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Synthesis and Pharmacological Screening of New Thienopyridines for the treatment of Alzheimer's disease

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ABSTRACT

*Alzheimer is a multitargeting brain-hitting disease. The need to prepare and screen new agents is endless. Novel thienopyridine-tacrine analogues **4a,b – 8a,b** and **9** have been designed, synthesized and biologically screened against cholinesterase inhibition activity. Two compounds **7a** and **7b** were found active. Compound **7b** exceeded tacrine as a cholinesterase inhibitor. The docking studies explained this activity as compound **7b** exhibited similar binding affinity to the acetylcholinesterase enzyme as with tacrine in addition to an extra hydrogen bonding.*

Keywords: Acetylcholine; Thienopyridines; Anti-Alzheimer's agents.

INTRODUCTION

Acetylcholine (ACh) is an important neurotransmitter produced in the human body. From the chemistry point of view, acetylcholine is the acetyl ester of choline molecule formed by the action of choline acetyltransferase (ChAT) enzyme found in the presynaptic cholinergic neurons (Figure 1).

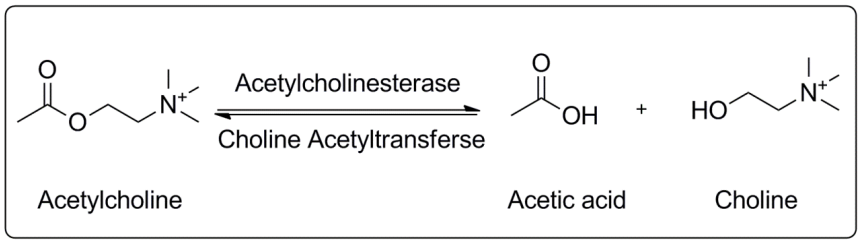


Figure 1. The dynamic equilibrium of (ACh)

Although this neurotransmitter is quite essential for normal human life, it is of a short duration of action. This short life time is attributed to the hydrolytic effect of acetylcholinesterase (AChE) enzyme on the formed ester bond leading to a straight forward splitting of acetylcholine to its original precursors [1]. This dynamic equilibrium between formation and hydrolysis of (ACh) is quite essential in maintaining a good balance for human biological functions. Any disturbance in such balance can lead to serious medical problems. Alzheimer's disease (AD) is a neurodegenerative disease associated with loss of memory, progressive impairment in cognitive functions and behavioural disturbances [2]. Unfortunately, the incidence of AD is now rising in both sexes and more over

exponentially with age [3]. The clear scientific explanation for the cause of this neurodegenerative disease is not clearly defined until now. Nevertheless, many hypotheses have been raised e.g. β-amyloid hypothesis [4,5], CDK5 inhibitors [6,7], oxidative stress hypothesis [8,9]. Cholinergic hypothesis is considered as one of the most important theories explaining this disease. The hypothesis is explaining (AD) due to the deficiency in (ACh) levels in brain. Consequently, one of the most important lines of treatment of (AD) is to try to elevate the levels of (ACh) levels in brain. Accordingly, acetycholinesterase inhibitors (AChEI) block the activity of the (AChE) enzyme leading to the elevated concentrations of (ACh) in brain and thus improving the case [10,11]. Tacrine and other related AChEI have been approved for the clinical treatment of AD. However, the serious side effects resulting from the use of such medications is always the main reason for ceasing the treatment. In order to obtain a new preclinical anti-AD drug candidate, we previously designed and already synthesised series of compounds (I – VI) (Figure 2) [12–14].

