

SAR Evaluation of Disubstituted Tacrine Analogues as Promising Cholinesterase and Carbonic Anhydrase Inhibitors

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ABSTRACT

Background: The inhibition of both hydrolysis products of acetylcholine (ACh), Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE), is essential for successful treatment of Alzheimer patients. **Objectives:** This study was investigated inhibition potentials of recently synthesized disubstituted tacrines derivatives on going our research against AChE, BChE and carbonic anhydrase cytosolic (hCA I and II) enzymes to explore the Structure activity relationship (SAR). **Methods:** Inhibitory activities of tested compounds against AChE and BChE were measured by spectrophotometric method, developed by Ellman *et al.* Furthermore, the disubstituted tacrines were determined as inhibitors of two physiologically relevant CA isoforms, the cytosolic hCA I and II by an esterase assay method. **Results:** The silyl, thiomethyl and cyano substituted seven membered hydrocycle tacrines (**9**, **11** and **14**) significantly inhibited AChE, compared with starting compound **3** (6,8-dibromo-2,3,4,5-tetrahydro-1H-cyclohepta[1,2-b]quinoline) and reference compounds, galantamine and tacrine, while methoxy substituted seven membered hydrocycle tacrine derivative **10** showed selective inhibition against BChE (IC₅₀ = 563 nM). Interestingly, disubstituted tacrines displayed higher or parallel inhibition to galantamine. Additionally, all these tacrine analogues were recorded to be powerful inhibitor compounds of the cytosolic isoenzyme hCA I with K_i in the range of 43.81–471.67 nM, as well as a moderate selectivity toward hCA II isoenzyme with K_i in the range from 87.14 to 614.68 nM compared with AZA, as standard. **Conclusion:** The disubstituted seven membered hydrocycle tacrine analogues **9-12** and **14** may have promising anti Alzheimer drug candidate and dibromo six membered hydrocycle **2** and dibromo seven membered hydrocycle **3** derivatives may be novel hCA I and II enzyme inhibitors.

Key words: Tacrine derivatives, Acetylcholinesterase, Butyrylcholinesterase, Carbonic anhydrase, Anticholinergic, SAR.

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INTRODUCTION

Alzheimer's disease (AD) is characterized as a neurological disorder and considered as lack of cognitive abilities in the elderly people.¹ AD starts with loss of memory accompanied by an increase of AChE activity that leads to run out of cholinergic and noncholinergic neurons in the brain.

Amyloid beta-protein plaques which form in the brain, are degradation product of Cholinesterase enzyme (ChE).² There are several suggestions to the treatment of AD. The most preferred medication is discovery of either new cholinesterase inhibitors (alternative to donepezil, rivastigmine,



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tacrine and galanthamine) or *N*-methyl-D aspartate (NMDA) receptor antagonists such as memantine.³ Adverse effects of some synthetic acetylcholinesterase inhibitors used for the treatment of AD such as tacrine and donepezil, leads to researchers to find innocent and most potent derivatives of these drugs.⁴⁻⁵ Therefore, it is very important to find new substances, more efficient and less expensive than the currently used drugs.

Tacrine was the first AChE inhibitor to be launched and approved by FDA as an AD drug.⁶ However, it exhibited several adverse effects which in some cases led to several problems.⁷ Due to that, many other AChE inhibitors have been studied and researchers have still continued to improve the pharmacological profile of the novel drug candidate.^{1,8} There are restricted reports about the synthesis of substituted tacrine displayed AChE inhibitory activity. Some reported studies showed halogenated tacrine analogues have better inhibitory activity compared with unsubstituted one.¹ In a work, the high inhibition of a set of 6- and 7-substituted with Cl and F atoms 9-amino-1,2,3,4-tetrahydroacridine-1-ols were determined.⁹ In a theoretical work, it was concluded that Cl atom at C-6 of tacrine increased the binding strength to AChE probably due to its electron withdrawing effect.¹⁰

