 8.0] containing 100 nM of the reference compounds (Fig. 1: tacrine, galanthamine, coumarin derivative, labetalol and ketamine), alone or in a mixture. The tube was mixed by vigorously shaking for 30 s, set for 90 s and placed into the magnetic separator for 120 s. The supernatant (S-1) was collected, and the *eel*AChE-MB was washed twice with 500 μL of ammonium acetate [15 mM; pH 8.0] by vigorously shaking for 10 s, placing into the magnetic separator for 120 s. The *eel*AChE-MB was then suspended in the elution buffer (500 μL of ammonium acetate [15 mM, pH 8.0] containing 20% of methanol and 1826 μM ACh) and was shaken at 300 rpm with a thermomixer model R (Eppendorf) at 25 °C for 10 min. The supernatant (S-2) was collected and the *eel*AChE-MB was washed twice. To the S-1 100 μL of methanol was added and to S-2 100 μL of ammonium acetate buffer [15 mM, pH 8.0] was added, to maintain a similar ratio of organic to aqueous. Nicotine at 20 μM was used as internal standard.

Calibration curves for all the tested compounds were prepared in serial dilution from 1.28 to 0.02 μM with 20 μM nicotine as the internal standard in ammonium acetate [15 mM, pH 8.0]: methanol (83:17 (v/v)) and 10 μL was injected in the LC–MS system. The samples were prepared in triplicate for the calibration curves that were constructed for each compound from a linear

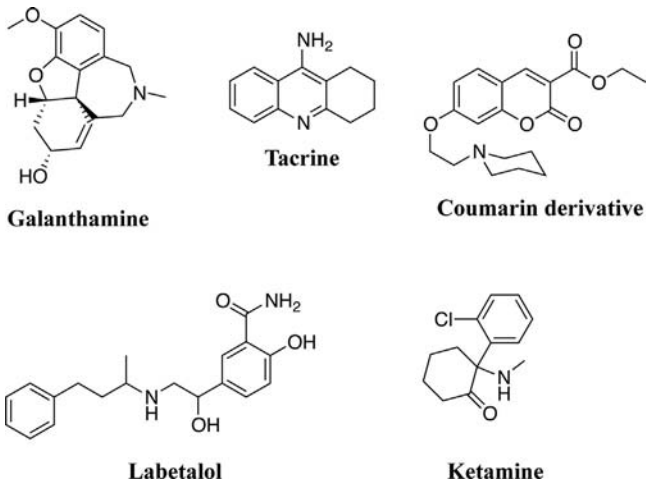

Fig. 1. Reference mixture of acetylcholinesterase ligands and non-ligands.

function by plotting the ratio of peak area and internal standard area against the concentration of compound.

The samples were injected in the LC–MS system eluting in an analytical Atlantis® HILIC column ($2.1 \times 150\ mm^2$; 5 μm) the mobile phase ACN:ammonium acetate buffer [10 mM, pH 4.5] (80:20, v/v), at 0.20 mL min^{-1}. The MS parameters were set at 10.0 L min^{-1} for the drying gas flow, 55.0 psig for the nebulizer pressure and 350 °C the drying gas temperature. The protonated molecular ion $[M+H]^+$ used for quantification for each of the tested compounds was: tacrine (*m*/*z* 199), galanthamine (*m*/*z* 288), coumarin derivative (*m*/*z* 346), labetalol (*m*/*z* 329), ketamine (*m*/*z* 238) and nicotine (*m*/*z* 162.4).