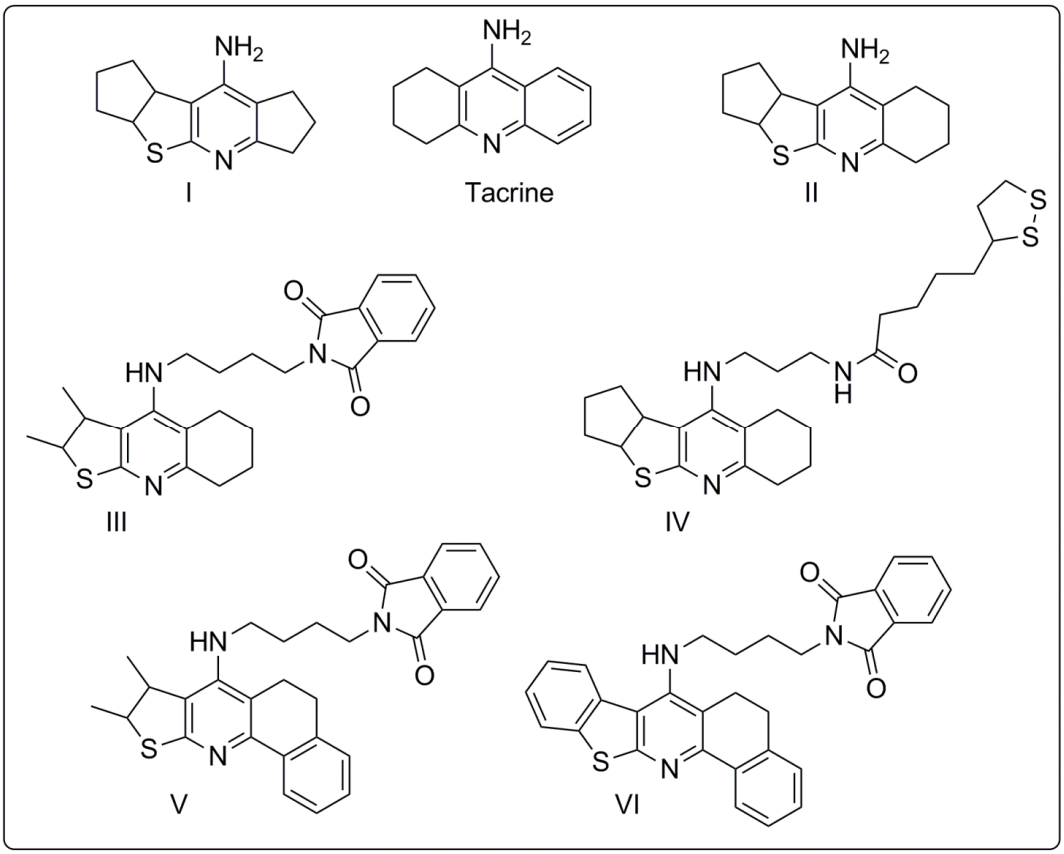


Figure 2. Thienopyridine-tacrine analogues

The thienopyridine nucleus was initially introduced as a bioisoster for the quinoline moiety of tacrine structure. Compounds (I, II) exhibited a promising anticholinesterase activity while compounds (III – VI) exceeded tacrine itself in the anticholinesterase activity. Based on our previous findings and in continuation to our efforts, we designed and synthesised new members **3a,b** – **8a,b**.

Moreover, the complexity of AD pathophysiology directed our sight towards multitargeting agents as a logistic strategy for drug development [15–18]. Lipoic acid is considered as a universal antioxidant through its reduction to its dihydro - reduced form (DHLA) (19). Moreover, the insertion of lipoic acid as an antioxidant moiety in many multipotent scaffolds has been reported (20). With this in mind, we aimed to the synthesis and screening of a thienopyridine-lipoic acid model that may possess both the cholinesterase inhibition and antioxidant dual activities (Figure 3).

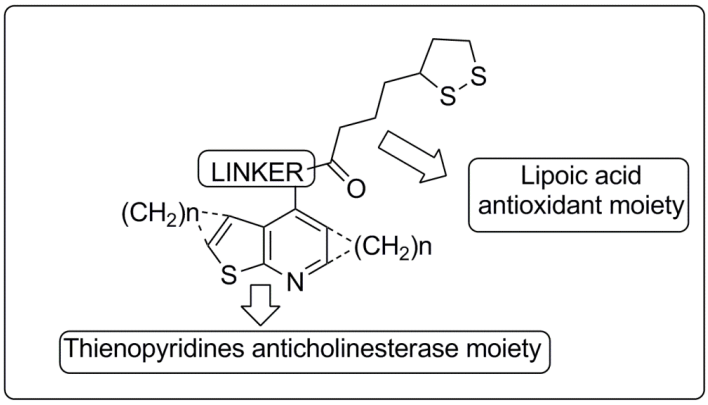
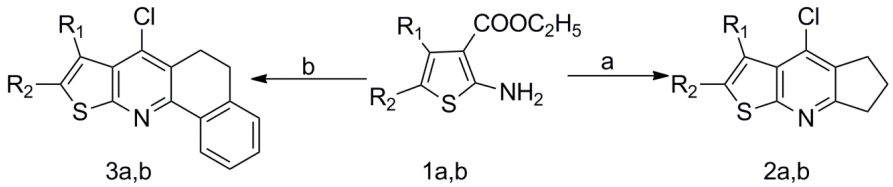