Carbonic anhydrase (CA, E.C.4.2.1.1) enzymes significantly exist in red blood cells and gastrointestinal tract of vertebrates and are class of zinc enzymes which perform catalyzing the reversible hydration of CO₂ into protons and HCO₃⁻ under physiological situations¹¹ and exist in blood pH homeostasis and play a key role in CO₂ transport to the lung cells.^{12,13} CA inhibitors (CAIs) have recently been common studying area due to their significant applications for the treatment and prevention of an extensive number of diseases. The inhibition and activation effects of CA by many chemicals and drugs have been reported in the literature.¹⁴ At least 16 CA isoforms were defined up to present in mammals, the scads active ones as catalyst agents for CO₂ hydration being CA II and IX.^{15,16} Additionally, the realization of the physiological outcomes of inhibiting particular CA isoforms is developing as the science base about subcellular positions building CA enzymes kinetics, expression levels and tissue repartition. Most of these isoforms have shown promise as medication aims with CAs now involved in regions where human treatments are extremely needed, for example, obesity-specific (CA V) pathways, in cancer-specific (CA IX and XII) and in brain function (CA XIV).¹⁶⁻¹⁷

Our group recently reported the preparation of novel disubstituted tacrine derivatives, including five, six and seven membered hydrocycles,¹⁸ through cyclodehydration reaction of several ketones and 2-amino-3,5-dibromoben-

zonitrile via Friedlander reaction furnished a series of dibromo tacrines. Then, bromo tacrine analogues were converted to corresponding disubstituted tacrine analogues by metal catalyzed or metal halogen exchange. The present work is a continuation of our ongoing research.¹⁸ Due to having the AChE inhibition potential of the tricyclic structure of the 1,2,3,4-tetrahydroacridine nucleus, we focused on investigation of anticholinergic activities of recently reported disubstituted tacrine as potential AChE, BChE, hCA I and hCA II inhibitors. Furthermore, to fill the gap in the SAR of substituted tacrine we focused on our work on tricyclic tacrine based AChE, BChE, hCA I and II inhibitors¹⁹⁻²⁰ by testing a series of disubstituted tacrine analogues. The IC₅₀ values for the inhibition of AChE and BChE were studied by means of SAR approach, in search for describing the dependence of AChE, BChE, hCA I and hCA II inhibitory activity on substituents of tacrine cycles.

MATERIALS AND METHODS

Chemistry

The synthesis of dibromo (**1-5**) tacrines via Friedländer reactions and disubstituted (**7-14**) tacrine derivatives via metal assisted electrophilic and nucleophilic substituted reactions and MeI salt form (**6**) of unsubstituted tacrine were reported recently by our previous publications.^{18,21} The isolated compounds (**1-14**) were fully characterized with melting point, HRMS analysis, infrared, ¹H, ¹³C, HMBC and HETCOR spectroscopy in this paper.¹⁸ This study was carried out with disubstituted tacrine derivatives (**1-14**) according to our previous paper.^{18,21,22}

Anticholinesterase Activity Assays

The inhibitory efficacy of disubstituted tacrine derivatives (**1-14**) on BChE/AChE activities was obtained conforming to the spectrophotometric Ellman's procedure.²³ Butrylcholine iodide (BChI) and also acetylthiocholine iodide (AChI) compounds were used as substrates of the both reactions.²⁴ In this part, 5,5'-dithio-bis(2-nitrobenzoic)acid (DTNB) was used for the estimation of the BChE/AChE activities.²⁴ Briefly, 130 µL of buffer solution (pH 8.0, Tris/HCl, 100 mM) and diverse concentration of sample solutions (50-200 µL) dissolved in deionised water were added to 50 µL of BChE/AChE solutions. Then, the mixture was incubated for 15 min at 25°C. Finally, 50 µL of DTNB (0.5 mM and 25 mL) of BChI/AChI was added to incubated mixture. Also, the reaction was initiated by the addition of 50 µL of BChI/AChI. The activities of these enzymes were evaluated spectrophotometrically at a wavelength of 412 nm.²⁵ The butrylcholinesterase enzyme obtained from *Equus caballus* and acetylcholinesterase enzyme

isolated from *Electrophorus electricus* were obtained from market. Galantamine and Tacrine compounds were used as control compound. Ethanol was used as a solvent to dissolve test compounds and the controls. The anticholinesterase activity of the fourteen tacrine derivatives were measured at different concentrations (10^{-8} -200 μ M) for calculation of the IC_{50} values. Each substance was tested three times in triplicate against AChE and BChE enzymes.

The percentage of inhibition of AChE or BChE was determined by a comparison of the rates of reaction of samples relative to blank sample (ethanol in phosphate buffer pH 8) using the formula;

$$[(E-S) / E] \times 100$$

where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample.

Carbonic anhydrase I and II isoenzymes purification and inhibition studies

The purification of both cytosolic CA isoforms (hCA I and II) of human blood cells was previously explained with a simple one-step procedure by a Sepharose-4B-L-Tyrosine-sulphanilamide affinity chromatography as described previously.²⁶ The protein quantity in the column effluents was evaluated spectrophotometrically at 280 nm previously described. For purification of both isoenzymes, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied. CA isoenzymes activities were obtained in conforming to Verpoorte procedure²⁷ explained previously in details.²⁸ Increasing absorbance of the reaction medium was spectrophotometrically obtained at 348 nm. We utilized the esterase activity procedure for ascertaining the inhibition agents by the Lineweaver–Burk procedure.²⁹ CA activity (%) versus inhibitory concentration and $1/V$ versus $1/[S]$ graphs were drawn. The quantity of protein was determined at 595 nm according to Bradford's procedure.³⁰ Bovine serum albumin was used as standard protein.³¹ For the designation of the inhibition efficacy of each novel disubstituted tacrine derivatives on both hCA isoenzymes, an activity (%)-[disubstituted tacrine derivatives] graph was drawn. The IC_{50} values were obtained from activity (%) versus compounds plots. For the calculation of K_i values, disubstituted tacrine derivatives concentrations were used.

RESULTS

Chemistry

In our previous papers,^{18,21-22} one-pot synthesis was described for bromo tacrine derivatives (**1-5**) (Figure 1)

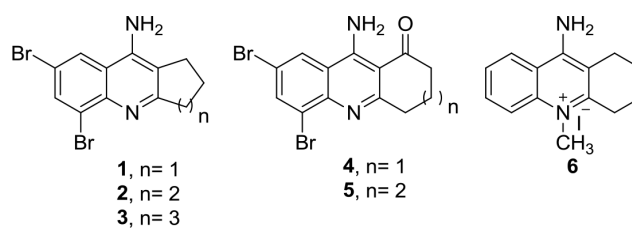
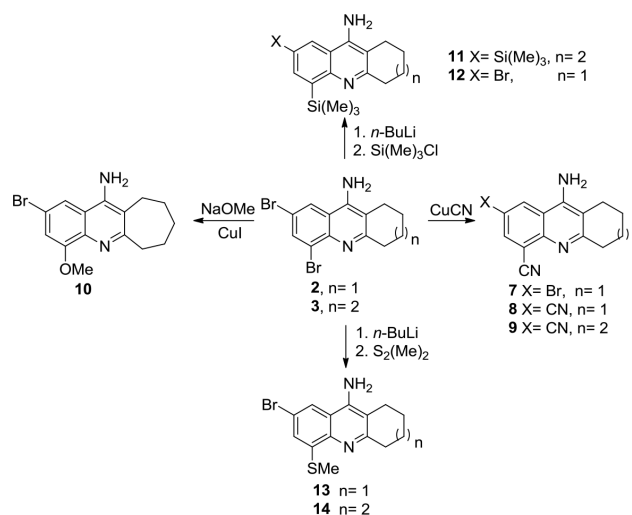


Figure 1: Structures of bromotacrine derivatives (1-5) and methyl iodine salt form tacrine (6)



Scheme 1: Synthesis of disubstituted tacrine derivatives (7-14)

using Friedländer reactions of several cyclic ketones and brominated 2-amino-3,5-dibromobenzonitrile (**17**) in the presence of some Lewis acids. To achieve the direct bromination, tacrine was treated to methyl iodine to protect of the N atom in the cycle. This reaction furnished the methyl iodine salt form (**6**) of unsubstituted tacrine.

