Med Chem Res (2013) 22:4087–4095
DOI 10.1007/s00044-012-0403-5

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design and synthesis of thienopyridines as novel templates for acetylcholinesterase inhibitors

Mohga M. Badran · Maha Abdel Hakeem · Suzan M. Abuel-Maaty · Afaf El-Malah · Rania M. Abdel Salam

Received: 15 July 2012 / Accepted: 6 December 2012 / Published online: 20 December 2012

Abstract New dual binding site acetylcholinesterase (AChE) inhibitors have been designed and synthesized as a new drug candidate for the treatment of Alzheimer's disease (AD) through the binding to both catalytic and peripheral sites of the enzyme. Therefore, a series of thienopyridine analogs of tacrine were synthesized and investigated for their ability to inhibit the activity of AChE in comparison with tacrine. All the compounds were found to inhibit AChE activity, especially compounds **7b** and **11a**, which were found to be more potent than tacrine.

Keywords Synthesis · Thienopyridines · AChE inhibitors · Alzheimer's disease

Introduction

Alzheimer's disease (AD) is a progressive and neurodegenerative disorder of the central nervous system that is most common cause of dementia among elderly people. The disease is characterized by neural loss, synaptic damage, neurotic, and vascular plaques (Bartus *et al*., 1982; Davis and Powchik, 1995). AChE inhibitors (Ruiz *et al*., 2005; Tariot and Federoff, 2003) represent a promising approach for the treatment of AD since AChE is the enzyme involved in the hydrolysis of neurotransmitter acetylcholine (ACh) at cholinergic synapse in the central and peripheral nervous system. Inhibitors of AChE activity promoted an increase in the concentration and duration of the action of synaptic ACh, thus causing an enhancement of cholinergic transmission through the activation of the nicotinic and muscarinic receptors. However, the achievement of potent inhibitors of AChE catalytic site will not represent a significant improvement unless there is a concomitant inhibition of the peripheral anionic site (PAS) of the enzyme, which is associated with the neurotoxic cascade of AD through AChE induced Aβ aggregation (Bolognesi *et al*., 2005; Kwon *et al*., 2007). The literature survey revealed also that new potent and selective AChE inhibitors such as tacrine–huperzine hybrid derivative (Badia *et al*., 1998; Camps *et al*., 1999) (a), bis-interacting galanthamine ligand (Mary *et al*., 1998) (b), and tacrine linked to lipoic acid fragment (Rosini *et al*., 2005) (c) have already been designed and synthesized in Fig. 1.

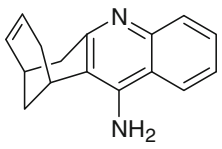
The first analog (a) is a hybrid of two well-known AChE inhibitors, tacrine and huperzine. Whereas, the second (b) represents an association of two pharmacophoric entities capable of interacting with the active and peripheral site of the enzyme (galantamine and phthalyl moiety). On the other hand, the introduction of lipoic acid moiety (LA) to tacrine afforded compound (c), in the third structure, combining the antioxidant properties of LA with the ability of tacrine to interact with the PAS. In addition, several tacrine-based compounds were also developed, among them is a compound containing two tacrine subunits (Butini *et al*., 2008; Carlier *et al*., 1999) (d) Fig. 2, the amino groups of which are connected with a hepta methylene side chain. This compound, designed taking in account the existence of two binding sites for tacrine in AChE, is about 149 times more potent than tacrine.

On the basis of this evidence, a new series of compounds containing two units of thienopyridine derivatives

M. M. Badran · M. A. Hakeem · S. M. Abuel-Maaty (✉) · A. El-Malah
Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Kaser El-Aini Street, P.O. Box 11562, Giza, Egypt
e-mail: suzanaboelnaga@gmail.com

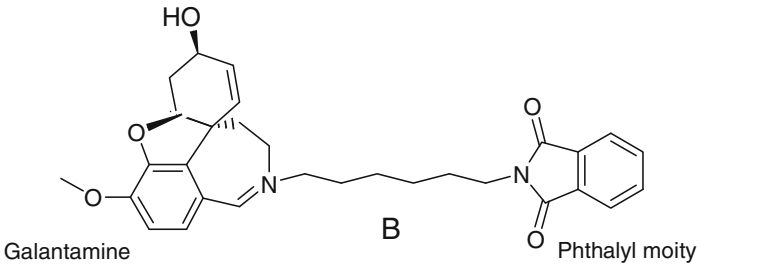
R. M. Abdel Salam
Pharmacology and Toxicology Department, Faculty of Pharmacy, Cairo University, Kaser El-Aini Street, Giza, Egypt