Figure 3. Thienopyridine-lipoic acid multitargeting design

Results and Discussion

The target compounds were prepared according to the five schemes outlined. Compounds **2a,b** and **3a,b** are considered the corner stone materials for this piece of work. In scheme 1, the appropriate ketone was reacted with the corresponding amino ester thiophene in the presence of excess phosphorous oxychloride to furnish the way for the preparation of the titled compounds **2a,b** and **3a,b**. Thus, refluxing cyclopentanone with ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (**1a**) in the presence of excess phosphorous oxychloride yielded 2a while refluxing 1-tetralone with ethyl 4,5,6,7-tetrahydrobenzothiophene-3-carboxylate (**1b**) resulted in the preparation of (**2b**).

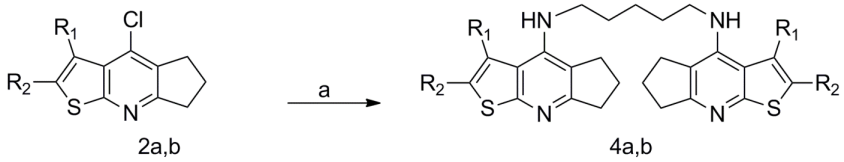


a; $R_1 = R_2 = CH_3$, b; $R_1 = R_2 = (CH_2)_4$

Reagents and conditions: a) cyclopentane, $POCl_3$, reflux 3 h; b) 1-tetralone, $POCl_3$, reflux 3 h.

Scheme 1. Preparation of compounds 2a,b and 3a,b

Consequently, scheme 2 outlines the preparation of the *bis* derivatives 4a,b.



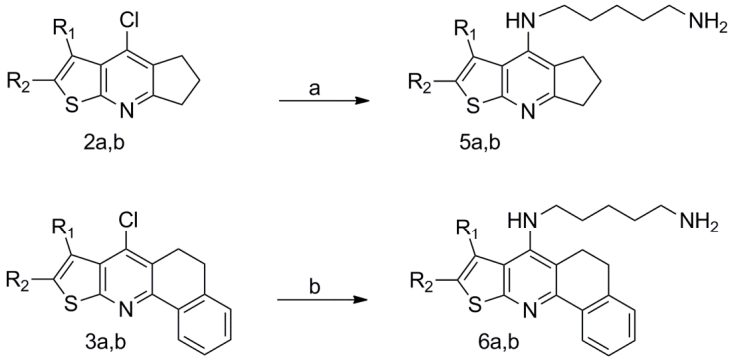
a; $R_1 = R_2 = CH_3$, b; $R_1 = R_2 = (CH_2)_4$

Reagents and conditions: a) 1,5-Diaminopentane (0.5 equiv.), 1-pentanol, reflux 72 h.

Scheme 2. Preparation of compounds 4a,b

Heating under reflux the 1,5-diaminopentane with chlorothienopyridines **2a,b** in a half molar ratio resulted in the formation of the desired compounds. Compounds 5a,b and 6a,b are considered as the starting materials for the continuation of plan of synthesis. Accordingly, scheme 3 outlines the synthesis of the compounds **5a,b** and **6a,b**.

The chlorothienopyridine derivatives **2a,b** and **3a,b** was reacted with the 1,5-diaminopentane in an equimolar ratio according to the reported procedures [12].

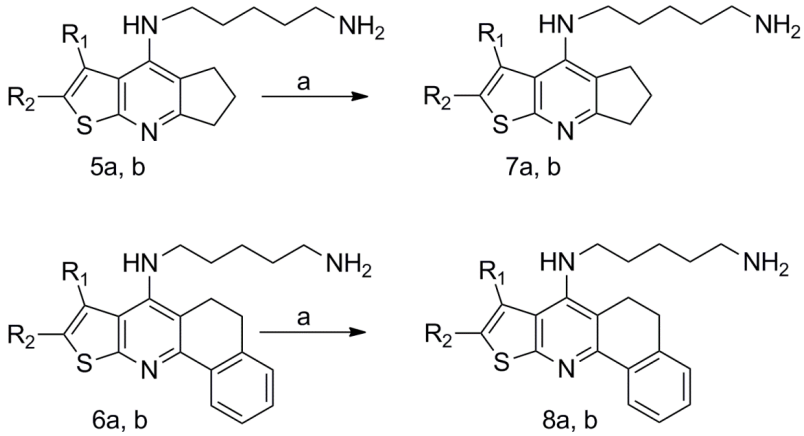


a; $R_1 = R_2 = CH_3$, b; $R_1 = R_2 = (CH_2)_4$

Reagents and conditions: a) 1,5-Diaminopentane (1.0 equiv.), ethylene glycol, Cu_2O, K_2CO_3, reflux 24 h.

