Full Paper

Novel Tacrine Analogs as Potential Cholinesterase Inhibitors in Alzheimer's Disease

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Acetylcholinesterase inhibitors (AChEIs) are used for the treatment of Alzheimer's disease (AD). The increase in ACh levels ameliorates the symptoms of the disease. Tacrine is the first clinically approved drug as AChEI used in the treatment of AD. In this paper, we synthesized new tacrine analogs to act on catalytic and peripheral sites of AChE. Their inhibitory activity was evaluated. All novel compounds except **7a** showed promising results toward AChE. Two compounds, **10b** and **11b**, are more potent than tacrine. Furthermore, molecular-modeling studies were performed for these two compounds to rationalize the obtained pharmacological activity. Moreover, various drug-likeness properties of the new compounds were predicted.

Keywords: Acetylcholinesterase / Alzheimer's disease / Drug-likeness / Molecular-modeling / Tacrine

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that accounts for most cases of dementia seen in the elderly population [1, 2]. Clinical manifestations of the disease start with minor lapses in episodic memory. As the disease progresses, problems with general cognitive functions such as intellectual abilities, memory, executive functions, and speech become more common [3, 4]. The gradual neuronal loss occurring in AD results in learning and memory impairments; this is thought to be largely due to deteriorating cholinergic neurotransmission [5, 6]. Acetylcholine (ACh) plays an important role in cognition; thus, maintaining its levels by reducing its degradation provides cognitive benefit. A number of inhibitors have been developed; their effect is to inhibit acetylcholinesterase (AChE), an enzyme that degrades ACh [7, 8].

Tacrine (A) (Fig. 1) was the first acetylcholinesterase inhibitor (AChEI) approved by the FDA for AD and sold under the name (Cognex®) [1, 9]. In addition comes galantamine (B) (Fig. 1), a reversible, competitive, and selective inhibitor of AChE that allosterically modulates nicotinic acetylcholine receptors, having thus a dual mode of action. It was presented to market under the name of Reminyl® [10, 11]. It was reported that compound (Fig. 1), which contains benzene ring fused to cyclohexyl ring of tacrine, was more active than tacrine as uptake inhibitor [12]. On the other hand, it was registered that association of two pharmacophoric entities, galantamine and phthalyl moiety (D) (Fig. 1), afforded a potent and selective AChE inhibitor that is able to interact with the catalytic and peripheral sites of the enzyme [13]. In addition, it was reported [14] that the choice of phthalimide moiety in the synthesis of this class of drugs is owing to the presence of two carbonyl groups, which may contribute to the enzyme inhibition by hydrogen bonding with peripheral sites of the enzyme. Based on these considerations, we designed a synthetic approach for the preparation of new compounds (**10a**,**b**, **11a**,**b**) where the tacrine analogs were linked to the phthalyl moiety and connected by methylene linkers, hoping that the new compounds may interact with both the catalytic and peripheral sites of AChE enzyme. Also, it is widely recognized that thiophene is a bioisostere of benzene [15], so we substitute the benzene ring in tacrine with the thiophene ring. In the view of the above-mentioned reason, we focused on the synthesis of certain tacrine analogs and substituted thiophene analogs of tacrine (compounds **7a**,**b**, **8a**,**b**) to be tested for their AChE inhibitory activity.

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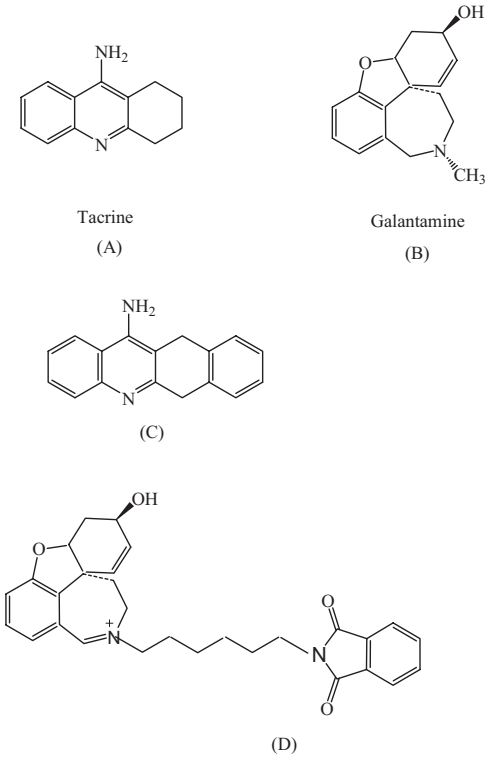
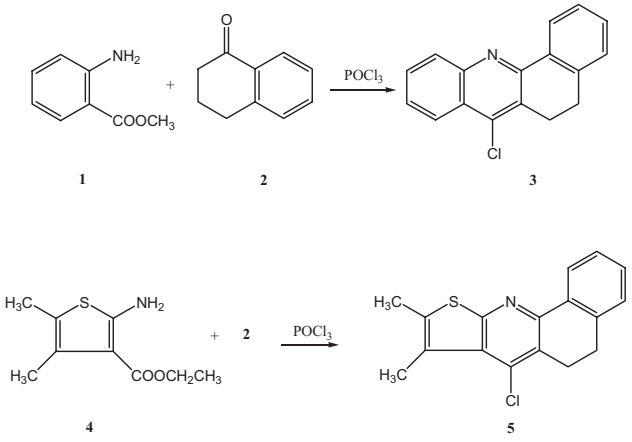


Figure 1. Potent AChE inhibitors.