Fig. 1 Tacrine based compounds

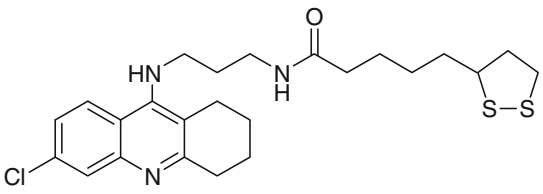


A

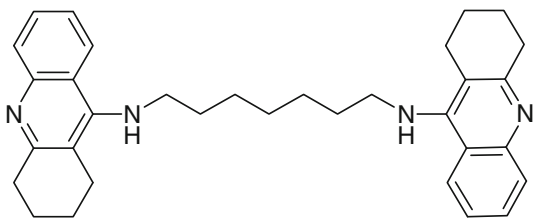
Tacrine-huperzine **A** hybrid



B



C



D

Fig. 2 Alkylene linked homodimeric tacrine

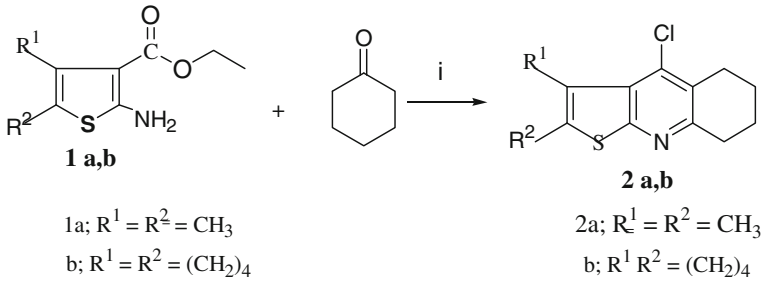
connected by methylene linkers **(5a–c)** is synthesized. In addition, another series of thienopyridine analogs of tacrine is synthesized applying a design strategy in which distinct pharmacophores of two different drugs were combined in the same structure leading to hybrid molecules. This is achieved through linking the thienopyridine analog of Tacrine by a linker of suitable length and nature to either phthalimide moiety which could interact with PAS **(7a–d)** and **(9a**, **b)** (Ishihara *et al*., 1991) or lipoic acid moiety, a universal antioxidant, **(11a–c)** (Rosini *et al*., 2005), to get new potent dual-binding site AChE inhibitors for enhancement of cholinergic transmission and inhibition of AChE-induced Aβ aggregation and oxidative stress, leading to a synergic and effective treatment of AD. On the other hand, exploratory replacement in successful drugs of benzene ring with thiophene moiety which is widely recognized as bioisostere of benzene has become a routine strategy in modern drug design. Encouraged by these findings, we substituted the benzene ring in tacrine with the thiophene ring in our study to investigate the biologic effects of these structural modifications of tacrine.

Results and discussion

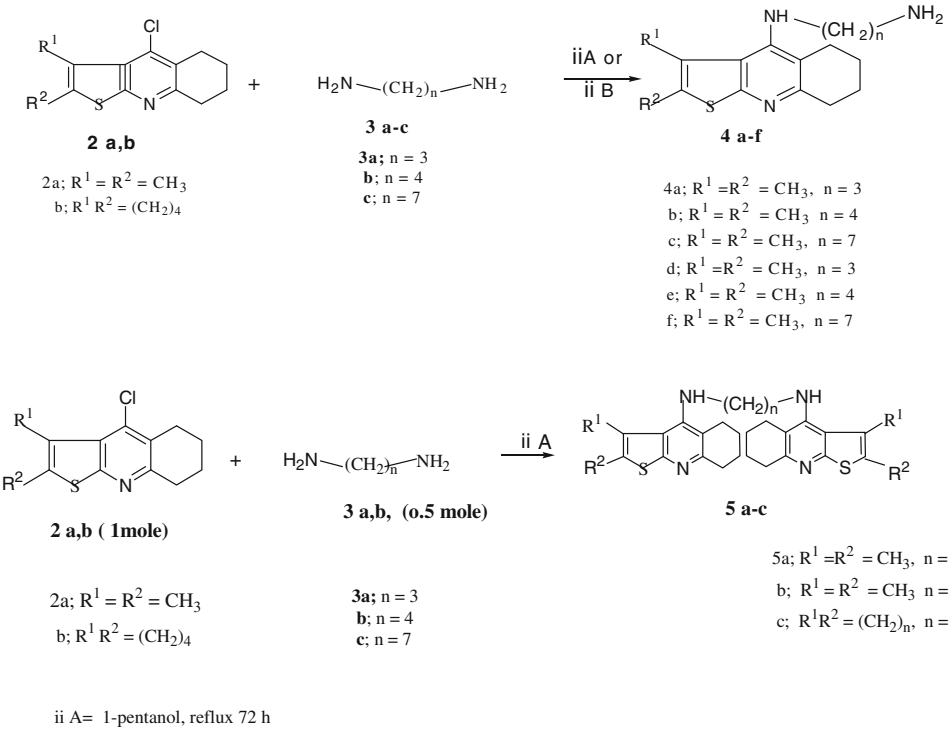
Chemistry

The synthetic methodology employed for the preparation of the new dual binding site heterodimers varied depending on the bond type in the linker which was inserted in the last

Scheme 1 Reagents and conditions: *i* = $POCl_3$, reflux 3 h



Scheme 2 Reagents and conditions: *ii A* = 1-pentanol, reflux 72 h; *ii B* = ethylene glycol, CuO/K_2CO_3, reflux 24 h

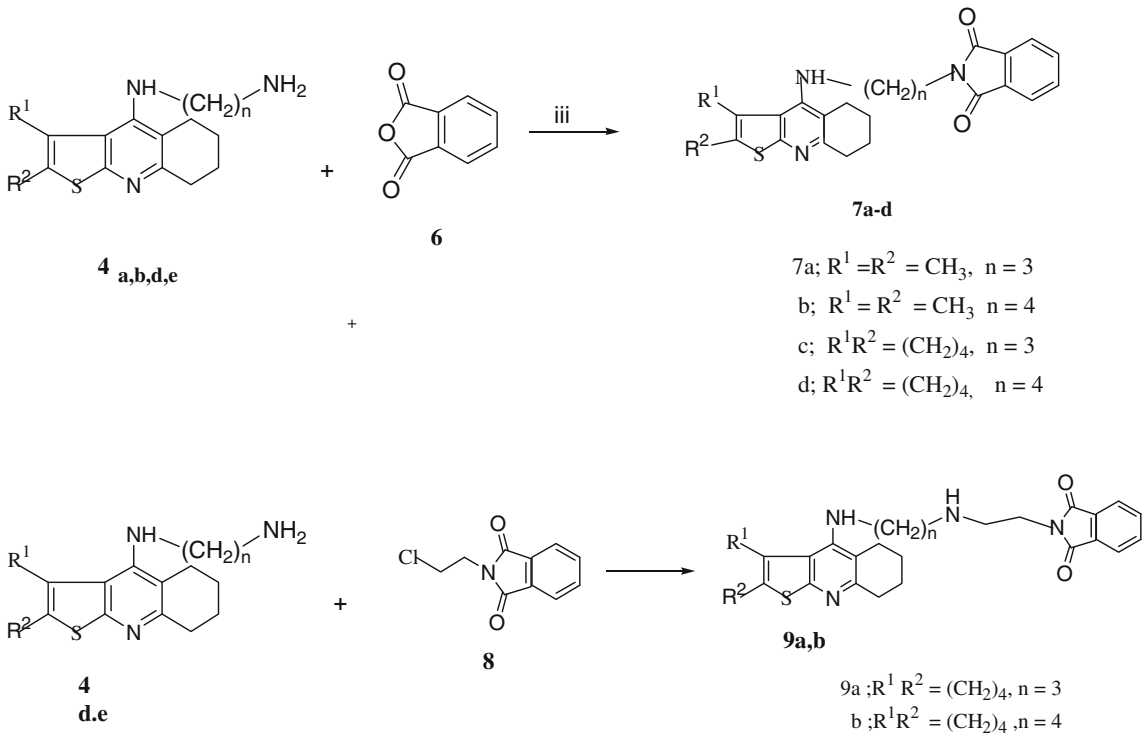


step of the synthetic pathway. The synthetic routes utilized for the preparation of these new compounds are depicted in Schemes 1, 2, 3, 4. The chlorothienopyridines **2a**, **b** were prepared in a one-step reaction by refluxing the aminothiophene esters **1a**, **b** with cyclohexanone in the presence of phosphorous oxychloride (Scheme 1).

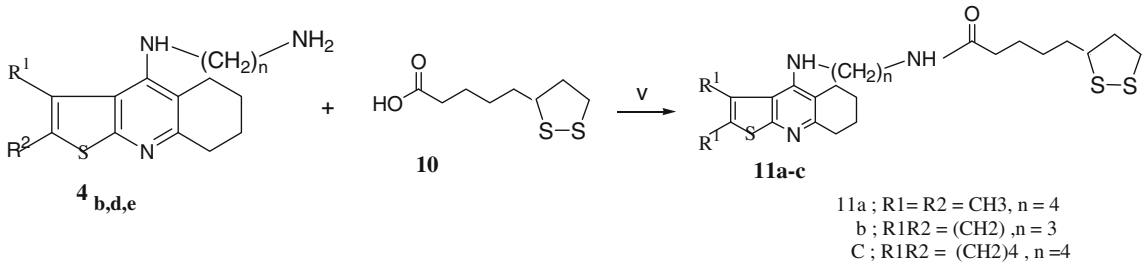
A search in the literature demonstrated that the synthesis of (9-aminoalkylamino)-1,2,3,4-tetrahydroacridine could be achieved in a good yield through the reaction of the chloro derivative of tacrine (9-chlorotetrahydroacridine) with the appropriate diamines in pentanol (Carlier *et al.*, 1999).

Applying the aforementioned method to the chloro derivatives **2a**, **b** afforded compounds **4a**–**f** in a poor yield. However, the synthesis of these compounds which could be considered adaptable key intermediates in our study, were accomplished in a satisfactory yield through their heating with the appropriate diamine in ethylene glycol, using Cu_2O (Lang *et al.*, 2001) as a catalyst. Amination of the chloro derivatives **2a**, **b** with a half equivalent of the diaminoalkanes **3a**–**c** afforded the alkylene-linked homodimeric derivatives **5a**–**c** (Scheme 2).

On the other hand, preparation of the new dual heterodimers containing the phthalimide moiety inserted in the aminothienopyridines **4a**–**e** was achieved through two routes. The first one was via heating the amino derivatives **4a**, **b**, **d**, **e** with phthalic anhydride **6** in glacial acetic acid to afford compounds **7a**–**d.** The second route was attained through the reaction of the appropriate aminothienopyridines **4d**, **e** with chloroethylphthalimide **8** in dry DMF in the presence of anhydrous potassium carbonate to yield the corresponding derivatives **9a**, **b** (Scheme 3). The IR spectra of compounds **7a**–**d** and **9a**–**e** revealed the presence of two overlapped C=O absorption bands at 1,705–1,720 cm^{-1}. Besides, the ^1H NMR spectra demonstrated the aromatic protons of the phthalimide moiety at 7.4–8 ppm. Nevertheless, the reaction of the



Scheme 3 Reagents and conditions: *iii* = glacial acetic acid, reflux 20 h; *iv* = K_2CO_3/DMF, reflux 24 h



Scheme 4 Reagents and conditions V = N_2/DMF, rt, 24 h

aminothienopyridines **4b**, **d**, **e** with lipoic acid **10** was fulfilled via stirring under nitrogen in the presence of 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) as dehydrating agent in dry DMF and at room temperature to furnish the corresponding thienopyridine-lipoic acid heterodimers **11a–c** (Scheme 4). The IR spectra of compounds **11a–c** showed C=O absorption bands at 1,647, 1,685, and 1,699 cm, respectively.

It is worth mentioning that all attempts to react compound **4f** which contains seven methylene groups with phthalic anhydride, chloroethylphthalimide, or lipoic acid were fruitless. This may be attributed to the nature of this compound, as it has rubber-like property and it is nearly insoluble in all solvents. All the new compounds have been fully characterized through their spectral data.

Conclusion

We reported here the synthesis of various thienopyridines as tacrine heterodimer analogs. The synthesized compounds were tested for their antiacetylcholinesterase activity. Results showed that all the compounds demonstrated significant activity in comparison with tacrine; they are more or less similar to each other and showed good to moderate inhibitory activity. The higher activity of compound **11b** might be attributed to the lipoic acid moiety which was reported to possess antioxidant effect (Rosini *et al*., 2005). Whereas, the higher activity of **7b** might be referred to the phthalimide (Ishihara *et al*., 1991) moiety that has two carbonyl groups which may contribute to the enzyme inhibition by hydrogen bonding with the enzyme.

Table 1 Inhibition of AChE activity of tacrine and synthesized thienopyridines

Compound	Cholinesterase content (U/gm net weight)	Inhibition (%)
Normal control (saline)	334.31 ± 26.49	0
Tacrine (10 mg/kg)	153,56 ± 12.38*	54.91
5a (10 mg/kg)	250.24 ± 28.19*	25.15
5b (10 mg/kg)	195.49 ± 11.59*	41.53
5c (10 mg/kg)	215.05 ± 14.09*	35.67
7a (10 mg/kg)	179.86 ± 7.82*	46.20
7b (10 mg/kg)	148.58 ± 7.82*	55.56
7c (10 mg/kg)	191.59 ± 18.59*	42.69
7d (10 mg/kg)	234.59 ± 33.18*	29.83
9a (10 mg/kg)	187.68 ± 20.98*	43.86
9b (10 mg/kg)	183.76 ± 11.19*	45.03
11a (10 mg/kg)	191.03 ± 20.75*	42.86
11b (10 mg/kg)	144.67 ± 16.49*	56.73
11c (10 mg/kg)	262.75 ± 27.16[@]	21.41

* Significantly different from normal control group at $P < 0.05$

[@] Significantly different from tacrine group at $P < 0.05$

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. 1H NMR spectra were recorded on a Mercury-300BB 300 MHZ and a Varian GEMINI-200 MHZ spectrometer using TMS as an internal standard. Mass spectra were recorded on a JEON JMS-AX 500 Mass Spectrometer. Elemental analyses for C, H, and N were within ±0.4 % of the theoretic values and were performed at the Micro Analytical Center, Cairo University. Progress of the reaction was monitored by TLC using pre-coated silica gel aluminum sheets MERCK 60 F 254 and was visualized by UV lamp. Compounds **1a** and **b** were prepared adopting Gewaled conditions (Gewald *et al*., 1966). All the chemicals were purchased from Aldrich and Sigma Companies.

*General procedures for the synthesis of compounds **2a** and **b***

Cyclohexanone (0.1 mol) was added portion wise to a slurry of 2-amino-3-ethoxycarbonylthiophenes **1a**, **b** (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 vacuum. Triturating of the residue with diethyl ether and crystallization from benzene yielded the title compounds **2a**, **b**.

*4-Chloro-2,3-dimethyl-5,6,7,8-tetrahydrothieno[2,3-b]quinoline (**2a**)* Yield 70 %; mp 138–140 °C; IR (KBr) cm^{-1}: 2,927, 2,856 (CH aliphatic), 1,683 (C=N); ^{1}H NMR ($CDCl_3$)δ: 1.66–1.74(m, 4H, $2CH_2$), 2.40(s, 3H, CH_3), 2.45(s, 3H, CH_3), 2.80–3.20(m, 4H, $2CH_2$); MS: *m/z* (% abundance) 251(M+) (27.86); Anal. Calcd for $C_{13}H_{14}Cl$ NS: C, 62.01; H, 5.60; N, 5.56. Found: C,61.98, H, 5.60; N, 5.56.

*11-Chloro-1,2,3,4,7,8,9,10-octahydro[1]benzothieno[2,3-quinoline (**2b**)* Yield 80 %; mp 160–162 °C; IR (KBr) cm^{-1}: 2,931, 2,858 (CH aliphatic), 1,682 (C=N); ^{1}H NMR($CDCl_3$)δ : 1.75–2.00(m, 8H, $4CH_2$), 2.75–2.89(m, 4H, $2CH_2$), 2.95–3.10(2 m, 4H, $2CH_2$); MS: *m/z* (% abundance) 277(M+) (100); Anal. Calcd for $C_{15}H_{16}Cl$ NS: C, 64.85; H, 5.81; N, 5.04. Found: C, 64.82, H, 5.80; N, 5.04.

General methods for alkylation reaction

Method A. A mixture chlorothienopyridines **2a** or **2b** (4.61 mmol), certain diaminoalkane **3a–c** (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 vacuum and crystallized from acetic acid to give the desired product.

Method B. To a solution of the appropriate chloro derivatives **2a** or **2b** (0.01 mol) in ethylene glycol l(10 ml), the selected diamine **3a–c** (0.03 mol) was added in the 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.

*4-(3-Aminopropyl)amino-2,3-dimethyl-5,6,7,8-tetrahydrothieno[2,3-b]quinoline (**4a**)* The title compound was prepared from the reaction of **2a** and 1,3-diamino propane (**3a**) adopting Method A or B to give **4a**. Yield 35 % (Method A), 64 % (Method B); mp 120–122 °C; IR (KBr) cm^{-1}: 3,383–3,273 (NH_2, NH), 2,927, 2,854 (CH aliphatic), 1,635 (C=N); ^{1}H NMR(DMSO-d6)δ: 1.20–1.32(m, 2H, CH_2), 1.49–1.76(2 m, 4H, $2CH_2$), 2.46(s, 3H, CH_3), 2.51(s, 3H, CH_3), 2.73–2.79(m, 4H, $2CH_2$), 3.65–3.70(m, 4H, $2CH_2$)); MS: *m/z* (% abundance) 289(M+) (11.94); Anal. Calcd for $C_{16}H_{23}N_3S$: C, 66.39; H, 8.01; N, 14.52. Found: C, 66.89, H, 8.02; N, 14.53.

*4-(4-Aminobutyl)amino-2,3-dimethyl-5,6,7,8-tetrahydrothieno[2,3-b]quinoline (**4b**)* The title compound was prepared from the reaction of **2a** and 1,4-diamino butane (**3b**) adopting Method A or B to give **4b.** Yield 21 % (Method A), 45 % (Method B); mp >300 °C; IR (KBr) cm^{-1}: 3,354–3,255 (NH_2, NH), 2,927, 2,856 (CH aliphatic), 1,635 (C=N); ^{1}H NMR(DMSO-d6)δ: 1.22–1.75(m, 8H, $4CH_2$), 2.36(s, 3H, CH_3), 2.44(s, 3H, CH_3), 2.65–2.97(m, 4H, $2CH_2$), 3.20–3.40(m, 4H, $2CH_2$); MS: *m/z* (% abundance) 303(M+) (18.13); Anal. Calcd for $C_{17}H_{25}N_3S$: C, 67.28; H, 8.30; N, 13.85. Found: C, 67.36, H, 8.31; N, 13.86.

*4-(7-Aminoheptyl)amino-2,3-dimethyl-5,6,7,8-tetrahydrothieno[2,3-b]quinoline (**4c**)* The title compound was prepared from the reaction of **2a** and 1,7-diamino heptane (**3c**) adopting Methods A or B to give **4c**. Yield 45 % (Method A), 50 % (Method B); mp >300 °C; IR (KBr) cm^{-1}: 3,450–3,300 (NH_2, NH), 2,924, 2,850 (CH aliphatic), 1,650 (C=N); ^{1}H NMR(DMSO-d6)δ: 1.15–1.29(m, 6H, $3CH_2$), 1.38–1.52 (2m, 4H, $2CH_2$), 1.82–1.85(m, 4H, $2CH_2$), 2.49(s, 3H, CH_3), 2.51(s, 3H, CH_3), 2.68–2.73(m, 4H, $2CH_2$), 3.25–3.43(m, 4H, $2CH_2$); MS: *m/z* (% abundance) 345(M +) (2.12); Anal. Calcd for $C_{20}H_{31}N_3S$: C, 69.52; H, 9.04; N, 12.16. Found: C, 69.48; H, 9.04; N, 12.15

*11-(3-Aminopropyl)amino-1,2,3,4,7,8,9,10-octahydro[1] benzothieno [2,3-b]quinoline (**4d**)* The title compound was prepared from the reaction of **2b** and **3a** adopting Method A or B to give **4d**. Yield 45 % (Method A), 53 % (Method B); mp >300 °C; IR (KBr) cm^{-1}: 3,400–3,300 (NH_2, NH), 2,941, 2,858 (CH aliphatic), 1,633 (C=N); ^{1}H NMR (DMSOd6)δ: 1.18–2.08(m, 10H, $5CH_2$), 2.63–2.91(m, 8H, $4CH_2$), 3.29–3.49(m, 4H, $2CH_2$), 4.40, 7.36(2s, 3H, NH, NH_2 D_2O exchangeable); MS: *m/z* (% abundance) 315(M +) (4.70); Anal. Calcd for $C_{18}H_{25}N_3S$: C, 68.53; H, 7.99; N, 13.32. Found: C,68.52; H, 7.99; N,13.32.

*11-(4-Aminobutyl)amino-1,2,3,4,7,8,9,10 octahydro[1] benzothieno [2,3-b]quinoline (**4e**)* The title compound was prepared from the reaction of **2b** and **3b** adopting Methods A or B to give **4e**. Yield 55 % (Method A), 66 % (Method B); mp >300 °C; IR (KBr) cm^{-1}: 3,390–3,273 (NH_2, NH), 2,926, 2,854 (CH aliphatic), 1,624 (C=N); ^{1}H NMR (DMSO) d6)δ: 1.42–1.75(m, 12H, $6CH_2$), 2.70–3.15(m, 8H, $4CH_2$), 3.40–3.70(m, 4H, $2CH_2$), 5.97, 7.80 (2s, 3H, NH, NH_2 D_2O exchangeable); MS: *m/z* (% abundance) 330(M+1) (0.43); Anal. Calcd for $C_{19}H_{27}N_3S$: C, 69.25; H, 8.26; N, 12.75. Found: C, 69.36; H, 8.27; N, 12.77.

*11-(7-Aminoheptyl)amino-1,2,3,4,7,8,9,10 octahydro[1] benzothieno[2,3-b]quinoline (**4f**)* The title compound was prepared from the reaction of **2b** and **3c** adopting Methods A or B to give **4f**. Yield 57 % (Method A), 66 % (Method B); mp >300 °C; IR (KBr) cm^{-1}: 3,404–3,300 (NH_2, NH), 2,929, 2,856 (CH aliphatic), 1,622 (C=N); ^{1}H NMR(DMSO-d6, TFAA-H)δ: 1.12–1.28(m, 6H, 3CH2), 1.55–1.76(2m, 12H, $6CH_2$), 2.72–2.92(m, 8H, $4CH_2$), 3.38–3.39 (m, 2H, CH_2), 3.49–3.50(m, 2H, CH_2); MS: *m/z* (% abundance) 371(M+) (5.15); Anal. Calcd for $C_{22}H_{33}N_3S$: C, 71.11; H, 8.95; N, 11.31. Found: C, 17.17; H, 8.96; N, 11.32.

*General procedure for synthesis of alkylene-linked dimers of thienopyridines **5a–c***

The title compound was prepared adopting Method A using chlorothienopyridines **2a** or **2b** (23.1 mmol), suitable diamine **3a** or **3b** (11.5 mmol), and 1-pentanol (30 ml).

*Propane-1,3-diamino-N,N′-diyl-4,4′-bis(2,3-dimethyl-5,6,7,8-tetrahydro thieno[2,3-b]quinoline) (**5a**)* Yield 80 %; mp >300 °C;); IR (KBr) cm^{-1}: 3,440–3,400 (2NH), 2,927, 2,854 (CH aliphatic), 1,620 (C=N); ^{1}H NMR(DMSO-d6)δ: 1.18–1.44(m, 2H, CH_2), 1.49–1.61(m, 4H, $2CH_2$), 1.77–2.04(m, 4H, $2CH_2$), 2.45 (s, 6H, $2CH_3$), 2.47(s, 6H, $2CH_3$), 2.75–2.79(m, 8H, $4CH_2$), 2.85–2.92(m, 4H, $2CH_2$); MS: *m/z* (%abundance) 504(M+) (1.06); Anal. Calcd for $C_{29}H_{36}N_4S_2$: C, 69.01; H, 7.19; N, 11.10. Found: C, 69.02; H, 7.19; N, 11.12.

*Butane-1,4-diamino-N,N′-diyl-4,4′-bis(2,3-dimethyl-5,6,7,8-tetrahydro thieno[2,3-b]quinoline (**5b**)* Yield 83 %; mp >300 °C; IR (KBr) cm^{-1}: 3,425–3,375(2NH), 2,927, 2,858 (CH aliphatic), 1,625 (C=N); ^{1}H NMR(DMSO-d6)δ: 1.23–1.52(m, 4H, $2CH_2$), 1.72–1.82(m, 8H, $4CH_2$), 2.38(s, 6H, $2CH_3$), 2.44(s, 6H, $2CH_3$), 2.80–2.90(2m, 12H, $6CH_2$), 4.90, 5.90(2s, 2H, 2NH D_2O exchangeable); MS: *m/z* (% abundance) 516(M−2) (3.10); Anal. Calcd for $C_{30}H_{38}N_4S_2$: C, 69.46; H, 7.38; N, 10.80. Found: C, 69.51; H, 7.39; N, 10.85

*Butane-1,4-diamino-N,N′-diyl-11,11′-bis(1,2,3,4,7,8,9,10-octahydro [1] benzothieno[2,3-b]quinoline (**5c**)* Yield 88 %; mp >300 °C; IR (KBr) cm^{-1}: 3,444–3,383 (2NH), 2,929, 2,856 (CH aliphatic), 1,645 (C=N); ^{1}H NMR(DMSO-d6)δ: 1.78–1.82(m, 20H, $10CH_2$), 2.75–2.85(m, 16H, $8CH_2$), 2.90–3.04(2m, 4H, $2CH_2$); MS:*m/z* (% abundance) 568(M−2) (2.13); Anal. Calcd for $C_{34}H_{42}N_4S_2$: C, 71.53; H, 7.42;N, 9.82. Found: C, 71.55; H, 7.45; N, 9.82.

*General procedure for synthesis of of compounds **7a–d***

To a solution of the desired aminothienopyridines **4a**, **b**, **d**, **e** (0.01 mol) in glacial acetic acid (10 ml) phthalic anhydride was added (**6**) (0.01 mol) and the mixture was heated under reflux for 20 h. The separated solid was filtered,

washed with water, dried, and crystallized from ethanol to afford the respective thienopyridine derivatives.

*4-[3-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)propyl]amino-2,3-dimethyl-5,6,7,8-tetrahydrothieno[2,3-b]quinoline (**7a**)* Yield 69 %; mp 190–192 °C; IR(KBr) cm^{-1}: 3,398(NH), 3,062(CH aromatic), 2,927, 2,858(CH aliphatic), 1,712(C=O), 1,654 (C=N); ^{1}H NMR(DMSO-d6)δ: 1.87–2.02(m, 6H, $3CH_2$), 2.49(s, 3H, CH_3), 2.51(s, 3H, CH_3), 2.72–2.73(m, 4H, $2CH_2$), 3.43–3.64(m, 4H, $2CH_2$), 7.56–7.68(2m, 4H, aromatic H); MS: *m/z* (% abundance) 419(M+) (0.15); Anal. Calcd for $C_{24}H_{25}N_3O_2S$: C, 68.71; H, 6.01; N, 10.02. Found: C, 68.75; H, 6.10; N, 10.22.

*4-[4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)butyl]amino-2,3-dimethyl-5,6,7,8-tetrahydrothieno[2,3-b]quinoline (**7b**)* Yield 83 %; mp >300 °C;); IR (KBr)cm^{-1}: 3,433(NH), 3,093, 3,062(CH aromatic), 2,927, 2,858(CH aliphatic), 1,716(C=O),1.612(C=N); ^{1}H NMR(DMSO-d6, TFAA-H)δ: 1.22–1.23(m, 8H, $4CH_2$), 2.38 (s, 3H,CH_3), 2.44(s, 3H, CH_3), 3.13–3.39(m, 4H, $2CH_2$), 3.41–3.75(m,4H,$2CH_2$), 7.44–7.63 (2m, 4H, aromatic H), 7.90(s, 1H, NH D_2O exchangeable); MS: *m/z* (% abundance) 433(M+) (1.07); Anal. Calcd for $C_{25}H_{27}N_3O_2S$: C, 69.25; H, 6.28; N, 9.96. Found: C,69.18; H, 6.27; N, 9.68.

*11-[3-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)propyl]amino-1,2,3,4,7,8, 9,10-octahydro[1] benzothieno[2,3-b]quinoline (**7c**)* Yield 85 %; mp 220–222 °C; IR (KBr)cm^{-1}: 3,433(NH), 3,066, 3,032(CH aromatic), 2,939, 2,862(CH aliphatic), 1,705(C=O), 1,627(C=N); ^{1}H NMR(DMSO-d6)δ: 1.16–1.31(m, 6H, $3CH_2$), 1.65–1.83(m, 4H, $2CH_2$), 1.96–2.03(m, 4H, $2CH_2$), 2.64–2.89(m, 4H, $2CH_2$), 3.60–3.64(m, 4H, $2CH_2$), 4.10(m, 1H, NH,D_2O exchangeable), 7.42–8.03(m, 4H, aromatic H); MS: *m/z* (% abundance) 445(M+) (0.92); Anal. Calcd for $C_{26}H_{27}N_3O_2S$: C, 70.08; H, 6.11; N, 9.43. Found: C,70.03; H, 6.10; N, 9.52.

*11-[4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)butyl]amino-2,3,4,7,8,9,10-octahydro[1] benzothieno[2,3-b]quinoline (**7d**)* Yield 85 %; mp >300 °C; IR (KBr) cm^{-1}:3,466 (NH), 3,095, 3,064(CH aromatic), 2,935, 2,868(CH aliphatic),1,720(2C=O), 1,647(C=N); ^{1}H NMR(DMSO-d6, TFAA-H)δ: 1.16–1.20(m, 4H, $2CH_2$), 1.56–1.62(m, 8H, $4CH_2$), 1.83–1.84(m, 2H, CH_2), 2.49–2.51(m, 8H, $4CH_2$), 3.52–3.60(m, 2H, CH_2), 7.70–7.78(m, 4H, aromatic H); MS: *m/z* (% abundance) 458(M−1) (8.10); Anal. Calcd for $C_{27}H_{29}N_3O_2S$: C, 70.56; H, 6.36; N, 9.14. Found: C, 70.49; H, 6.35; N, 9.15.

*General procedure for synthesis of compounds **9a, b***

The selected aminothienopyridines **4d**, **e** (0.01 mol), *N*-(2-chloroethyl) phthalimide (**8**) (0.01 mol), catalytic amount of potassium carbonate, and dimethylformamide (10 ml) were heated under reflux for 24 h. After cooling to room temperature, the separated solid was filtered, washed with water, and crystallized from 1,4-dioxane

*11-{3-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethylamino] propyl} amino-1,2,3,4,7,8,9,10-octahydro[1] benzothieno [2,3-b]quinoline (**9a**)* Yield 68 %; mp 100–102 °C; IR (KBr) cm^{-1}: 3,381, 3,244(2NH), 3,088, 3,062(CH aromatic), 2,931, 2,858(CH aliphatic),1,734(2C=O), 1,618(C=N); ^{1}H NMR(DMSO-d6, TFAA-H)δ: 1.62–1.78(m, 10H, $5CH_2$), 2.46–2.51(m, 4H, $2CH_2$), 2.62–2.70(m, 4H, $2CH_2$), 2.88–2.98(m, 4H, $2CH_2$), 3.60(t, 2H, CH_2, $J = 6$ Hz), 3.77(t, 2H, CH_2, $J = 6$ Hz), 7.57–7.62(m, 4H, aromatic H); MS: *m/z* (% abundance) 488(M+) (2.21); Anal. Calcd for $C_{28}H_{32}N_4O_2S$: C, 68.82; H, 6.60; N, 11.47. Found: C, 68.80; H, 6.65; N, 11.20.

*11-{4-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl) ethyl amino] butyl} amino-1,2,3,4,7,8,9,10-octahydro[1] benzothieno [2,3-b]quinoline (**9b**)* Yield 90 %; mp 210–212 °C; IR (KBr) cm^{-1}: 3,400, 3,398(2NH), 3,050(CH aromatic), 2,927, 2,854(CH,aliphatic), C=O), 1,662(C=N); ^{1}H NMR (DMSO-d6)δ: 1.70–1.90(m, 12H, $6CH_2$), 2.79–2.90 (2m, 8H, $4CH_2$), 3.03–3.31(m, 4H, $2CH_2$), 3.85(t, 2H, CH_2, $J = 6$ Hz), 3.91(t, 1,716(22H, CH_2, $J = 6$ Hz), 7.80–7.90 (m, 4H, aromatic H); MS: *m/z* (% abundance) 502(M+) (0.14); Anal. Calcd for $C_{29}H_{34}N_4O_2S$: C, 69.29; H, 6.82; N, 11.15. Found: C, 69.33; H, 6.90; N,11.25.

*General procedure for synthesis of compounds **11a–c***

A mixture of the appropriate aminothienopyridines **4b**, **d**, **e** (0.1 mol), lipoic acid (**10**) (0.15 mol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.12 mol) in dry DMF (15 ml) was stirred at room temperature under nitrogen for 24 h. The mixture was poured into ice/water. The separated solid was filtered and crystallized from ethanol.

*4-{4-[(5-{1,2-Dithiolan-3-yl}-1-oxopentyl)amino]butyl} amino-2,3-dimethyl-5,6,7,8-tetrahydrothieno[2,3-b]quinoline(**11a**)* Yield 25 %; mp 180–182 °C; IR (KBr)cm^{-1}: 3,446, 3,419(2NH), 2,924, 2,852 (CH aliphatic),1,647(C=O),

1,608(C=N); ^{1}H NMR(DMSO-d6)δ: 1.30–1.37(m, 6H, $3CH_2$), 1.47–1.54(m, 8H, $4CH_2$), 1.80–1.86(m, 2H, CH_2), 2.02–2.07(m, 4H, $2CH_2$), 2.38–2.40(m, 2H, CH_2), 2.66(s, 3H, CH_3), 2.87(s, 3H, CH_3), 3.01–3.14(m, 6H, $3CH_2$), 3.55–3.60(m, 1H, CH), 7.90(m, 1H, NH D_2O exchangeable); MS: *m/z* (% abundance) 491(M+) (3.70); Anal. Calcd for $C_{25}H_{37}N_3OS_3$: C, 61.06; H, 7.59; N, 8.54. Found: C, 16.13; H, 7.55; N, 8.50

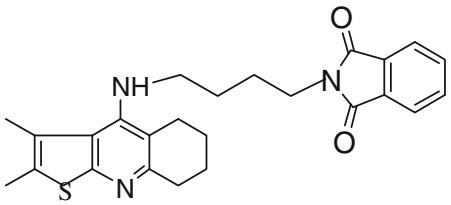
*11-{3-[(5-{1,2-Dithiolan-3-yl}-1-oxopentyl)amino]propyl} amino-1,2,3,4,7,8,9,10- octahydro[1] benzothieno[2,3-b]quinoline (**11b**)* Yield 40 %; mp 155–157 °C; IR (KBr) cm^{-1}: 3,425, 3,350(2NH), 2,931, 2,866 (CH aliphatic), 1,685(C=O), 1,624(C=N); ^{1}H NMR(DMSO-d6)δ: 1.17–1.37 (m, 8H, $4CH_2$), 1.52–1.83(m, 8H, $4CH_2$), 1.85–1.87(m, 2H, CH_2), 2.21–2.27(m, 2H, CH_2), 2.66–2.89(2m, 8H, $4CH_2$), 3.13–3.31(m, 6H, $3CH_2$), 3.58–3.65(m, 1H, CH), 7.95(s, 1H, NH D_2O exchangeable; MS: *m/z* (% abundance) 503(M+) (10.79); Anal. Calcd for $C_{26}H_{37}N_3OS_3$: C, 61.98; H, 7.40; N, 8.34. Found: C, 61.70; H, 7.45; N, 8.55.

*11-{4-[(5-{1,2-Dithiolan-3-yl}-1-oxopentyl)amino]butyl} amino-1,2,3,4,7,8,9,10-octahydro[1] benzothieno [2,3-b] quinoline (**11c**)* Yield 67 %; mp 138–140 °C; IR (KBr) cm^{-1}: 3,400, 3,388(2NH), 2,926, 2,854 (CH aliphatic), 1,699(C=O), 1,610(C=N); ^{1}H NMR(DMSO-d6, TFAA-H)δ: 1.17–1.25(m, 4H, $2CH_2$), 1.27–1.33(m, 4H, $2CH_2$),1.46–1.63(m, 6H, $3CH_2$), 1.78–1.84(m, 6H, $3CH_2$), 2.13–2.18 (m, 2H, CH_2), 2.33–2.39(m, 2H, CH_2), 2.69–2.84(m, 4H, $2CH_2$), 2.82–2.98(m, 2H, CH_2), 3.02–3.14(m, 6H,$3CH_2$), 3.41–3.54(m, 1H, CH); MS: *m/z* (% abundance) 517(M+) (3.53); Anal. Calcd for $C_{27}H_{39}N_3OS_3$: C, 62.63; H, 7.59; N, 8.12. Found: C, 62.69; H, 7.60; N, 8.15.

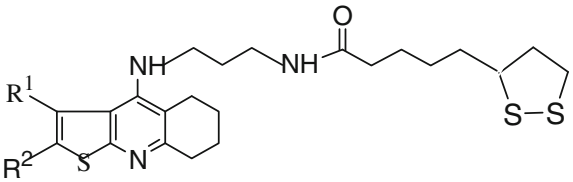
Results

Biologic evaluation

The preliminary anti-acetylcholine esterase activity for the synthesized thienopyridines derivatives was assessed according to Ellman's method (Ellman *et al*., 1961) using tacrine as a reference compound. AChE was obtained from homogenates of rat brain. Results of anti-acetylcholine esterase activity of the tested compounds as well as tacrine are shown in (Table 1). The screening results showed that all the compounds exhibited significant inhibitory activity ($p < 0.05$) against AChE in comparison with tacrine. From the biologic activity studies, we could conclude that compounds **7b** and **11b** are the most active compounds; they demonstrated inhibitory activity higher than tacrine (55.56 and 56.73 %, respectively), while compounds **5b** and **c**, **7a** and **c**, **9a** and **b**, and **11a** showed moderate activity. In addition, compounds **5a**, **7d**, and **11c** showed the lowest activity (Table 1).