Scheme 3. Preparation of compounds 5a,b and 6a,b

It is worth mentioning that using Cu_2O / K_2CO_3 with the high-boiling point ethylene glycol have been used to improve the yield [21]. Furthermore, the reaction of either **5a,b** or **6a,b** with phthalic anhydride under reflux resulted in the preparation of **7a,b** and 8a,b respectively (scheme 4).

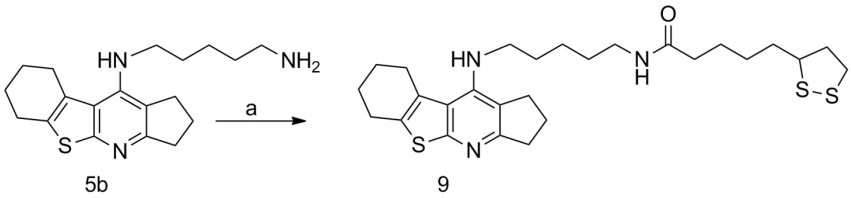


a; $R_1 = R_2 = CH_3$, b; $R_1 = R_2 = (CH_2)_4$

Reagents and conditions: a) Phthalic anhydride, acetic acid, reflux 18 h.

Scheme 4. Preparation of compounds 7a,b and 8a,b

Finally, compound **9** has been prepared through the reaction of the aminothienopyridine derivative **5b** with lipoic acid. This coupling reaction has been stirred at room temperature in the presence of the coupling reagent carbonyl diimidazole (CDI). It is worth mentioning that lipoic acid is a thermo labile chemical, easily affected with oxidizing agents and heat. Consequently, the coupling reaction with **5b** has to be run under nitrogen gas as an inert atmosphere and at ambient temperature. Moreover, the reactants have to be completely soluble in the reaction solvent. This was a limitation resulted in the preparation of only one member in this designed motif.



Reagents and conditions: a) Lipoic acid, DMF, CDI, stirring at R.T. 24 h.

Scheme 5. Preparation of compounds 9

MATERIALS AND METHODS

Chemistry

Melting points were obtained on Griffin apparatus and the values given were uncorrected. IR spectra were recorded on a Shimadzu 435 spectrometer, using KBr discs. ^{1}HNMR spectra were recorded on a Mercury-300MHz spectrometer using TMS as an internal standard. Mass spectra were recorded on a JEON JMS-AX 500 mass spectrometer. Element analysis for C, H and N were within 0.4% of the theoretical values and were performed at the regional center for Mycology and Biotechnology, Al-Azhar University. Progress of the reactions were monitored by TLC using precoated aluminum sheets silica gel MERCK 60 F 254 and was visualized by UV lamp. All chemicals were purchased from Sigma-Aldrich company.

General procedure for preparation of 2a,b and 3a,b:

The appropriate ketone (0.1 mol.) was added portionwise to a slurry of ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (1a) or ethyl 2-amino-4,5,6,7-tetrahydrobenzothiophene-3-carboxylate (1b) (0.1 mol.) and phosphorous oxychloride (75 ml). The reaction mixture was stirred under reflux for 3h 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* and the formed residue was triturated with diethyl ether. The residue was recrystallized from the appropriate solvent to yield the titled compounds.

4-Chloro-2,3-dimethyl-6,7-dihydro-5*H*-cyclopenta[*b*]thieno[3,2-*e*]pyridine (2a): Yield: 80%; acetonitrile; mp 159 °C; IR cm^{-1}: 2922 - 2850 (CH aliphatic), 1683 (C=N); ^{1}HNMR ($CDCl_3$) δ: 1.26 – 1.33 (m, 2H, CH_2), 1.69 – 2.10 (m, 4H, $2CH_2$), 2.39 (s, 3H, CH_3), 2.48 (s, 3H, CH_3); MS: m/z (% abundance) 237 (M^+) (6.34); