

The physicochemical properties and the *in vivo* AChE inhibition of two potential anti-Alzheimer agents, bis(12)-hupyrindone and bis(7)-tacrine

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Abstract

The lipophilicity and solubility profiles of bis(12)-hupyrindone (B12H) and bis(7)-tacrine (B7T), two novel acetylcholinesterase inhibitors dimerized from huperzine A fragments and tacrine, respectively, were investigated over a broad pH range. Lipophilicity was assessed by both shake flask method with 1-octanol–water system and a reverse-phase HPLC system with methanol–water as mobile phase. The former method was used for determining the lipophilicities of the ionized forms ($\log D$) of the dimers while the latter method was used for that of the neutral forms ($\log P$). The $\log P$ values for B12H and B7T were found to be 5.4 and 8.2, respectively, indicating that the two dimers are highly lipophilic. The solubilities of both dimers were found to be affected by pH. The solubility of B12H was >1.41 mg/ml when the pH was <7 , but <0.06 mg/ml when the pH was >8 . The solubility of B7T was >0.26 mg/ml when the pH was <9 , but <0.005 mg/ml when the pH was >12 . The ionic strength of a solution could affect the solubilities considerably (11.16 mg/ml for B12H and 12.71 mg/ml for B7T in water; 2.07 mg/ml for B12H and 0.36 mg/ml for B7T in saline). The ionization constants (pK_a) of the two dimers were determined by UV spectrophotometry. Both dimers were found to have two pK_a values: 7.5 ± 0.1 (pK_{a1}) and 10.0 ± 0.2 (pK_{a2}) for B12H; and 8.7 ± 0.1 (pK_{a1}) and 10.7 ± 0.4 (pK_{a2}) for B7T. Furthermore, an *in vivo* pharmacological assay conducted in mice showed that a maximum AChE inhibition occurred 15 min after the single-dose and intraperitoneal administration of either dimer. This indicates that the two dimers may easily cross the blood–brain barrier. In summary, these physicochemical characteristics suggest that the two dimers may be promising candidates for the development of better drugs for Alzheimer's disease.

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1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease that attacks the brain and results in impaired memory, thinking and behaviour in the elderly [1]. There are many different theories on the causes of AD and one of the well-established theories suggests that the neurotransmitter acetylcholine lev-

els are too low in the brains of AD patients [2,3]. Although several acetylcholinesterase (AChE) inhibitors have been developed, they are only useful for treating patients with mild to moderate AD [4,5]. Tacrine (TAC, Fig. 1A), the first approved AChE inhibitor for AD, has been found to have potential hepatotoxicity [6] and is therefore seldom used nowadays. As a result, the development of more effective anti-AD agents is still an important area of AD-associated research. With the identification of the three-dimensional structure of AChE and the assistance of computer docking programs, a series of new dimers has been designed based on the structures of tacrine [7,8] and huperzine A (Fig. 1B, extracted from the Chinese

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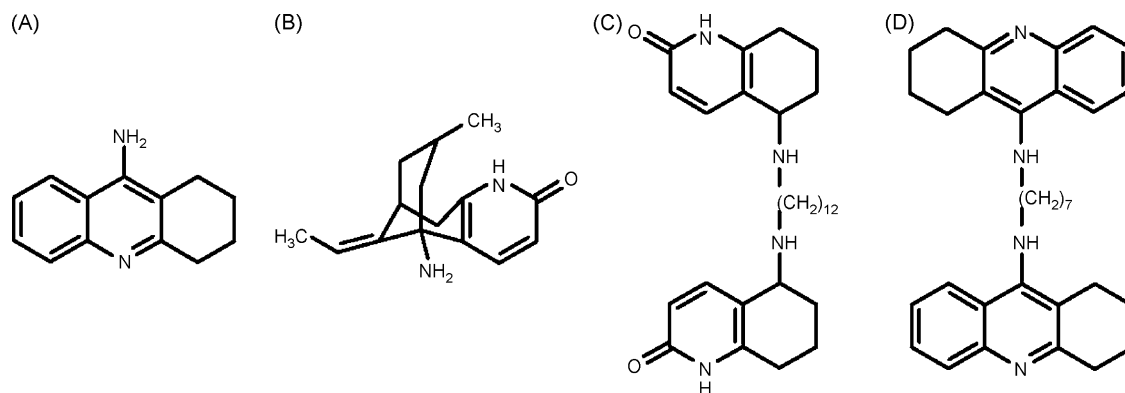