Tacrine bromides were converted corresponding thiomethyl (**13-14**) and silyl (**11-12**) derivatives by the treatment of dibromo **1** and **2** derivatives with *n*-BuLi followed by trapping with an electrophile [Si (Me)₃Cl, S₂(Me)₂] (Scheme 1). Copper-assisted nucleophilic substitution reactions of brominated tacrines furnished cyano (**7-9**) and methoxide (**10**) derivatives (Scheme 1).¹⁸

Anticholinesterase activity results

Tacrine (THA, 1,2,3,4-tetrahydroacridine) is one of the most important inhibitor of both AChE and BChE enzymes.^{4,20,32-34} Despite the lack of its selectivity and hepatotoxicity and limitation of its medical therapy, tacrine remains a reference compound (IC_{50} = 59 nM for AChE and IC_{50} = 23 nM for BChE, taken in our study) in the design and development of promising ChEs inhibitors candidates. The high AChE and BChE

inhibitory feature of tacrine has encouraged us to development of the effective tacrine derivatives.

We have just reported the inhibition potential of disubstituted tacrine derivatives against AChE and BChE as IC_{50} values in Table 1. The inhibitory activities of tested molecules are compared with unsubstituted tacrine and galantamine ($IC_{50} = 1280$ nM for AChE and $IC_{50} = 19530$ nM for BChE, taken in our study) as standard compound.

The disubstituted tacrine derivatives were showed to be potent inhibitor compounds of cholinesterases with IC_{50} values in nanomolar concentration scale. The excellent AChE inhibitor in the assayed series of disubstituted tacrine is dicyano substituted seven membered hydrocycle tacrine **9** with $IC_{50} = 68$ nM. The compound **11** has selectivity to AChE than BChE comparison to standard compounds galantamine and tacrine. Also, disilyl **11** ($IC_{50} = 454$ nM) and thiomethyl **14** ($IC_{50} = 405$ nM) seven membered hydrocycle tacrines and monosilyl **12** ($IC_{50} = 233$ nM) six membered hydrocycle tacrine significantly inhibited AChE enzyme. The compounds **9**,

11, **12** and **14** proved to be the most selective AChE inhibitor. Also these compounds are more selective than galantamine and unsubstituted tacrine. The substituted tacrine derivatives, except of compounds **2** ($IC_{50} = 6140$ nM), **4** ($IC_{50} = 8480$ nM) and **5** ($IC_{50} = 7460$ nM) showed good inhibition as galantamine against AChE in a range of 68-3780 nM.

To the BChE inhibition results, among the all tacrine derivatives, methoxy substituted seven membered tacrine **10** was the most active and more selective in inhibiting BChE in IC_{50} of 536 nM. Despite the high inhibition of **10** against BChE enzyme, this compounds moderately inhibited AChE enzyme. Moreover, all compounds, except of **11**, have good inhibitory effect against to BChE compared with galantamine as standard and clinical cholinergic enzymes inhibitor. It was interested that compound **11** significantly inhibited to AChE with IC_{50} value of 454 nM while it was not active against BChE (Table 1).

These results indicated that the position and kinds of substituents are important to anti AChE and BChE

Table 1: AChE and BChE inhibitory activity (IC_{50}) of the disubstituted tacrine derivatives.

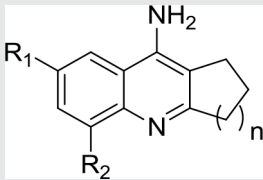
								
Comp.	n	R ₁	R ₂	Bounded other groups	AChE $IC_{50} \pm SD$ (nM)	r ²	BChE $IC_{50} \pm SD$ (nM)	r ²
1	1	Br	Br	-	1890±530	0.996	2080±670	0.992
2	2	Br	Br	-	6140±148	0.998	6930±1720	0.999
3	3	Br	Br	-	3780±201	0.999	4280±161	0.999
4	2	Br	Br	Ketone form at C1	8480±836	0.994	11140±658	0.996
5	3	Br	Br	Ketone form at C1	7460±502	0.997	6400±181	0.999
6	2	H	H	Mel salt form at N of ring	1470±358	0.990	1860±103	0.987
7	2	Br	CN	-	2980±640	0.999	4740±810	0.993
8	2	CN	CN	-	2750±320	0.998	2360±118	0.994
9	3	CN	CN	-	68±12.3	0.999	3720±208	0.984
10	3	Br	OCH ₃	-	2340±240	0.989	563±32.5	0.999
11	3	Si(CH ₃) ₃	Si(CH ₃) ₃	-	454±23.5	0.999	732520±3540	0.999
12	2	Br	Si(CH ₃) ₃	-	233±11.8	0.982	9100±260	0.996
13	2	Br	SCH ₃	-	2010±470	0.992	2030±530	0.998
14	3	Br	SCH ₃	-	405±22.1	0.986	2980±129	0.998
Tacrine (Reference Compound A)					59±14.7	0.999	23±10.6	0.999
Galantamine (Reference Compound B)					2280±106	0.997	19530±1740	0.999

Table 2: hCA I and hCA II inhibitory activity (IC₅₀ and K_i) of the disubstituted tacrine derivatives.

Compounds	IC ₅₀ (nM)				K _i (nM)	
	hCA I	r ²	hCA II	r ²	hCA I	hCA II
1	358.51	0.9996	500.36	0.9329	300.04±7.51	554.36±228.18
2	76.15	0.9628	99.06	0.9440	111.76±45.03	114.44±51.05
3	33.03	0.9877	85.77	0.9615	43.81±11.98	87.14±34.23
6	408.12	0.9880	551.75	0.9330	380.15±90.21	614.68±153.91
7	379.11	0.9973	563.86	0.9712	330.17±108.95	603.43±227.47
8	392.85	0.9827	565.71	0.9437	303.31±61.82	434.51±200.98
9	414.22	0.9522	460.46	0.9631	315.04±48.37	454.08±147.66
10	443.66	0.9814	462.92	0.9593	471.67±201.35	422.58±161.62
11	354.31	0.9813	420.76	0.9707	297.63±40.02	421.26±99.96
12	389.54	0.9805	456.82	0.9728	320.67±24.34	424.23±83.54
13	399.65	0.9776	474.65	0.9461	382.60±93.32	509.73±182.84
14	412.52	0.9863	475.31	0.9695	323.21±85.56	455.12±94.14
AZA	3745.94	0.9653	3347.82	0.9899	3320.46±716.02	718.92±531.18

activities, especially silyl and cyano substituted seven membered hydrocycle tacrines showed the highest AChE inhibitory activity compared with bromo tacrine analogues and galantamine. Also, these compounds showed to close to unsubstituted tacrine's inhibitory activity against AChE (Table 1).

Carbonic anhydrases inhibition activity results

The disubstituted tacrine derivatives reported here were determined as inhibitors of two physiologically relevant CA isoforms, the cytosolic hCA I, hCA II by an esterase assay method.^{35,36} Acetazolamide (AZA), a clinically used sulfonamide CAI was used in the assays as a standard drug. The results are shown in Table 2 (IC₅₀ and K_i values expressed as nanomolar (nM)). This study clearly indicated that disubstituted tacrine derivatives (**1-3**, **7-14**) and its methyl iodine salt form (**6**) showed good hCA I and hCA II inhibitory effects. The slow cytosolic isoform hCA I (take part in important physiological and pathological processes in many tissues and organs), was significantly inhibited by most of the disubstituted tacrine derivatives with K_is ranging between 43.81 and 471.67 nM. The best inhibitions for this isoform were determined by dibromo six membered **2** and dibromo seven membered **3** tacrine derivatives, with K_i values of 111.76 and 43.81 nM, respectively. Additionally, acetazolamide (AZA) as sulphonamide compound was recorded for broad-specificity CA inhibitor which obtained K_i value of 3320.46 nM against hCA I.

CA II is often associated with several diseases such as glaucoma, osteoporosis and renal tubular acidosis.³⁷ For

hCA II, disubstituted tacrine derivatives (**1-3**, **7-14**) had K_i values from 87.14 to 614.68 nM. Moreover, AZA (5-aceta-mido-1,3,4-thiadiazole-2-sulfonamide), which is used for the treatment of altitude sickness, cystinuria, idiopathic intracranial hypertension, glaucoma, epileptic seizure, dural estasia and central sleep apnoea, had a medium potency hCA II inhibition for this isoform,³⁸ with a K_i value of 2718.92 nM. Also, dibromo six membered hydrocycle **2** and dibromo seven membered hydrocycle **3** tacrine derivatives have the highest inhibition effects against hCA II with K_i values of 114.44 and 87.14 nM, respectively.

DISCUSSION

These compounds were defined in synthetically available position molecules of the aryl rings of tacrine analogues bearing six and seven membered hydrocycle as well as on primary amino group in order to evaluate the SAR mechanism of this series. The structural of disubstituted tacrine analogues of AChE and BChE enzymes inhibition data expressed as IC₅₀ values in nM level which are shown in Table 1. Also hCA I and hCA II inhibitory potentials' data expressed as IC₅₀ and K_i values in nM concentrations are shown in Table 2.

It is evident that both nature and positions of the substituents at C-5 and C-7 for six membered hydrocycle tacrine and the substituents at C-6 and C-8 for seven membered hydrocycle tacrine analogues are able to vary the AChE and BChE inhibition activity of tacrine. However, we did not observe the variation and selectivity

of hCA I and hCA II inhibitory potentials of disubstituted tacrine derivatives. All compounds have good inhibitory effect of hCA I and hCA II enzymes, compared standard and clinical compound of AZA. Especially, 5,7-dibromo six membered hydrocycle tacrine **2** and 6,8-dibromo seven membered hydrocycle tacrine **3** showed the best inhibition against hCA I (IC_{50} = 76.15 and 33.03 nM, respectively) and against hCA II (IC_{50} = 99.06 and 85.77 nM, respectively).

The results of AChE and BChE inhibition activity, we introduced the electron attracting groups CN and Br and the electron donating groups, $Si(CH_3)_3$, SCH_3 and OCH_3 in positions of C-6 and C-8 of seven membered hydrocycle, C-5 and C-7 of six membered hydrocycle tacrines. The nitrile (CN), silyl ($Si(CH_3)_3$) and thiomethyl (SCH_3) substituents in seven membered hydrocycle tacrine (**9**, **11** and **14**) increase the AChE inhibitory activity, compared with starting material **3** (IC_{50} = 3708 nM). Despite these results, **9**, **11** and **14** showed poor inhibitory activities against BChE inhibitory activities, especially for compound **12** (IC_{50} = 732520 nM) in Table 1. Similarly, silyl, electron donating group, at C-5 in six membered hydrocycle tacrine **11** (IC_{50} = 233 nM) significantly increased the inhibition activity against AChE enzyme. However, the other substituents in six membered hydrocycle tacrine, Br, CN and SCH_3 substituted at positions of C-5 and C-7, analogues **2**, **7**, **8**, **13** showed moderate activity against AChE with IC_{50} ranging between 2010 and 6140 nM and BChE enzymes with IC_{50} ranging between 2030 and 6930 nM, compared with galantamine (IC_{50} = 2280 nM against AChE, IC_{50} = 19530 nM against BChE). Interestingly, the electron donating groups SCH_3 and OCH_3 in C-6 position of seven membered hydrocycle tacrines display selective inhibitory activity. While the bromine changed with methoxide at C-6 of compound **10** blocked the increasing of BChE enzyme with IC_{50} = 563 nM, thiomethyl at C-6 of compound **14** showed selectively AChE inhibitory activity with IC_{50} = 405 nM. The bromine group in all dibromo tacrine derivatives (**1-5**) did not show significant inhibitory activity against AChE and BChE enzymes compared with unsubstituted one. The other interesting result is that tacrine in unsubstituted form, used as reference compound in anticholinergic studies, have excellent inhibition effect against AChE and BChE enzymes while the AChE (IC_{50} = 1470 nM) and BChE (IC_{50} = 1860 nM) inhibitory activity of MeI salt form (**6**) of this compound significantly decreased, which is evidence that N atom of tacrine in unsubstituted form could be responsible for inhibitory effect of tacrine against AChE and BChE enzymes. Moreover, the decreasing of inhibitory activity of MeI salt form (**6**)

of this compound compared tacrine's inhibitory activity possibly may cause diminish some serious side effects of tacrine on AD patients because the cytotoxicity of MeI salt form of tacrine has promising acceptable results according to preresults of ongoing research.