2.4. Preparation of Melodinus fusiformis extract

The dried and powdered twigs and leaves of *M. fusiformis* (10.4 kg) were extracted with 95% aq. ethanol ($30\ L \times 3$) at room temperature, and the solution was concentrated under reduced pressure (40 °C) to give a residue (JSC, 970 g). The concentrated ethanol extract was suspended in water (5.0 L) and then successively extracted with petroleum ($4 \times 4\ L^2$), ethyl acetate ($4 \times 4\ L^2$), and n-butyl acetate ($4 \times 4\ L^2$). Each solution was concentrated under reduced pressure (40 °C) to give petroleum fraction (JSC-P, 210 g), ethyl acetate fraction (JSC-E, 260 g) and n-butanol fraction (JSC-B, 400 g), respectively. For the total alkali, the crude extract was dissolved in 1% hydrochloric acid and filtered. The filtered acidic solution was basified to pH 10 using ammonium hydroxide and re-extracted with ethyl acetate ($0.5\ L \times 2$) to afford the ethyl acetate alkaloid fraction (JSC-E total alkali, 0.36 g).

2.4.1. Screening assay for M. fusiformis extracts

Stock solution of the fractions of *M. fusiformis* ethyl acetate (JSC-E and total alkali JSC-E), butanol (JSC-B and total alkali JSC-B) and petroleum ether (JSC-P) were prepared at 1.0 mg mL^{-1} in DMSO. A working solution of 1.0 μg mL^{-1} was prepared in water. Samples at 1, 10 and 250 ng mL^{-1} of each fraction were prepared in water containing 490 μM ACh and 10 μL of each sample was injected in the LC–MS system. The area ratio of Ch/ACh was determined and, the extract that presented the lowest ratio (largest inhibition) was selected for analysis.

2.4.2. Ligand fishing assay and screening for total alkali JSC-E

The validated method for ligand fishing assays using *eel*AChE-MB was applied to total alkali JSC-E (T.A.-JSC-E) at 2.0 μg mL^{-1}. The *eel*AChE-MB was suspended in the extract and the supernatants $S_{T.A.}$-1 and $S_{T.A.}$-2 were collected. An injection of 10 μL of each supernatant collected was carried out in the LC–MS system using the fingerprint method developed for T.A.-JSC-E. Based on the compounds that were identified (*m*/*z* and retention time) in $S_{T.A.}$-2, five fractions are collected (Table 1), stream dried under nitrogen and resuspended in 100 μL of water containing 490 μm ACh and injected in the *eel*AChE-ICER using the LC–MS system. For each tested fraction sample, a negative control (absence of ACh) and a positive control samples (ACh and absence of ligand) were analyzed. The area ratio of choline to acetylcholine was calculated and the fraction sample that presented the lowest ratio (strongest inhibition) value over positive control sample was selected and injected (10 μL) in the LC–MS system for fingerprint assay.

2.4.3. Fingerprint for total alkali JSC-E

The analysis was carried out on the analytical Zorbax Eclipse XDB-C_{18} column ($4.60 \times 150\ mm^2$, 5 μm) with the following conditions: flow rate 0.8 mL min^{-1} and injection volume 10 μL, with the mobile phase consisting of 0.05% aqueous formic acid (v/v) (A) and methanol (B). The gradient elution was 20–25% (B) in 20 min, 25–40% (B) in 0.01 min, 40–70% in 40 min and 70–90% (B) in 0.01 min with the total run time equal to 100 min. The MS parameters were set at 10.0 L min^{-1} for the drying gas flow, 55.0 psig for the nebulizer pressure and 350 °C for the drying gas temperature. Scans from 100.0 to 400*m*/*z* and 400–800*m*/*z* were carried out in both the negative and positive ionization modes.

2.5. Isolation of compound (156m/z) from total alkali JSC-E

The isolation was carried out injecting 20 μL of T.A.-JSC-E extract solution (10 mg mL^{-1}) prepared in DMSO:H_2O (20:80, v/v) in a semi-preparative Zorbax Eclipse XDB-C_{18} ($9.60 \times 250\ mm^2$, 5.00 μm) column with the following conditions: flow rate 1.5 mL min^{-1}, mobile phase consisting of water (A) and acetonitrile (B). The gradient elution was from 30% to 50% (B) in 30 min, collecting fractions from 6 to 7 min. The column was cleaned after each run with 90% (B) and equilibrates with 30% (B) for 90 min. The MS parameters were set at 12.0 L min^{-1} for the drying gas flow, 55.0 psig for the nebulizer pressure and 350 °C the drying gas temperature.

A total of 24 fractions were collected and stream dried with nitrogen, to provide a weight equal to 1.0 mg. A stock solution at 1.0 mg mL^{-1} was prepared in methanol, an aliquot of 20 μL was added to 180 μL methanol to prepare a sample solution at 200 μg mL^{-1} and, 10 μL was injected in the LC–MS fingerprint method to verify the compound purity.

2.6. Non-linear chromatography assays

The NLC assays were carried out as previously described [24]. The method was validated, using the reference inhibitors of AChE enzyme. The K_d of tacrine, galanthamine and coumarin derivative

Table 1
Overview of range times and the ions collected in each fraction. Results of *eel*AChE-ICER screening for fractions 1–5 collected from *Melodinus fusiformis* total alkali JSC ethyl acetate extract (T.A.-JSC-E).

Time range (min)	Fraction	Ions (*m*/*z*)	Ratio ($Area_{ACh}/Area_{Ch}$)	Inhibition percentage
1.0–10	1	156.2	6.6	40
45–50	2	363.7; 368.7	10	9.0
52–56	3	300.7; 484.0; 489.0	8.6	21
62–80	4	206.4; 358.7; 363.7; 368.7; 369.0; 374.8; 390.8; 400.0; 406.0; 406.8; 412.8; 420.8; 428.9; 436.0; 436.9; 484.0; 489.0; 522.4; 550.0; 579.2; 581.2; 582.2; 583.0; 641.2	15	−0.36
83–90	5	368.8; 337.9; 402.8	9.9	10
	Positive control		11	–

were determined using 0.50 serial dilutions from 20 nM to 1.25 nM for tacrine; 4–0.25 nM for the coumarin derivative, and 100–6.25 nM for galanthamine. The samples were injected in the LC–MS system with the *eel*AChE-ICER.

The shape of the peaks were used to determine the parameters a_1, a_2 and a_3 for each concentration (Eq. (1)) and then to calculate the kinetics parameters: $k_{off}=1/a_2/t_0$, $k_{on}=a_3/C_0$ and $K_a=k_{on}/k_{off}$, where C_0 is the concentration of compound injected multiplied by the injected volume and t_0 is the dead time of the column [25].

$$y=\frac{a_0}{a_3}\left[1-\exp\left(-\frac{a_3}{a_2}\right)\right]\left[\frac{\sqrt{(a_1/x)I_1}(2\sqrt{a_3x}/a_2)\exp(-x-a_1/a_2)}{1-T[(a'_1/a_2)(x/a_2)][1-\exp-(a_3/a_2)]}\right] \quad (1)$$

The validated method was applied to calculate the kinetic parameters for the isolated ligand. A working solution of the isolated ligand at 62 ng mL^{-1} was prepared from the stock solution. The K_d of the isolated ligand was determined with the following equation: $1/K_a$, using 0.75 serial dilutions from 27 to 3 nM, assuming the molecular weight of the compound is 155 g mol^{-1}. The chromatograms were analyzed using the PeakFit Software version 4.12.

3. Results and discussion

3.1. Validation of ligand fishing assays

Calibrations curves were done before and after the samples using the reference compounds. Both were linear in the range studied for each compound, with mean correlation coefficients ($n=3$) of 0.99 or higher, accuracy values between 80.0% and 116% and RSD in the range of 0.260–7.65. In order to validate the ligand fishing assay, a series of experiments were carried out using the reference compounds individually and as a mixture (Fig. 2a and b, respectively). As expected, ketamine and labetalol were only present in S-1, demonstrating that the non-binders were not retained by the *eel*AChE-MB. Further, galanthamine, tacrine and the coumarin derivative, known binders, although also present in some cases in S-1, were predominantly present in S-2. In fact, the fishing capacity is associated with the enzyme affinity and inhibitory constants. Subsequently, the experiment was repeated on the control magnetic beads, end-capped with Tris without the presence of *eel*AChE. In these studies, greater than 90% of all the compounds tested were present in S-1 and wash supernatant, with no presence in S-2, with the exception of the coumarin derivative which had 13% present in S-2 and 87% in S-1 (data not shown). Although this was the largest amount present in S-2 for the control magnetic beads, the *eel*AChE-MB had greater than 95% present in S-2; therefore, the retention on the control beads is of no concern.

The results of the individual fishing experiments were then compared to the fishing experiments using the mixture of compounds and similar results were obtained, where the inhibitor with the strongest affinity, the coumarin derivative, was predominantly present in S-2, followed by tacrine and galanthamine.

3.2. Screening assay for M. fusiformis extracts

Fig. 3 exemplifies the workflow for the screening of five extracts of *M. fusiformis* leaves. Samples at 1.0 ng mL^{-1}, 10 ng mL^{-1} and 250 ng mL^{-1} of each plant extract were screened (Fig. 3 – Part I) to determine which extract could inhibit AChE in a concentration-dependent manner. The peak area ratio of enzymatic catalysis product and remaining substrates were calculated, and the results are illustrated in Fig. 4. For that, the inhibition percentage was obtained based on the average ratio of the five extracts, in which T.A.-JSC-E had a 76% inhibition rate.

Based on these results, the T.A.-JSC-E was selected for the ligand fishing experiment. Prior to the ligand fishing experiments, a fingerprint of the T.A.-JSC-E extract was carried out. The fingerprint was monitored in both positive and negative mode with molecular weight ranges from 100 to 400m/z and 400–700m/z.

3.3. Screening assays for total alkali JSC-E extract

A final concentration of 2.0 μg mL^{-1} of T.A.-JSC-E extract was used for the ligand fishing experiment (Fig. 3—Part II). The supernatants $S_{T.A.}$-1 and $S_{T.A.}$-2 were collected and each fraction was fingerprinted in both negative and positive modes (Fig. 3—Part III). For $S_{T.A.}$-2, while no m/z ions peaks were observed in the negative ionization mode, there were over 30m/z ions detected in the positive ionization mode. In order, to determine whether the fractions could inhibit the *eel*AChE-ICER, five fractions (Table 1) were collected based on the retention times. For the assay, after stream drying, they were resuspended in 100 μL of water containing 490 μm ACh. The area ratio of choline produced to remaining acetylcholine was calculated and the inhibition percentages were established using a positive control sample (Table 1).

Of the five fractions assayed, Fraction 1 had the inhibitory activity greater than 40%. Therefore, a semi-preparative LC method was developed in order to isolate a sufficient amount for initial

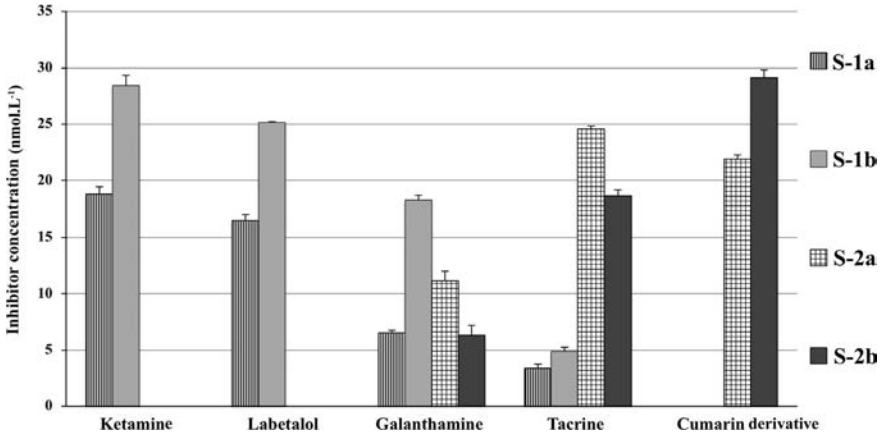

Fig. 2. Inhibitor concentration present on samples S-1 and S-2. (a) Assay with a unique reference compound, and (b) assay with a reference mixture.

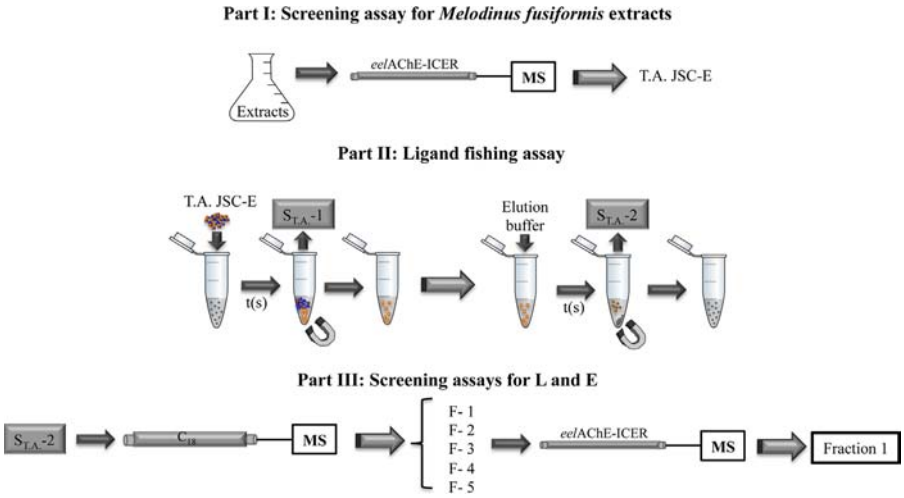

Fig. 3. Workflow for the screening assay with *Melodinus fusiformis* extracts.

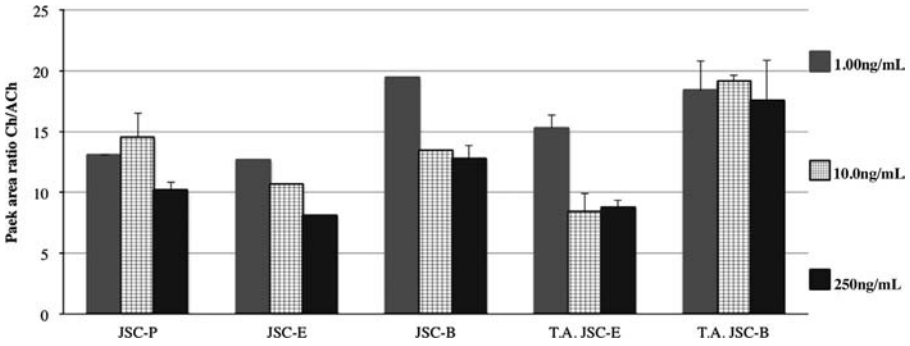

Fig. 4. Screening assay for the extracts of *Melodinus fusiformis* species.

Table 2
Non-linear chromatography and kinetics parameters determined for tacrine, galanthamine and the coumarin derivative in *eel*AChE–ICER.

Inhibitors	Concentration (nM)	k_{off} (s^{-1})	k_{on} ($s^{-1}nM^{-1}$)	K_d (nM)
Tacrine	2.50	1.43	0.149	10.49
	5.00	1.32	0.125	
	20.0	1.56	0.141	
Galanthamine	25.0	1.21	0.013	198
	50.0	1.14	0.006	
	100	1.18	0.004	
Coumarin derivative	0.250	0.98	1.22	4.30
	1.00	1.03	0.585	
	2.00	0.99	0.303	
	4.00	1.05	0.167	

characterization using the *eel*AChE-ICER prepared. The purity of the isolated compound (156m/z) was verified by LC–MS.

3.4. Calculation of association/dissociation constant (k_{on} and k_{off}) and equilibrium dissociation constant (K_d)

Although, the affinity of an inhibitor for the *eel*AChE can be determined using the frontal bioaffinity chromatography, it requires a sufficiently large amount of sample (>2.0 mg). In order to circumvent this limiting step, an alternative is to determine the k_d values using non-linear chromatography, which would require a much smaller sample volume. Non-linear chromatography can be used to determine the k_{on}, k_{off} and K_a of a compound. In this approach, the Impulse Input Solution program is used to fit the experimentally obtained peak profiles. The shape of the peaks were used to determine the parameters a_1, a_2 and a_3 for each concentration Eq. ((1)) and then to calculate the kinetics parameters: k_{on}, k_{off} and K_a, where a_2 is the peak's broadness. Initially, the method is validated using the reference acetylcholinesterase inhibitors: tacrine, galanthamine and the coumarin derivative (Table 2).

The K_d values obtained for the reference inhibitors in *eel*AChE-ICER are in agreement with the previously determined K_i values [13], with the same relative order of coumarin derivative as the strongest and galanthamine as the weakest inhibitor. Based on these results, NLC was carried out with the isolated compound (Fig. 5) and its K_d determined as 0.82 μm, four times weaker than the calculated binding affinity of the reference inhibitor, galanthamine (K_d=0.19 μm).

4. Conclusion

The AChE coated magnetic beads were prepared and the application of protein coated magnetic beads for 'fishing' out and identification of an active compound from a complex matrix was carried out. The data presented suggests that the protein-coated magnetic beads could be a very useful tool in the isolation of an active compound from complex matrices. The novel acetylcholinesterase inhibitor isolated had a K_d of 800 nM and may present a novel scaffold for AChE inhibitors; however, full characterization is required.

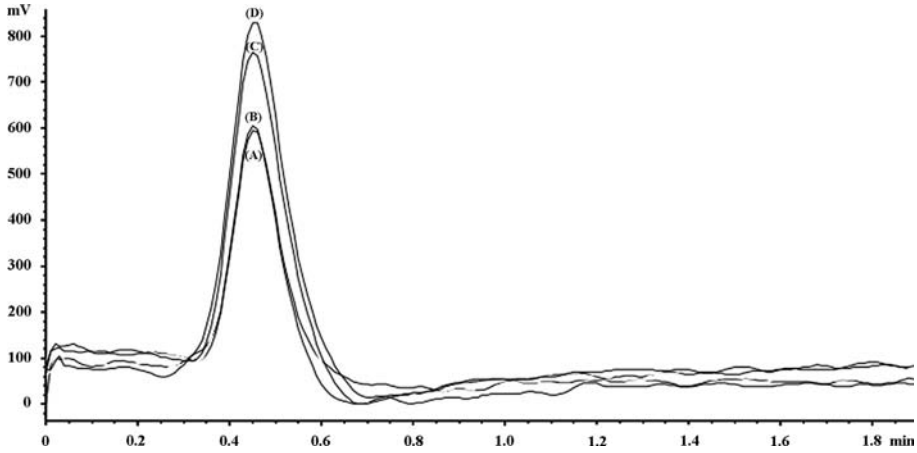

Fig. 5. Extracted ion chromatograms (EICs) for 156*m*/*z* at 3.0 (A), 8.0 (B), 20 (C) and 27 nmol L^{-1}(D) by non-linear chromatography assays.

Acknowledgments

This research was supported by the Intramural Research Program of the National Institute of Aging NIH, by the Brazilian Agency for support and Evaluation of Graduate Education (CAPES). The authors also thank the National Institute of Science and Technology (INCT), Controle Biorracional de Insetos Praga (CBIP), and National Council for Scientific and Technological Development (CNPq).

References

[1] I. Silman, C.B. Millard, A. Ordentlich, H.M. Greenblatt, M. Harel, D. Barak, A. Shafferman, J.L. Sussman, Chem. Biol. Interact. 119–120 (1999) 43–52.
[2] D.J. Newman, G.M. Cragg, J. Nat. Prod. 70 (2007) 461–477.
[3] C.V. Junior, V.D.S. Bolzani, M. Furlan, C.A.M. Fraga, E.J. Barreiro, Quím. Nova 27 (2004) 655–660.
[4] Y. Yang, L. Xi-Qiang, T. Chun-Ping, Y. Sheng, Chin. J. Nat. Med. 10 (2012) 1–12.
[5] Z.-J. Zhan, Q. Yu, Z.-L. Wang, W.-G. Shan, Bioorg. Med. Chem. Lett. 20 (2010) 6185–6187.
[6] Y.-P. Liu, Y. Li, X.-H. Cai, X.-Y. Li, L.-M. Kong, G.-G. Cheng, X.-D. Luo, J. Nat. Prod. 75 (2012) 220–224.
[7] M. Lambert, J.-L. Wolfender, D. Stærk, S.B. Christensen, K. Hostettmann, J. W. Jaroszewski, Anal. Chem. 79 (2007) 727–735.
[8] I.K. Rhee, N. Appels, T. Luijendijk, H. Irth, R. Verpoorte, Phytochem. Anal. 14 (2003) 145–149.
[9] E.M. Forsberg, J.R.A. Green, J.D. Brennan, Anal. Chem. 83 (2011) 5230–5236.
[10] W. Min, W. Wang, J. Chen, A. Wang, Z. Hu, Anal. Bioanal. Chem. 404 (2012) 2397–2405.
[11] V. Andrisano, M. Bartolini, R. Gotti, V. Cavrini, G. Felix, J. Chromatogr. B Biomed. Sci. Appl. 753 (2001) 375–383.
[12] J.I. da Silva, M.C. de Moraes, L.C.C. Vieira, A.G. Corrêa, Q.B. Cass, C.L. Cardoso, J. Pharm. Biomed. Anal. 73 (2013) 44–52.
[13] K.L. Vanzolini, L.C.C. Vieira, A.G. Corrêa, C.L. Cardoso, Q.B. Cass, J. Med. Chem. 56 (2013) 2038–2044.
[14] R. Moaddel, K. Jozwiak, R. Yamaguchi, I.W. Wainer, Anal. Chem. 77 (2005) 5421–5426.
[15] C. Temporini, S. Ceruti, E. Calleri, S. Ferrario, R. Moaddel, M.P. Abbracchio, G. Massolini, Anal. Biochem. 384 (2009) 123–129.
[16] M.A. Nelson, A. Moser, D.S. Hage, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 878 (2010) 165–171.
[17] R. Moaddel, M.P. Marszałł, F. Bighi, Q. Yang, X. Duan, I.W. Wainer, Anal. Chem. 79 (2007) 5414–5417.
[18] M.P. Marszałł, R. Moaddel, S. Kole, M. Gandhari, M. Bernier, I.W. Wainer, Anal. Chem. 80 (2008) 7571–7575.
[19] L. Pochet, F. Heus, N. Jonker, H. Lingeman, A.B. Smit, W.M.A. Niessen, J. Kool, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 879 (2011) 1781–1788.
[20] M. Yasuda, D.R. Wilson, S.D. Fugmann, R. Moaddel, Anal. Chem. 83 (2011) 7400–7407.
[21] N.B. Oujji, I. Bakas, G. Istamboulié, I. Ait-Ichou, E. Ait-Addi, R. Rouillon, T. Noguer, Sensors 12 (2012) 7893–7904.
[22] F. Hu, H. Zhang, H. Lin, C. Deng, X. Zhang, J. Am. Soc. Mass Spectrom. 19 (2008) 865–873.
[23] L.C.C. Vieira, Chemistry, Federal University of São Carlos, São Carlos, 2010, p. 141.
[24] K. Jozwiak, J. Haginaka, R. Moaddel, I.W. Wainer, Anal. Chem. 74 (2002) 4618–4624.
[25] M. Sanghvi, R. Moaddel, I.W. Wainer, J. Chromatogr. A 1218 (2011) 8791–8798.