Fig. 1. Structures of tacrine (A), huperzine A (B), bis(12)-hupyridone (C) and bis(7)-tacrine (D).

medicinal plant *Huperzia serrata* and is now in Phase II clinical trial for AD treatment in the USA [9]). Of the newer drugs, bis(12)-hupyridone (B12H, Fig. 1C) is a selective and potent AChE inhibitor *in vitro* while bis(7)-tacrine (B7T, Fig. 1D) has been demonstrated to have similar effects both *in vitro* and *in vivo* [10–14]. B7T has also been shown to have multiple actions such as antagonizing *N*-methyl-D-aspartate (NMDA) receptors [15] and gamma-aminobutyric acid (GABA_A) receptors [16] and inhibiting nitric oxide synthase [17], which may play synergistic roles in the treatment of AD [17,18].

Although these new dimers may offer superior therapeutic potentials, their physicochemical properties such as lipophilicities ($\log P$ or $\log D$), ionization constants (pK_a) and solubilities remain unknown. Determination of aqueous solubility as a function of pH along with pK_a is important to the understanding of the dissolution abilities of the dimers (for solid dosage forms) and their subsequent permeability through cell membrane [19]. Further, the 1-octanol/water partition coefficients may help understand the lipophilicity of the compounds, thus predicting their absorption pathways and even their sites of action [20]. An investigation of the physicochemical properties of the dimers is also an essential step in understanding the pharmacokinetic profiles *in vivo*. In this study, we determined the various physicochemical properties of B12H and B7T—partition coefficients, ionization constants and solubilities. In addition, we also examined the *in vivo* AChE inhibitory ability by B12H and compared that with B7T.

2. Materials and methods

2.1. Chemicals

Bis(12)-hupyridone dihydrochloride (B12H, Fig. 1B) and bis(7)-tacrine dihydrochloride (B7T, Fig. 1C) were synthesized as described previously [21,22] and their purities were up to 99.9% (detected by HPLC analysis). Tacrine hydrochloride (TAC, Fig. 1A) and other chemicals (all in analytical-grade) were purchased from Sigma Chemicals Ltd. (St. Louis, MO, USA). Water was prepared with an EASYpure UV system (model D7401; Barnstead Thermolyne Co. Dubuque, IA, USA).

2.2. Animals

Male ICR mice (25–35 g, 6 weeks of age) supplied by the Animal and Plant Care Facility of the Hong Kong University of Science and Technology were fed on a standard laboratory diet with free access to water at a controlled temperature of 20–22 °C and relative humidity of 50% with a 12 h light/dark cycle prior to the study. Before experiments, all the mice were fasted but were allowed to have free access to water overnight.

2.3. Analytical method

About 1.0 mg, 1.5 mg and 1.0 mg of TAC, B12H and B7T, respectively, were accurately weighed and dissolved in water in 25 ml volumetric flasks. 10 ml of each solution was transferred to a 50 ml volumetric flask and then diluted to the mark with water. Certain volumes of the stock solutions (0.1–0.7 ml) were then transferred into 1 ml volumetric flasks and diluted to the marks with water. The final concentrations of the solutions were analyzed in triplicate using a DU 640 UV/Vis Spectrophotometer (Beckman Coulter, Fullerton, CA, USA) at wavelengths of 240, 229 and 244 nm for TAC, B12H and B7T, respectively. Calibration curves were constructed by plotting the concentrations as a function of UV absorbance values.