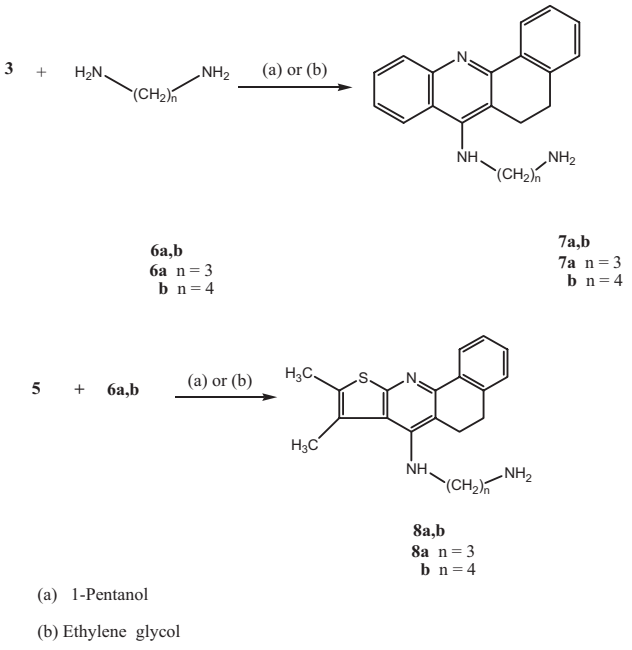
Results and discussion

Chemistry

The synthetic routes adopted in this work for constructing the required tacrine analogs and the new dual binding site compounds are all illustrated in Schemes 1–3. The 4-chloropyridine derivatives (**3**) and (**5**) are considered as the key compounds in this part of study, the synthesis of which was accomplished in good yield adopting phosphorus oxychloride-mediated cyclodehydration reaction between aminoester compound (**1**) or (**4**) and 1-tetralone (**2**) [16, 17]. Surveying the literature disclosed a number of different routes for the amination of 9-chlorotetrahydroacridine with various diamines. The most common one was achieved by heating the selected chloroacridines and the appropriate diamine in pentanol for a long period of time (18–40 h) [16–20]. Another approach was *via* fusion of the selected chloro derivative and different diamines using phenol as a catalyst [21–23]. In the present study, the reaction of the chloropyridines (**3**) and (**5**) and the selected diamines in pentanol for 72 h afforded aminopyridines **7a**,**b** and **8a**,**b**. In an attempt to increase the yield of these compounds, the amination procedure was carried out using ethylene glycol as a solvent of high boiling point and catalytic amounts of cuprous oxide and potassium carbonate. Preparation of the new dual heterodimers



Scheme 1. The synthetic pathway for starting materials.

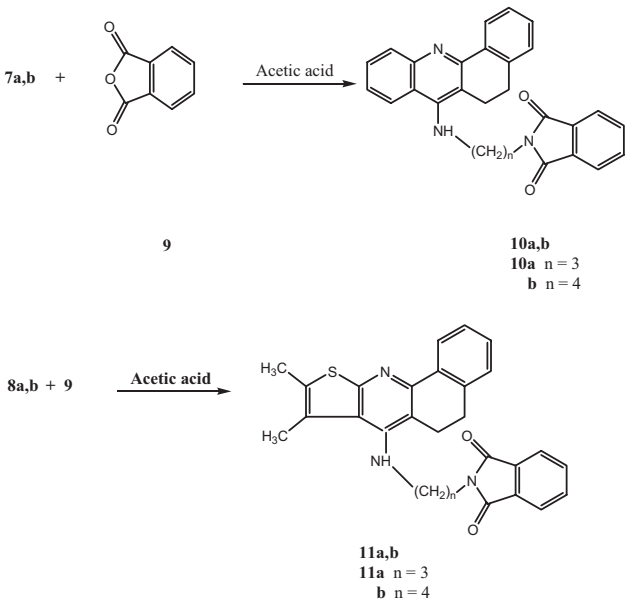


Scheme 2. The synthetic pathway for compounds **7** and **8**.

containing phthalimide moiety inserted in the aminopyridines **7a**,**b** and **8a**,**b** was achieved *via* boiling of these compounds with phthalic anhydride in glacial acetic acid to give the respective isoindolyl derivatives **10a**,**b** and **11a**,**b**. The IR spectra of compounds **10a**,**b** and **11a**,**b** revealed the presence of two C=O absorption bands at 1770–1700 cm^{-1}. All the new compounds have been fully characterized through their spectral data (Schemes 1–3).

Biological evaluation

The preliminary anti-AChE activity for the synthesized pyridine derivatives was assessed according to Ellman's



Scheme 3. The synthetic pathway for compounds **10** and **11**.

method [24], using tacrine as a reference compound. AChE was obtained from homogenates of rat brain. Results of anti-AChE activity of the tested compounds as well as tacrine are shown in Table 1. The screening results showed that all compounds exhibited significant ($p < 0.05$) inhibition against AChE, compared with tacrine. The biological activity results revealed that compounds **10b** and **11b** have the highest inhibitory activity among the synthesized compounds; they are more potent than tacrine. Furthermore, compounds **7b** and **8b** showed AChE inhibitory activity nearly similar to tacrine.