7b



11b

Based on the just-mentioned results, it was speculated that compounds **7b** and **11b**, which are linked to phthalimide and lipoic acid moieties, respectively, were the most active among the tested compounds. Moreover, other compounds that are bound to phthalimide moiety (**7a** and **c**) and (**9a** and **b**) showed moderate inhibitory activity. Only compound **7d** revealed low inhibitory activity. Once more, compound **11a**, where the thienopyridine is linked to lipoic acid, showed moderate activity, while compound **11c** of the same series showed the lowest activity. Regarding the bis-thienopyridins **5a–c**, compounds **5b** and **c** showed moderate activity, while compounds **5a** had low activity. It is worth mentioning that we could not observe adaptable correlation between structures and biologic activities.

Materials and methods

Adult male albino Wister rats weighing 180–200 g were used in the present 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. 30 min later, rats were killed, decapitated, and the brains were carefully removed and homogenized in normal saline (pH 7.4).

AChE inhibition assay in vitro

Inhibitory activity against AChE was evaluated at 37 °C by the colorimetric method reported by (Ellman *et al*., 1961). The final concentration containing test compound of the assay solution consisted of 0.1 M sodium phosphate buffer (pH 8.0), 0.3 mM 5,5-dithiobis-2-nitobenzoic acid (DTNB, Ellman's reagent), 0.02 U of AChE from electrophorus lectricus, and 0.5 mM acetylthiocholine iodide as substrate of the enzymatic reaction. The assay solutions except substrate were pre-incubated with enzyme for 10 min at 37 °C. Following pre-incubation, the substrate was added. The absorbance changes at 405 nm were recorded for 5 min, using 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 of animal experimentation at the Faculty of pharmacy, Cairo University.

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 ± S.E. ($n = 6$–8 rats).

Acknowledgments The authors are very grateful to the staff members of the Department of Pharmacology, Faculty of Pharmacy, Cairo University, for carrying out the pharmacological screening of the newly synthesized compounds.

References

Badia A, Banos JE, Camps P, Contreras J, Gorbig DM, Torrero DM, Simon M, Vivas NM (1998) Synthesis and evaluation of tacrine–huperzine a hybrids as acetylcholinesterase inhibitors of potential interest for the treatment of Alzheimer's disease. Bioorg Med Chem 6:427–440

Bartus RT, Dean RL, Beer B, Lippa AS (1982) The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408–414

Bolognesi ML, Andrisano V, Bartolini M, Banzi R, Melchiorre C (2005) Propidium-based polyamine ligands as potent inhibitor of acetylcholinesterase and acetylcholinesterase-induced amyloid-β aggregation. J Med Chem 48:24–27

Butini S, Guarino E, Campiani G, Brindisi M, Coccone SS, Fiorini I, Novellin o E, Belinskaya T, Saxena A, Gemma S (2008) Tacrine based human cholinesterase inhibitors: synthesis of peptidic-tethered derivatives and their effect on potency and selectivity. Bioorg Med Chem Lett 18:5213–5216

Camps P, Achab RE, Gorbig DM, Morral J, Torrero DM, Badia A, Banos JE, Vivas NM, Barril X, Orozco M, Luque FJ (1999) Propidium-based polyamine ligands as potent inhibitor of acetylcholinesterase and acetylcholinesterase-induced amyloid-β aggregation. J Med Chem 42:3227–3242

Carlier PR, Han YF, Chow ESH, Wang CPLLH, Lieu TX, Wong HS, Pang P (1999) Evaluation of short-tether bis-THA AChE inhibitors. A further test of the dual binding site hypothesis. Bioorg Med Chem 7:351–357

Davis KL, Powchik P (1995) Tacrine. Lancet 345:625–630

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new andrapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Gewald K, Schinke E, Böttcher H (1966) 2-Amino-thiophene aus methylenaktiven nitrilen, carbonylverbindungen and schwefel. Chem Ber 99:94–100

Ishihara Y, Kato K, Goto G (1991) Central cholinergic agents. I. Potent acetylcholinesterase inhibitors, 2-[ω-[*N*-akyl-*N*-(ω-phenyl-alkyl)amino]alkyl]-1*H*-isoindole-1,3(2*H*)-diones, Based on a new hypothesis of the enzymes active site. Chem Pharm Bull 39:3225–3235

Kwon YE, Park JY, TaiNo K, Shin JH, Lee SK, Eun JS, Yang JH, Shin TY, Kim DK, Chae BS, Leem J-Y, Kim KH (2007) Synthesis, in vitro assay, and molecular modeling of new piperidine derivatives having dual inhibitory potency against acetylcholinesterase and Abeta1-42 aggregation for Alzheimer's disease therapeutics. Bioorg Med Chem 15:6596–6607

Lang F, Zewge D, Houpis IN, Volante RP (2001) Amination of aryl halide using copper catalysis. Tetrahedron Lett 42:3251–3254

Mary A, Renko DZ, Guillou C, Thal C (1998) Potent acetylcholinesterase inhibitors: design, synthesis, and structure-activity relationships of bis-interacting ligands in the galanthamine series. Bioorg Med Chem 6:1835–1850

Rosini M, Andrisano V, Bartolini M, Balognesi ML, Hrelia P, Minarini A, Tarozzi A, Melchiorre C (2005) Rational approach to discover multipotent anti-Alzheimer drugs. J Med Chem 48:360–363

Ruiz PM, Rubio L, Palomero EG, Dorronsoro I, Millan MM, Valenzuela R, Usan P, Austria C, Bartolini M, Andrisano V, Chanal AB, Orozco M, Luque FJ, Medina M, Martinez A (2005) Design synthesis, and biological evaluation of dual binding site acetylcholinesterase inhibitors: new disease-modifying agents for Alzheimer's disease. J Med Chem 48:7223

Tariot PN, Federoff HJ (2003) Current treatment for Alzheimer disease and future prospects. Alzheimer Dis Assoc Disord 17:105–113