For method validation, intra-day precision was determined by analyzing six replicates of control samples at low, medium and high concentrations of the calibration range within the same day. The inter-day precision was determined by using samples prepared in a similar manner on five separate days. Precisions were reported as the relative standard deviations (R.S.D.) and accuracies expressed as $[(1 - (\text{mean concentration measured} - \text{concentration added}) / \text{concentration added})] \times 100\%$.

2.4. Measurement of partition/distribution coefficients

The lipophilicities for neutral forms of TAC, B12H and B7T were measured by an HPLC method recommended by the “Organization for Economic Co-operation and Development (OECD)” [23]. The analytical process was carried out with an Agilent HP1100 system (Hewlett-Packard, Palo Alto, CA, USA) equipped with an Alltech 2000 Evaporative Light Scattering

Detecting (ELSD) system coupled with an Alltech lower temperature adaptor (LTA) (Alltech, Deerfield, IL, USA). The column used for elution was an Agilent Extend C₁₈ (4.6 mm × 150 mm, I.D. 5 μm). The mobile phase was water:methanol/25:75 (v/v) and the pH was adjusted to 11.2. The flow rate was 1.0 ml/min. Since the log *P* value of a compound is related to its retention time in HPLC analysis using the water–methanol system under isocratic condition, log *P* values may be calculated from Eq. (1):

$$\log P = \log \frac{t_R - t_0}{t_0} + C \quad (1)$$

in which *t_R* is the retention time of the test compound, *t₀* is the dead time, and *C* is the intercept on the *y*-axis (log *P*). Quantitation is not required and only the determination of retention times is necessary.

A calibration curve was established by plotting the *t_{RS}* to log *P* values of six chemicals (cimetidine, clonidine, nifedipine, dexetimide, pimozone and cholesterol) and the *t_{RS}* of TAC, B12H and B7T were determined for the calculation of log *P* values.

1-Octanol–water distribution coefficients were determined by the shake flask method as described previously [24]. Phosphate buffer in the pH range of 2.0–8.0 were used as the aqueous phase. The two phases were mutually saturated before the experiment. About 1.0 mg, 2.0 mg, 2.0 mg of TAC, B12H and B7T, respectively, were weighed accurately and dissolved in 50 ml of buffers previously saturated with 1-octanol. 4.0 ml of the solution and 4.0 ml of 1-octanol were mixed together in a 25 ml conical flask. The conical flasks were stoppered to prevent solvent evaporation. The flasks were then shaken horizontally at 25 °C on a rotator (DS-500 VWR orbital shaker, Bridgeport, NJ, USA) with 130 cycles/min for 3 h and then allowed to stand for another 30 min. The mixtures from the flasks were then centrifuged for 15 min at 3000 rpm. The aqueous phases were analyzed before and after equilibration as described in Section 2.3. Dilution of the samples was made if the values of absorbance were over 0.9. The distribution coefficients were calculated according to Eq. (2):

$$D = \frac{C_0 - C_1}{C_1} \times \frac{v_{\text{aq}}}{v_{\text{octanol}}} \quad (2)$$

in which *C₀* and *C₁* are the drug concentrations before and after equilibration, and *v_{aq}* and *v_{octanol}* are the volumes of water and 1-octanol used.

Each determination was performed at least six times and the mean values were used to produce the log *D* versus pH profile.

2.5. Solubility measurements

The solubilities of the two dimers in water and various buffer solutions were determined at a constant temperature of 25.0 ± 1.0 °C and a constant ionic strength of 0.15 M NaCl in 50 mM phosphate buffers. Appropriate amounts of the compounds were added to 2 ml of water or buffers in glass vials. The vials were then capped and placed in a shaking bath for

at least 12 h. Preliminary experiments showed that equilibrium was achieved within that period of time. Samples were filtered through a milipore nylon filter (0.22 μm) (Millipore Corporation, Bedford, USA). B7T and B12H in the filtrates were further diluted and determined spectrophotometrically at 229 or 244 nm, respectively.

The effects of ionic strength ([Na⁺] and [Cl⁻]) on the solubilities of B12H and B7T were also determined. Solutions containing different [Cl⁻] (0.05, 0.10 and 0.15 M of HCl) or [Na⁺] (0.05, 0.10 and 0.15 M with constant [Cl⁻] at 0.15 M) were prepared and the procedures described above were followed.

2.6. Determination of ionization constants

The measurement of p*K_a* was based on a method described previously [25]. TAC, B12H and B7T were freshly dissolved in water at concentrations of less than 0.02 mM. The pH of the solutions was adjusted by adding 0.5 M HCl or 0.5 M NaOH standard solutions while the ionic strength of each solution was maintained below 0.001. The pH values were measured with an Orion 420A Benchtop pH Meter and an Orion 9157BN Triode™ 3-in-1 combination pH electrode (Orion, New Hyde Park, NY, USA). UV absorbance was determined at 12 random wavelengths near to the maximum absorption wavelength and the p*K_a* values were calculated from Eq. (3):

$$\text{p}K'_a = I - \log \frac{(10^b - 10^a)A_1 + (1 - 10^b)A_2 + (10^a - 1)A_3}{(10^a - 10^b)A_1 + (10^{a+b} - 10^a)A_2 + (10^b - 10^{a+b})A_3} \quad (3)$$

in which *A₁*, *A₂* and *A₃* (*A₁* ≠ *A₂* ≠ *A₃*) are the UV absorbance values measured at three different pH values (*I*, *I* + *a* and *I* + *b*, *a* ≠ *b* ≠ 0).

2.7. Inhibition of AChE activity in mice cortex

ICR mice (25–35 g) were randomly assigned into three groups (I: saline control, II: B12H, 5.28 μmol/kg and III: B7T, 3.54 μmol/kg; intraperitoneal). Whole cortex was dissected on ice at 5, 15, 30, 60, 120 and 240 min (*n* = 6) after drug administration and then homogenized in 40 vol (w/v) ice-cold PBS (0.1 mM, pH 7.4). The homogenate was then centrifuged at 3000 rpm for 15 min at 4 °C and then pre-incubated at 37 °C for 5 min with ethopropazine (0.1 mM), a selective inhibitor of BChE. The AChE activity assay was performed using a colorimetric method [26] with minor modifications. A mixture of 2 ml in volume which contained 0.1 ml acetylcholine iodide (12 mM), 1.8 ml sodium phosphate buffer (0.1 mM, pH 7.4), and 0.1 ml homogenate was incubated at 37 °C for 8 min. The reaction was terminated by adding 1 ml of 3% (w/v) sodium lauryl sulphate (SDS), and then 1 ml of 0.2% (w/v) 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB) to produce a yellow complex. The colour production was measured spectrophotometrically at 420 nm. At the same time, the protein content was measured with the Coomassie blue protein-binding method (Bio-Rad Labora-

Table 1
The retention times ($n=6$), $\log(t_R - t_0)/t_0$ and $\log P$ values of the reference compounds

Compounds	t_R (min)	$\frac{t_R - t_0}{t_0}$ *	$\log \frac{t_R - t_0}{t_0}$	$\log P^{**}$
Cimetidine	1.370	0.231	-0.636	0.40 a
Clonidine	1.593	0.431	-0.366	1.59 a
Nifedipine	1.919	0.724	-0.140	2.20 b
Dexetimide	3.442	2.093	0.321	3.55 a
Pimozide	8.693	6.810	0.833	6.30 a
Cholesterol	30.718	26.599	1.424	8.74 c

* t_0 was 1.113 min which was determined by using copper sulphate.

** $\log P$ values were taken from references [28] (a), [29] (b) and [30] (c).

tories, Hercules, CA, USA) [27], using bovine serum albumin as standard. The AChE inhibition was calculated by Eq. (4).

$$\text{AChE inhibition} = - \left(1 - \frac{\text{OD}_{(\text{test})}}{\text{OD}_{(\text{control})}} \right) \times 100\% \quad (4)$$

in which $\text{OD}_{(\text{test})}$ and $\text{OD}_{(\text{control})}$ are the absorbance values of the samples obtained from the tested and control groups, respectively.

3. Results and discussion

3.1. Analytical method

The derived calibration curves exhibited a good linear response within the concentration ranges of 0.744–5.208 $\mu\text{g/ml}$ for TAC ($y = 6.9346x - 0.0561$, $r^2 = 0.9999$), 1.384–9.568 $\mu\text{g/ml}$ for B12H ($y = 15.912x - 0.0547$, $r^2 = 0.9999$) and 0.888–6.126 $\mu\text{g/ml}$ for B7T ($y = 11.487x - 0.0676$, $r^2 = 0.9999$). Method validation demonstrated good overall intra-day variation ($\leq 1.00\%$) and inter-day variation ($\leq 1.71\%$). The accuracies of all tests were 99.0–103.5%.

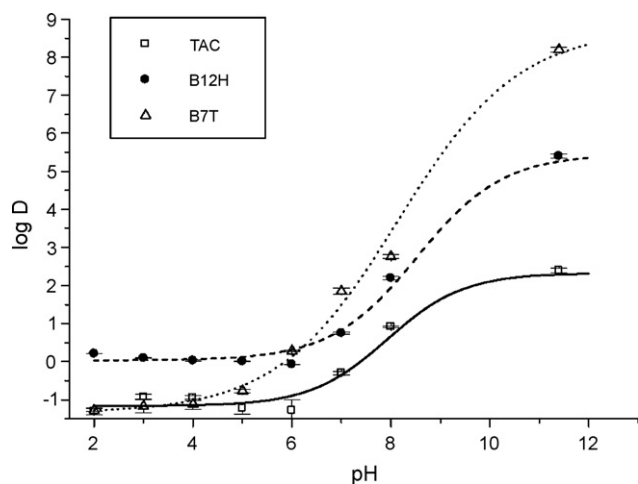


Fig. 2. Experimental $\log D$ (mean \pm S.D., $n=6$) vs. pH profiles of TAC (solid line), B12H (dashed line) and B7T (dotted line). The $\log D$ values in phosphate buffers at pH below 8 were determined by shake flask method while those values at pH 11.2 were measured using HPLC method.

Table 2
Determination of pK_a values for TAC, B12H and B7T ($n=3$)

Compounds	Selected pH for pK_a calculation ^a		Wavelength range ^b		pK_a (mean \pm S.D.) ^c
TAC					
pK_{a1}	8.58	9.54	9.99	210–320	9.94 ± 0.22
B12H					
pK_{a1}	6.19	7.18	8.50	225–320	7.46 ± 0.11
pK_{a2}	9.42	10.02	10.50	220–335	10.00 ± 0.16
B7T					
pK_{a1}	6.49	7.63	8.95	220–340	8.70 ± 0.09
pK_{a2}	9.55	10.47	11.51	220–340	10.66 ± 0.41

^a Various pH values were selected for pK_a determination. Both B12H and B7T are dibasic salts and therefore two sets of pH values were used for the determination of pK_{a1} and pK_{a2} .

^b The wavelength range was selected based on the results obtained from the UV absorption spectra.

^c The pK_a values at 12 random wavelengths near to the maximum absorption wavelength were calculated by using Eq (3).

3.2. Lipophilicity

The distribution coefficients were determined by a shake flask method and an HPLC method over the pH range of 2.0–11.2.

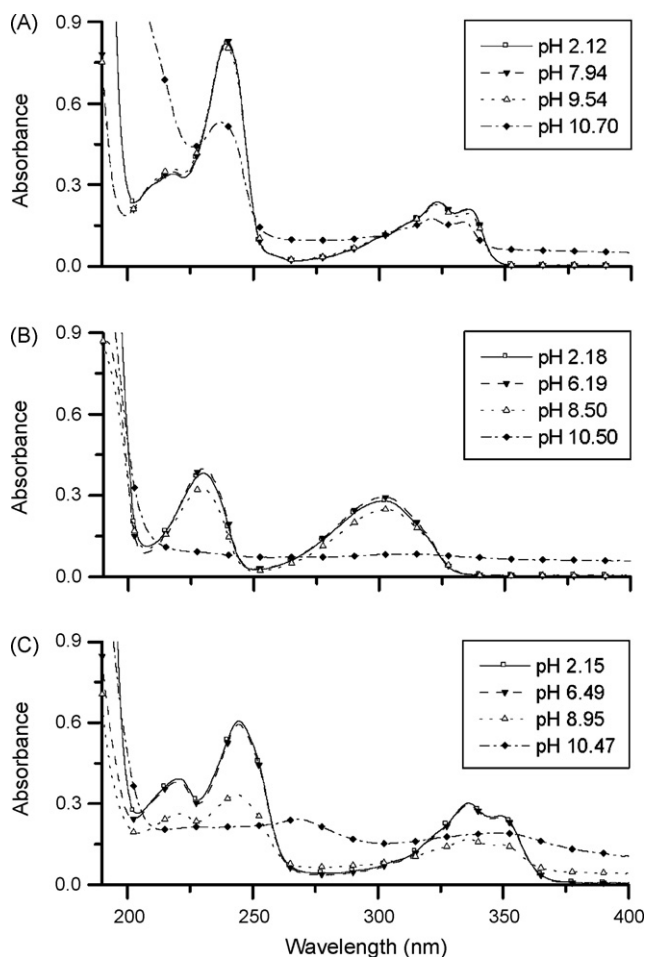


Fig. 3. Effects of pH values on UV absorbance for TAC (A), B12H (B) and B7T (C). During the experiments, buffers with pH values between 2 and 11.5 were used. In each graph, only few representative curves are shown in order to illustrate the trend of the pH effects.

Table 3
Effect of ionic strength on solubilities (mean \pm S.D., mg/ml, $n=6$) for B12H and B7T

Compounds	[Cl ⁻] (mol/L) ^a			[Na ⁺] (mol/L) ^b		
	0.05	0.10	0.15	0.05	0.10	0.15
B12H	6.38 \pm 0.06	2.98 \pm 0.12	1.95 \pm 0.02	2.19 \pm 0.02	2.42 \pm 0.05	2.36 \pm 0.01
B7T	2.30 \pm 0.01	0.55 \pm 0.02	0.28 \pm 0.01	0.29 \pm 0.01	0.40 \pm 0.01	0.35 \pm 0.03

^a [Cl⁻] was adjusted by addition of different concentrations of HCl.

^b [Na⁺] was adjusted by addition of different concentrations of NaCl, and the [Cl⁻] was fixed at 0.15 M by addition of HCl.

Since the partition of the chemicals between the mobile phase and the hydrocarbon stationary phase is closely related to their partition coefficients, only the retention times are required in the HPLC method while quantitative analysis is not necessary. Moreover, as the evaporative light scattering detector (ELSD) relies on the existence of the particles (i.e. the compounds going to be detected) instead of a particular wavelength of UV absorption, we could determine all compounds without changing the detection wavelengths. The log D -pH profiles of B12H and B7T are illustrated in Fig. 2. The flat regions of the curves, which correspond to the log D values of partitioning cation in the form of an ion pair, were found to be approximately -1.0 for TAC (at pH < 6), approximately 0.0 for B12H (at pH < 6) and approximately -1.0 for B7T (at pH < 5). By plotting the log P values against log $(t_R - t_0)/t_0$ values of the known compounds (Table 1), the following calibration curve was obtained with $r=0.9910$:

$$\log P = 4.027 \times \log \frac{t_R - t_0}{t_0} + 2.833 \quad (5)$$

in which t_0 was 1.113 min as determined by using copper sulphate.

The retention times of TAC, B12H and B7T were found to be 1.96, 5.96 and 24.48 min, respectively and the distribution coefficients of the neutral molecules, expressed as log P values, were 2.4, 5.4 and 8.2 for TAC, B12H and B7T, respectively. The differences between log D and log P values (diff(log $P - \log D$)) were 3.4 (TAC), 5.4 (B12H) and 9.2 (B7T). Soluble drugs usually have higher diff(log $P - \log D$) values and exist either in ionic and/or neutral forms in tissue fluids, which contribute to higher permeability through biological barriers *in vivo*.

3.3. Ionization constants

As the neutral forms of B12H and B7T were sparingly soluble, the apparent ionization constants (pK_a), were determined by the UV spectrophotometry method as previously described [25]. TAC was included in these tests for method validation. Fig. 3 shows the UV absorbance profiles (from 190 to 400 nm) of TAC, B12H and B7T in various solutions with pH values ranging from approximately 2.0 to 11.5. Only one apparent isosbestic point could be found for all three compounds although both B12H and B7T are dibasic salts. The wavelengths selected for the final determination of pK_a values were: 210–320 nm for TAC; 225–320 nm (pK_{a1}) and 220–335 nm (pK_{a2}) for B12H; and 220–340 nm (pK_{a1} and pK_{a2}) for B7T. The results of pK_a values for TAC, B12H and B7T based on their absorbance values at different pH and different wavelengths are given in Table 2. The pK_a

value for TAC was found to be 9.9 ± 0.2 , which is very close to the previously reported value of 9.8 ± 0.2 [31]. The method used in our study is thus valid and reproducible. As calculated from the absorbance and pH values, both B12H and B7T were demonstrated to have two pK_a values: 7.5 ± 0.1 (pK_{a1}) and 10.0 ± 0.2 (pK_{a2}) for B12H; and 8.7 ± 0.1 (pK_{a1}) and 10.7 ± 0.4 (pK_{a2}) for B7T, which were exhibited by the dihydrochloride salts of both dimers.

3.4. Solubility

The solubilities of B12H and B7T were measured in water, saline and 50 mM sodium phosphate buffers (pH 2.0–6.0). B12H and B7T were both found to be poorly soluble in water (11.16 and 12.71 mg/ml, respectively), and sparingly soluble in saline (2.07 and 0.36 mg/ml, respectively) and phosphate buffers (2.01–2.48 and 0.33–0.47 mg/ml, respectively). In the study of the relationships between ionic strength and the solubilities of the dimers, both B12H and B7T were found to be more sensitive to [Cl⁻] (6.38–1.95 mg/ml for B12H and 2.30–0.28 mg/ml for B7T when [Cl⁻] varying from 0.05 to 0.15 M) than to [Na⁺] (2.19–2.36 mg/ml for B12H and 0.29–0.35 mg/ml for B7T when [Na⁺] varying from 0.05 to 0.15 M with constant [Cl⁻] at 0.15 M) (Table 3). The solubility–pH profiles of B12H and B7T were also established in the pH range 2.0–12.0 (at a constant ionic strength of 0.15 M NaCl) and illustrated in Fig. 4. For B12H, the flatter region of the curve at pH < 6 (solubility between 2.02 and 2.38 mg/ml) corresponds to the dication form, while the flat region at pH > 8 (solubility between 0.002 and 0.06 mg/ml) corresponds to the monocation and neutral forms.

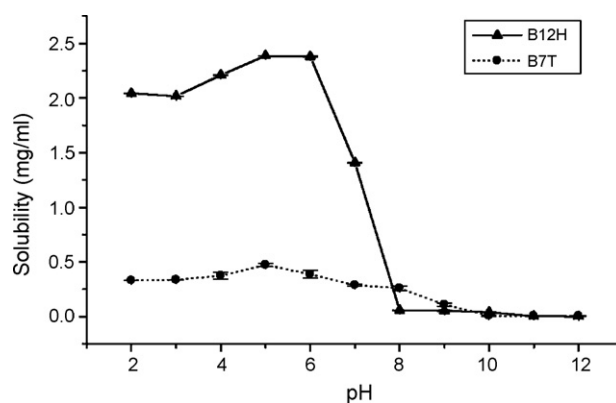


Fig. 4. The graph of solubility (mean \pm S.D., $n=6$) vs. pH values (2–12) for B12H (solid line) and B7T (dotted line) in isotonic buffer (containing 0.15 M NaCl).

The significant difference in the solubility is due to the degree of the ionization and the differences in lipophilicity between the dication and the monocation of B12H at different pH values. However, the solubility of B7T was shown to be less sensitive to pH change than that of B12H—the maximum solubility was 0.29–0.47 mg/ml at pH < 7 and the minimum solubility was 0.005–0.008 mg/ml at pH > 10. The effective solubility values for B12H and B7T measured in phosphate buffer (pH 7.4) were 0.96 and 0.26 mg/ml, respectively. Although both dimers had lower solubilities in phosphate buffers than in water, the concentrations were still much higher than that required for pharmacological effects to take place [32].

3.5. *In vivo* pharmacological study

Generally, the data of lipophilicity are crucial for assessing the abilities of chemicals to permeate through the blood–brain barrier. This is especially true for centrally acting drugs [33]. The predominant target sites for anti-AD drugs are in brain areas such as hippocampus [34,35], cortex [36] and amygdala [37]. Therefore, studying the lipophilicity is important in the evaluation of anti-AD drugs. In our previous study, B7T (38 $\mu\text{mol/kg}$) showed a much higher AChE inhibition in rat cortex than TAC (120 $\mu\text{mol/kg}$) after intraperitoneal administration; and B7T showed quite similar anti-cholinesterase effects after either intraperitoneal or oral administration [11]. In this study, B12H has been shown to have similar physicochemical properties to those of B7T. An *in vivo* evaluation based on inhibition of AChE in brain was therefore performed in order to compare the potency of these two dimers. Fig. 5 shows the time-course of AChE inhibition in mice cortex from 5 to 240 min after a single-dose, intraperitoneal administration of B12H and B7T. Both B12H (5.28 $\mu\text{mol/kg}$) and B7T (3.54 $\mu\text{mol/kg}$) showed maximum AChE inhibition (approximately 35.5% for B12H and 46.3% for B7T) at 15 min after the drug administration, suggesting that both dimers could be rapidly absorbed from the peritoneal blood vessels into the brain. This finding may be associated with the physicochemical properties of the dimers. In the brain tissue fluids where the pH values ranges from 7.4 to 7.6,

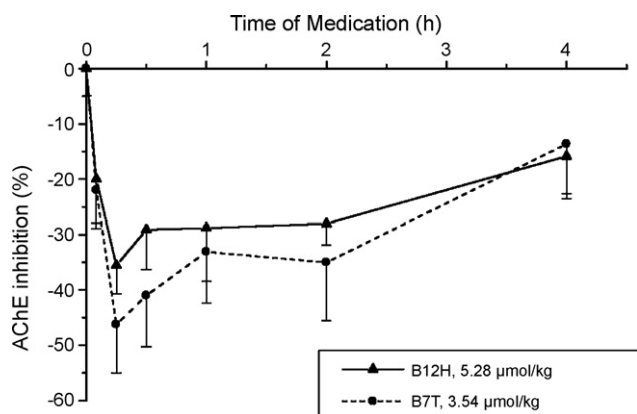


Fig. 5. Time-course of AChE inhibition in cortex after intraperitoneal administration of B12H (5.28 $\mu\text{mol/kg}$) and B7T (3.54 $\mu\text{mol/kg}$) to mice. Values are calculated using Eq. (4) and expressed as percentage of inhibition \pm S.E.M. ($n=6$).

both dimers should exist mainly as the monocations or as the neutral forms which contribute to their high lipophilicities. The drugs may therefore permeate the blood–brain barrier effectively and exert their pharmacological effects.

4. Conclusion

In summary, both B12H and B7T possess higher solubility in water than in physiological solutions; display two pK_a values; and have high $\log P$ values. The high $\log P$ values of both B12H and B7T suggest that both dimers are very lipophilic, which should give rise to high membrane permeability. The rapid and potent anti-AChE effects of the dimers are confirmed by the *in vivo* study in mice, which suggests that they are potential agents for the treatment of AD.

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