Table 1. Inhibition of AChE activity of tacrine and synthesized pyridine derivatives.

Compound (dose)	Cholinesterase content (U/g wet weight)	Inhibition %
Normal control (Saline)	312.79 ± 22.39	0
Tacrine (10 mg/kg)	$222.87 \pm 11.73^*$	28.74
7a (10 mg/kg)	$314.36 \pm 15.91^*$	0
7b (10 mg/kg)	$244.10 \pm 30.18^*$	21.96
8a (10 mg/kg)	$281.52 \pm 14.84^*$	10
8b (10 mg/kg)	$243.98 \pm 15.91^*$	21.99
10a (10 mg/kg)	$281.52 \pm 12.85^*$	10
10b (10 mg/kg)	$197.07 \pm 24.15^*$	36.99
11a (10 mg/kg)	$253.37 \pm 18.77^*$	18.99
11b (10 mg/kg)	$168.13 \pm 16.49^*$	46.25

Statistical analysis was carried out by one-way ANOVA followed by Tukey–Kramer multiple comparisons test for comparison of means of different groups.
Each value represents mean ± SE ($n = 6$–8 rats).
* Significantly different from normal control group at $p < 0.05$.

On the other hand, compounds **8a**, **10a**, and **11a** demonstrated mild inhibitory activity. Finally, compound **7a** has no inhibitory activity.

In conclusion, the highest activity of compounds **10b** and **11b** may be attributed to the peripheral inhibition of AChE enzyme by phthalimide moiety.

Furthermore, compounds **10a** and **11a** showed mild inhibitory activity due to the same explanation. Unfortunately, there is no obvious correlation between structures of other synthesized compounds and their biological activities.

Molecular-modeling study

Molecular-modeling studies of the synthesized compounds were performed in order to gain an insight into the mechanisms of enzyme inhibition. Besides, molecular docking studies helped in understanding the various interactions between ligands and enzyme active site in detail. Docking studies of inhibitors were performed on Molecular Operating Environment (MOE-2008) on the basis of the existing X-ray crystal structure of tacrine–AChE complex (code: 1ACJ) [25]. The binding model suggests that tacrine is sandwiched between the rings of Phe 330 and Trp-84; its aromatic phenyl and pyridine rings showed parallel π–π interaction with the phenyl ring of Phe 330.

In addition, both rings showed interaction with the five-membered ring of indole of Trp-84. The amino group of tacrine is bonded to water molecule, which is bonded to Ser 122.

The proposed binding model of tacrine with amino acid residue of AChE is shown in Fig. 2. Moreover, tacrine docking conformation within AChE demonstrating its interaction to the aromatic residue in the active site gorge of AChE is shown in Fig. 3.

Docking of compound **10b** showed π–π interaction of its phenyl and pyridine rings with the phenyl ring of Phe 330. In addition, both rings showed π–π interaction with the five-

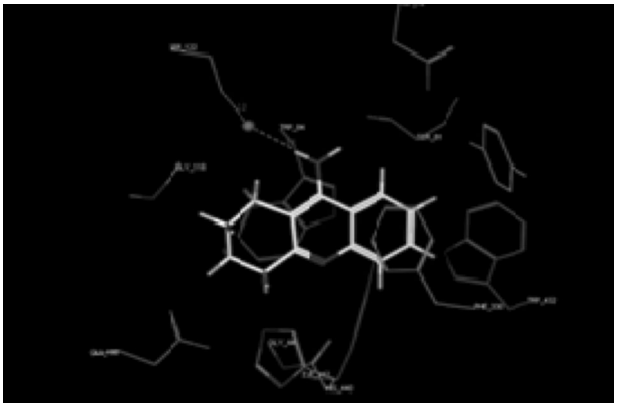


Figure 2. Proposed binding model of tacrine with the main amino acid residues of AChE; other amino acids are hidden for clarity.

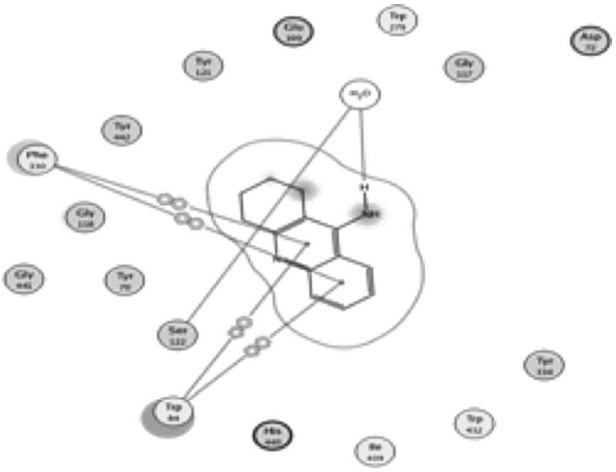


Figure 3. Interaction between ligand (tacrine) and the aromatic residues in the active site gorge of AChE.

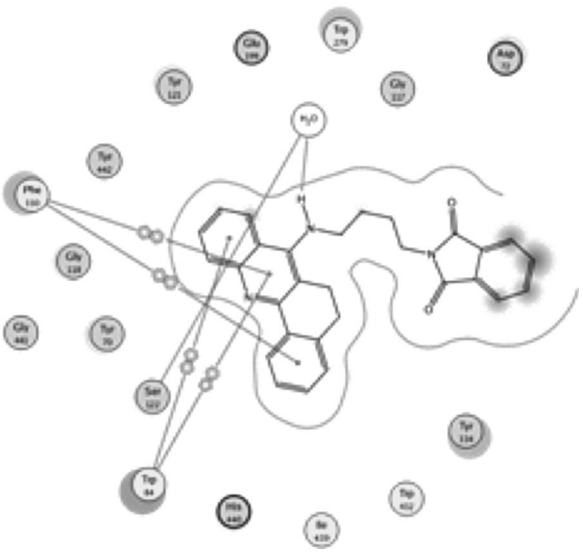


Figure 5. Interaction between ligand (**10b**) and the aromatic residues in the active site gorge of AChE.

membered ring of indole of Trp-84. The amino group is bonded to water molecule, which is bonded to Ser 122. Conformational superposition of tacrine (from X-ray crystal structure of tacrine–AChE complex) with compound **10b** (from docking simulation) is shown in Fig. 4. The superposition showed their hydrophilic and hydrophobic groups overlapping with each other. Moreover, **10b** docking conformation within AChE demonstrating their interaction to the aromatic residue in the active site gorge of AChE is shown in Fig. 5.

Docking of compound **11b** showed π–π interaction of its thiophene and pyridine rings with the phenyl ring of Phe 330. Furthermore, its phenyl, thiophene, and pyridine rings showed π–π interaction with the five-membered ring of the indole of Trp-84. The amino group is bonded to water molecule, which is bonded to Ser 122. Conformational superposition of tacrine (from the X-ray crystal structure of tacrine–AChE complex) with compound **11b** (from docking simulation) is shown in Fig. 6. The superposition showed their hydrophilic and hydrophobic groups overlapping with each other. In addition, **11b** docking conformation within AChE demonstrating their interaction to the aromatic residue in the active site gorge of AChE is shown in Fig. 7.

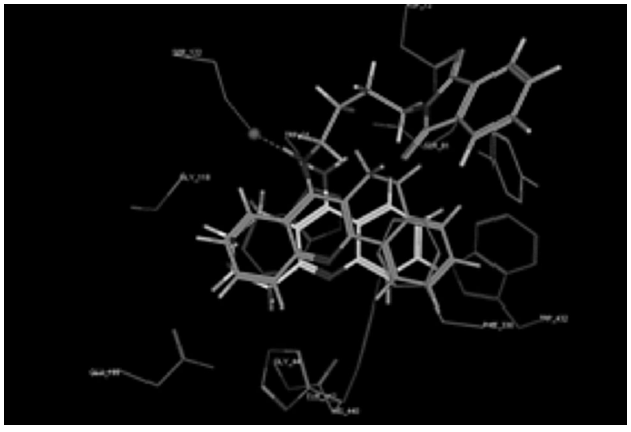


Figure 4. Proposed binding model of compound **10b** and tacrine with AChE. Tacrine represented as ball and stick (white), compound **10b** represented as pink sticks, and other amino acid residues as stick model (color of carbon atom is green, nitrogen is blue, hydrogen is white, oxygen is red, and sulfur is yellow).

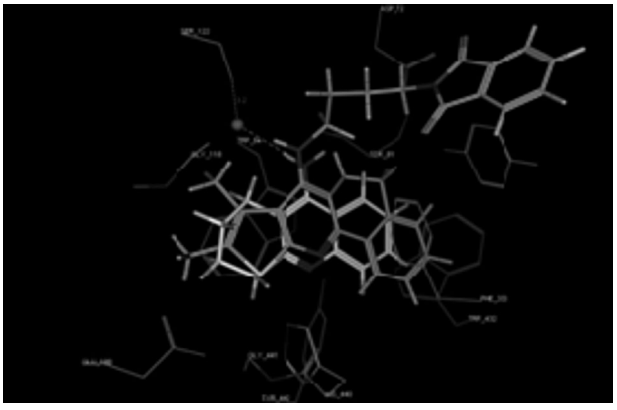


Figure 6. Proposed binding model of compound **11b** and tacrine with AChE. Tacrine represented as ball and stick (white), compound **11b** represented as pink sticks, and other amino acid residues as stick model (color of carbon atom is green, nitrogen is blue, hydrogen is white, oxygen is red, and sulfur is yellow).

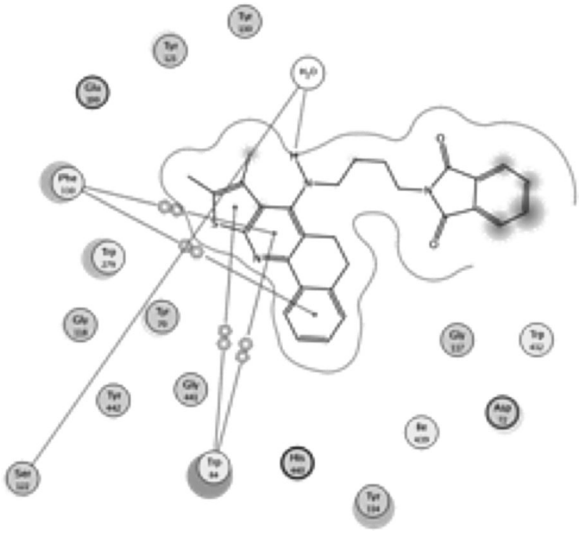


Figure 7. Interaction between ligand (**11b**) and the aromatic residues in the active site gorge of AChE.

Analyzing results

AChE has catalytic and peripheral sites. Compounds **10b** and **11b** showed good fitting to the catalytic active site of AChE by interactions with Phe 330 and Trp-84. It is worth mentioning that there is no interaction of compounds **10b** and **11b** at peripheral active site (Trp 279) of AChE [25]. Examination of the literature revealed [26] that derivatives with five-carbon spacer or less interact only with catalytic active site, but the shorter tether precludes sandwich interaction in the peripheral active site. This seemed to be the reason for the lack of interaction of newly synthesized compounds **10b** and **11b** at peripheral site of AChE.

Drug-likeness

The computed molecular properties are shown in Table 2. The degree of absorption is expressed by the percentage of absorption. Absorption percent (%ABS) was calculated by using $\%ABS = 109 - (0.345 \times PSA)$ [27, 28]. Molecular polar surface area (PSA), log P, number of rotatable bonds, and number of hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) atoms of Lipinski's rule of five [29] were calculated using Molinspiration online property calculation toolkit [30]. Pharmacokinetic property optimization is a rather complex undertaking that is likely to require changes in those molecular determinants that are responsible for binding affinity and specificity like hydrogen bonds. HBA and HBD groups in the compound optimize the drug receptor interaction. Number of HBA ($\leq$10) and HBD ($\leq$5) in the proposed compounds obeys Lipinski's rule of five, so we may have compounds with good absorption or permeability properties through the biological membrane. Dissolution is highly interdependent on aqueous solubility, ionizability (pK_a), and lipophilicity (log P). Furthermore, log P is a crucial factor governing passive membrane partitioning, influencing permeability opposite to its effect on solubility. The log P values of the synthesized compounds lie in between 3.10 and 6.13. In addition, molecular weight of the compound is important in drug action; if the molecular weight of the compound is increased beyond a limit, the bulkiness of the compounds also increases, which will affect the drug action (affect the drug receptor/DNA interaction). Molecular weight of compounds like **7a**,**b**, **8a**,**b**, **10a**,**b**, and **11a**,**b** lies between 303.41 and 481.62, which shows that these compounds follow Lipinski's rule of five. Molecular PSA is a sum of surfaces of polar atoms (usually nitrogen and oxygen attached hydrogen) in a molecule. PSA is a very useful parameter for the prediction of drug transport property. PSA is inversely proportional to %ABS.

Conclusion

We prepared here various benzopyridines and thienopyridines as tacrine analogs. AChE inhibitory activity of new compounds was tested. Screening results showed that eight of compounds exhibited significant ($p < 0.05$) inhibition against AChE, compared with tacrine. It is worth to mention that two of compounds (**10b** and **11b**) were more potent than tacrine. In addition, molecular-modeling study of these compounds revealed that the highest biological activity may be attributed to their proper fitting to the active site of AChE enzyme.

Table 2. Calculated absorption (% ABS), polar surface area (PSA), Lipinski parameters of title compounds **7a**,**b**, **8a**,**b**, **10a**,**b**, and **11a**,**b**.

Compound	%ABS	Volume	PSA	NROTB	HBA	HBD	Log P, calcd	Formula weight
7a	91.43	292.15	50.94	4	3	3	3.10	303.41
7b	91.43	308.95	50.94	5	3	3	3.37	317.44
8a	91.43	315.98	50.94	4	3	3	3.46	337.49
8b	91.43	332.78	50.94	5	3	3	3.73	351.52
10a	86.92	392.18	63.99	5	5	1	5.51	433.51
10b	86.92	408.98	63.99	6	5	1	5.78	447.54
11a	86.92	416.01	63.99	5	5	1	5.86	467.59
11b	86.92	432.81	63.99	6	5	1	6.13	481.62

Experimental

Chemistry

Melting points are obtained on Griffin apparatus and the values given are uncorrected, IR spectra were recorded on a Shimadzu 435 spectrometer, using KBr disks. ^{1}H NMR spectra were recorded on a Mercury-300BB 300MHz spectrometer using TMS as internal standard. Mass spectra were recorded on a JEOL JMS-AX 500 mass spectrometer. Elemental analyses for C, H, and N were within ±0.4% of the theoretical values and were performed at the Microanalytical Center, Cairo University. Progress of the reaction was monitored by TLC using precoated aluminum sheets silica gel Merck 60 F 254 and visualized by UV lamp. Compound **4** was prepared adopting Gewald conditions [31]. All the chemicals were purchased from Aldrich and Sigma Company.

*General procedure for the synthesis of compounds **3** and **5***

1-Tetralone (**2**) (0.1 mol) was added portionwise to a slurry of methyl anthranilate (**1**) or ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (**4**) (0.1 mol) and phosphorus oxychloride (75 mL). The reaction mixture was stirred under reflux for 3 h and concentrated under reduced pressure. The residue was dissolved in chloroform and poured carefully into a mixture of ice and ammonium hydroxide. The aqueous phase was separated and extracted with methylene chloride. The extract was concentrated *in vacuo*. Trituration of the residue with diethyl ether and crystallization from benzene yielded the title compounds **3** and **5**.

*7-Chloro-5,6-dihydrobenzo[c]acridine (**3**)*

Yield: 67%; mp 148°C; IR (KBr) cm^{-1}: 3062–3050 (CH aromatic), 2947–2885 (CH aliphatic), 1685 (C=N); ^{1}H NMR ($CDCl_3$) δ: 2.14–3.48 (m, 4H, $2CH_2$), 6.96–8.41 (m, 8H, CH aromatic); MS: *m/z* (% abundance) 265 (M^+) (56.40); Anal. calcd. for $C_{17}H_{12}ClN$: C, 76.83; H, 4.55; N, 5.27. Found: C, 76.59; H, 4.54; N, 5.25.

*7-Chloro-8,9-dimethyl-5,6-dihydrothieno[2,3-b]benzo[h]-quinoline (**5**)*

Yield: 70%; mp 210°C; IR (KBr) cm^{-1}: 3100–3050 (CH aromatic), 2962–2870 (CH aliphatic), 1681 (C=N); ^{1}H NMR ($CDCl_3$) δ: 1.24–1.38 (m, 2H, CH_2), 2.40 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 3.03–3.72 (m, 2H, CH_2), 7.12–7.54 (m, 4H, CH aromatic); MS: *m/z* (% abundance) 299 (M^+) (83.01); Anal. calcd. for $C_{17}H_{14}ClNS$: C, 68.10; H, 4.71; N, 4.67. Found: C, 68.09; H, 4.70; N, 4.67.

General methods for the alkylation reaction

Method A

A mixture of chloropyridines **3** or **5** (4.61 mmol), certain diaminoalkane **6a** or **6b** (13.8 mmol), and 1-pentanol (5 mL) was heated under reflux for 72 h. After cooling to room temperature, the mixture was diluted with methylene chloride (50 mL) and then washed with 10% NaOH (1 × 50 mL) and water (2 × 40 mL). The organic layer was concentrated *in vacuo* and crystallized from *N*,*N*-dimethylformamide to give the desired product.

Method B

To a solution of the appropriate chloro derivatives **3** or **5** (0.01 mol) in ethylene glycol (10 mL), the selected diamine **6a** or **6b** (0.03 mol) was added in presence of catalytic amounts of cuprous oxide and potassium carbonate. The mixture was heated under reflux for 24 h, filtered, and the filtrate was left aside to cool and poured on ice-cooled water. The separated solid was filtered, dried, and crystallized from ethanol.

*7-(3-Aminopropyl)amino-5,6-dihydrobenzo[c]acridine (**7a**)*

The title compound was prepared from the reaction of (**3**) and 1,3-diaminopropane (**6a**) adopting method A or B to give **7a**. Yield: 55% (method A), 62% (method B); mp >300°C; IR (KBr) cm^{-1}: 3390–3278 (NH_2, NH), 3059–3040 (CH aromatic), 2927–2862 (CH aliphatic), 1625 (C=N); ^{1}H NMR (DMSO-d_6) δ: 1.15–1.25 (m, 2H, CH_2), 1.60–1.75 (m, 4H, $2CH_2$), 2.49–2.51 (m, 2H, CH_2), 3.35–3.57 (m, 2H, CH_2), 3.58–3.65 (m, 3H, NH, NH_2 D_2O exchangeable), 7.70–7.81 (m, 4H, CH aromatic), 7.82–7.85 (m, 4H, CH aromatic); MS: *m/z* (% abundance) 304 $(M+1)^+$ (0.74); Anal. calcd. for $C_{20}H_{21}N_3$: C, 79.17; H, 6.98; N, 13.85. Found: C, 79.18; H, 6.97; N, 13.85.

*7-(4-Aminobutyl)amino-5,6-dihydrobenzo[c]acridine (**7b**)*

The title compound was prepared from the reaction of (**3**) and 1,4-diaminobutane (**6b**) adopting method A or B to give **7b**. Yield: 70% (method A), 75% (method B); mp >300°C; IR (KBr) cm^{-1}: 3450–3170 (NH_2, NH), 3066–3050 (CH aromatic), 2927–2862 (CH aliphatic), 1610 (C=N); ^{1}H NMR (DMSO-d_6) δ: 1.55–1.65 (m, 6H, $3CH_2$), 1.91–1.95 (m, 2H, CH_2), 2.49–2.51 (m, 2H, CH_2), 3.25–3.45 (m, 2H, CH_2), 3.59–3.70 (m, 3H, NH, NH_2 D_2O exchangeable), 7.70–7.82 (m, 4H, CH aromatic), 7.83–7.95 (m, 4H, CH aromatic); MS: *m/z* (% abundance) 316 $(M-1)^+$ (0.38); Anal. calcd. for $C_{21}H_{23}N_3$: C, 79.46; H, 7.30; N, 13.24. Found: C, 79.45; H, 7.30; N, 13.23.

*7-(3-Aminopropyl)amino-8,9-dimethyl-5,6-dihydrothieno-[2,3-b]benzo[h]quinoline (**8a**)*

The title compound was prepared from the reaction of **5** and **6a** adopting method A or B to give **8a**. Yield: 60% (method A), 65% (method B); mp >300°C; IR (KBr) cm^{-1}: 3363–3271 (NH_2, NH), 3062–3050 (CH aromatic), 2924–2854 (CH aliphatic), 1630 (C=N); ^{1}H NMR (DMSO-d_6) δ: 1.23–1.54 (m, 4H, $2CH_2$), 2.47 (s, 3H, CH_3), 2.60–2.65 (m, 2H, CH_2), 2.87 (s, 3H, CH_3), 3.19–3.35 (m, 4H, $2CH_2$), 4.34, 5.26 (2s, 3H, NH, NH_2 D_2O exchangeable), 7.27–8.17 (m, 4H, CH aromatic); MS: *m/z* (% abundance) 337 (M^+) (1.19); Anal. calcd. for $C_{20}H_{23}N_3S$: C, 71.18; H, 6.87; N, 12.45. Found: C, 71.16; H, 6.86; N, 12.44.

*7-(4-Aminobutyl)amino-8,9-dimethyl-5,6-dihydrothieno-[2,3-b]benzo[h]quinoline (**8b**)*

The title compound was prepared from the reaction of **5** and **6b** adopting methods A or B to give **8b**. Yield: 70% (method A), 80% (method B); mp 201°C; IR (KBr) cm^{-1}: 3430–3250 (NH_2, NH), 3055–3040 (CH aromatic), 2927–2866 (CH aliphatic), 1654 (C=N); ^{1}H NMR (DMSO-d_6) δ: 1.39–1.74 (m, 4H, $2CH_2$), 2.30–2.34 (m, 2H, CH_2), 2.39 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.76–2.78 (m, 2H, CH_2), 3.13–3.33 (m, 4H, $2CH_2$), 4.43–4.60 (m, 3H, NH, NH_2 D_2O exchangeable), 7.19–8.18 (m, 4H, CH aromatic); MS: *m/z* (% abundance) 351 (M^+) (0.34); Anal. calcd. for $C_{21}H_{25}N_3S$: C, 71.75; H, 7.17; N, 11.95. Found: C, 71.76; H, 7.17; N, 11.96.

*General procedure for the synthesis of compounds **10a,b** and **11a,b***

To a solution of the desired amino pyridines **7a** or **7b** or **8a** or **8b** (0.01 mol) in glacial acetic acid (10 mL) phthalic anhydride (**9**) (0.01 mol) was added and the mixture was heated under reflux for

18 h. The separated solid was filtered, washed with water, dried, and crystallized from ethanol to afford the pyridine derivatives.

*7-[3-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)propyl]-amino-5,6-dihydrobenzo[c]acridine (**10a**)*

Yield: 83%; mp 237–238°C; IR (KBr) cm^{-1}: 3470–3400 (NH), 3100–3050 (CH aromatic), 2941–2862 (CH aliphatic), 1770, 1700 (2C=O), 1616 (C=N); ^1H NMR (DMSO-d_6) δ: 1.56–1.76 (m, 6H, $3CH_2$), 2.49–2.51 (m, 2H, CH_2), 3.40–3.45 (m, 2H, CH_2), 3.58 (s, 1H, NH D_2O exchangeable), 7.65–7.95 (m, 12H, CH aromatic); MS: *m/z* (% abundance) 432 $(M-1)^+$ (21.64); Anal. calcd. for $C_{28}H_{23}N_3O_2$: C, 77.58; H, 5.35; N, 9.69. Found: C, 77.58; H, 5.34; N, 9.69.

*7-[4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)butyl]amino-5,6-dihydrobenzo[c]acridine (**10b**)*

Yield: 87%; mp 219–220°C; IR (KBr) cm^{-1}: 3460–3400 (NH), 3090–3064 (CH aromatic), 2935–2867 (CH aliphatic), 1769, 1712 (2C=O), 1607 (C=N); ^1H NMR (DMSO-d_6) δ: 1.61–1.75 (m, 6H, $3CH_2$), 1.90–1.95 (m, 2H, CH_2), 2.50–2.60 (m, 2H, CH_2), 3.29–3.40 (m, 2H, CH_2), 3.59 (s, 1H, NH D_2O exchangeable), 7.50–8.10 (m, 12H, CH aromatic); MS: *m/z* (% abundance) 447 (M^+) (0.90); Anal. calcd. for $C_{29}H_{25}N_3O_2$: C, 77.83; H, 5.63; N, 9.39. Found: C, 77.78; H, 5.63; N, 9.38.

*7-[3-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)propyl]-amino-8,9-dimethyl-5,6-dihydrothieno[2,3-b]benzo[h]-quinoline (**11a**)*

Yield: 85%; mp 190°C; IR (KBr) cm^{-1}: 3500–3400 (NH), 3056–3036 (CH aromatic), 2941–2869 (CH aliphatic), 1771, 1710 (2C=O), 1613 (C=N); ^1H NMR (DMSO-d_6) δ: 1.96–2.01 (m, 6H, $3CH_2$), 2.49–2.51 (m, 2H, CH_2), 3.26 (s, 1H, NH D_2O exchangeable), 3.59 (s, 3H, CH_3), 3.60–3.62 (m, 2H, CH_2), 3.64 (s, 3H, CH_3), 7.81–7.84 (m, 8H, CH aromatic); MS: *m/z* (% abundance) 467 (M^+) (0.34); Anal. calcd. for $C_{28}H_{25}N_3O_2S$: C, 71.92; H, 5.38; N, 8.99. Found: C, 71.90; H, 5.39; N, 8.98.

*7-[4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)butyl]amino-8,9-dimethyl-5,6-dihydrothieno[2,3-b]benzo[h]-quinoline (**11b**)*

Yield: 90%; mp 227–228°C; IR (KBr) cm^{-1}: 3500–3350 (NH), 3050–3025 (CH aromatic), 2950–2875 (CH aliphatic), 1770, 1712 (2C=O), 1612 (C=N); ^1H NMR (DMSO-d_6) δ: 1.51–1.70 (m, 6H, $3CH_2$), 1.90–1.95 (m, 2H, CH_2), 2.77–2.82 (m, 4H, $2CH_2$), 2.89 (s, 1H, NH D_2O exchangeable), 3.30(s, 3H, CH3), 3.59(s, 3H, CH3), 7.43–7.91(m, 8H, CH aromatic); MS: *m/z* (% abundance) 481 (M^+) (29.43); Anal. calcd. for $C_{29}H_{27}N_3O_2S$: C, 72.32; H, 5.65; N, 8.73. Found: C, 72.25; H, 5.65; N, 8.72.

Animals and methods

Adult male albino Wister rats weighing 180–200 g were used in the current study. Rats were purchased from the animal house of El-Nile Company (Cairo, Egypt). Rats were kept under constant laboratory conditions and were allowed free access to food and water throughout the period of investigation. The tested compounds were orally administered once. After 30 min the rats were killed by decapitation, and then the brains were carefully removed and homogenized in normal saline (pH 7.4).

AChE inhibition assay *in vitro*

Inhibitory activity against AChE was determined using cholinesterase kits (purchased from Gamma Trade Company) at 37°C according to colorimetric method reported by Ellman et al. [24]. Cholinesterase kits consist of 0.1 M sodium phosphate buffer (pH 8.0), 0.3 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent), 0.02 U of AChE from *Electrophorus electricus*, and 0.5 mM acetylthiocholine iodide as substrate of the enzymatic reaction. Briefly, the method used was as follows: AChE of rat brain homogenates, either without any drugs (control group) or containing tested compounds (AChE inhibitors) mixed with cholinesterase kit assay solution without the substrate, were preincubated with enzyme for 10 min at 37°C. After preincubation, the substrate was added. The absorbance changes at 405 nm were recorded for 5 min with a microplate reader GENios FI29004 (Tecan Ltd., Austria). The AChE inhibition was determined for each compound. Each assay was run in triplicate and each reaction was repeated at least three independent times.

The study was carried out according to international guidelines and approved by the ethical committee animal experimentation at the Faculty of Pharmacy, Cairo University (serial number of the protocol: OC (422) in April 30, 2012).

Statistical analysis

Statistical analysis was carried out by one-way ANOVA followed by Tukey–Kramer multiple comparisons test for comparison of means of different groups. Each value represents mean ± SE ($n = 6–8$ rats).

Results of anti-AChE activity of the tested compounds as well as tacrine are shown in Table 1.

Molecular docking

All the molecular-modeling studies were carried out on Intel Pentium 1.6 GHz processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment (MOE 2008.10; Chemical Computing Group, Canada) (Molecular Operating Environment (MOE 2008.10); C.C.G., Inc., 1255 University St., Suite 1600, Montreal, Quebec, Canada H3B 3×3, 2005, www.Chemcomp.com) as the computational software.

All the minimizations were performed with MOE until a RMSD gradient of 0.05 kcal mol^{-1} A^{-1} with MMFF94x force-field and the partial charges were automatically calculated.

The X-ray crystallographic structure of AChE complexed with tacrine (PDB ID: 1ACJ) was obtained from the protein data bank available at the RCSB Protein Data Bank (www.rcsb.org) with 2.80 Å resolution.

Enzyme structures were checked for missing atoms, bonds, and contacts; hydrogens and partial charges were added to the system using Protonate 3D application; water molecules were manually deleted; the active site was generated using the residues close to the tacrine atoms; the ligand molecules were constructed using the builder module and were energy-minimized; all antagonist structures were docked into the active site by using the MOE Dock tool.

This method is divided into a number of stages:

a. *Conformational analysis of ligands*: The algorithm-generated conformation from a single 3D conformation by conducting a systematic search. In this way, all combinations of angles were created for each ligand.

b. *Placement*: A collection of poses was generated from the pool of ligand conformations using the Triangle Matcher placement method. Poses were generated by superposition of ligand atom triplets and triplets of points in the receptor binding site in a systematic way.
c. *Scoring*: Poses generated by the placement methodology were scored using the London dG scoring function implemented in MOE, which estimates the free energy of binding of the ligand from a given pose. The top ten poses for each ligand were the output in a MOE database. Each resulting ligand pose was then subjected to MMFF94x energy minimization.

The authors have declared no conflict of interest.

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