CONCLUSION

In this work, disubstituted tacrine analogues, recently synthesized by our group,^{18,21} were found to be potent and selective inhibitors of AChE and BChE with IC_{50} in nanomolar concentration scale. Moreover, we investigated and assessed as inhibitors of two physiologically related and important CA isoforms, the cytosolic, hCA I and hCA II. Then, we attempted to determine the SAR of the effects of the introduction of substituents in some position of tacrine. The substituted seven membered hydrocycle tacrine analogues selectively showed the effective inhibitory activity against AChE and BChE enzymes. The cyano at C-6 and C-8, silyl at C-6 and C-8 and thiomethyl at C-6 substituted seven membered hydrocycle tacrine derivatives (**9**, **11** and **14**) have the good inhibitions against AChE (IC_{50} in the range of 68-454 nM) while methoxy at C-6 substituted seven membered hydrocycle tacrine **10** showed the best inhibitory activity against BChE (IC_{50} = 563 nM). All the disubstituted tacrine derivatives (**1-3**, **6-14**) used in the present study described effective inhibition profiles against both hCA isoenzymes compared with acetazolamide (AZA), which used as a medication used to treat glaucoma, altitude sickness, epilepsy, idiopathic intracranial hypertension, periodic paralysis and heart failure.³⁹ In conclusion, disubstituted tacrine analogues, especially seven membered hydrocycle, have being a potential of cholinesterase enzymes inhibitor and both cytosolic CA isoenzymes. These results suggest that the dibromo tacrine derivatives may lead to synthesis of novel and effective CA inhibitors. In our ongoing research, we have been studying on cytotoxic profiles of substituted tacrine analogues in progress and we have obtained promising results. This present study and our ongoing research can contribute to the investigation of a cure for AD or therapy in the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

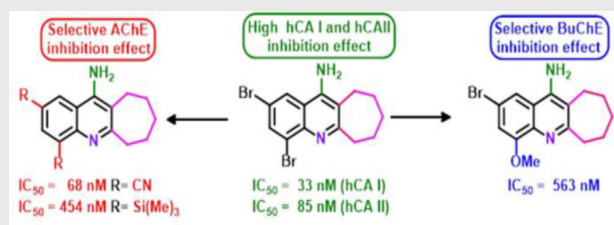
ACh: Acetylcholine; **AChE:** Acetylcholinesterase; **BChE:** Butyrylcholinesterase; **SAR:** Structure Activity Relationship; **CA:** Carbonic Anhydrase; **AD:** Alzheimer's disease; **ChE:** Cholinesterase Enzyme; **A beta:** Amyloid beta-protein; **NMDA:** N-methyl-D aspartate; **FDA:** Food and Drug Administration; **HRMS:** High Resolution Mass Spectroscopy; **¹H NMR:** Proton Nuclear Magnetic Resonance Spectroscopy; **¹³C NMR:** Carbon 13 Nuclear Magnetic Resonance Spectroscopy; **HMBC:** Heteronuclear Multiple Bond Correlation; **HETCOR:** Heteronuclear Correlation; **DTNB:** 5,5'-Dithio-bis(2-nitro-benzoic)acid; **BChI:** Butrylcholine Iodide; **AChI:** Acetylthiocholine Iodide; **MeI:** Methyl Iodide; **SDS-PAGE:** Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis; **THA:** Tacrine; **IC₅₀:** The Half Maximal Inhibitory Concentration; **K_i:** Inhibition Constant; **AZA:** Acetazolamide.

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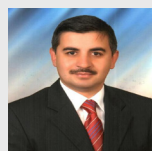
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PICTORIAL ABSTRACT



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SUMMARY

- Disubstituted tacrine derivatives have selective inhibitory effects against cholinesterase enzymes. Bromotacrines bearing six or seven membered hydrocycle displayed high inhibition effect against cytosolic hCA I and hCA II enzymes. The cyano, silyl and thiomethyl groups at C-6 and C-8 positions have significantly increased inhibitory effect of seven membered hydrocycle tacrines against AChE enzyme.

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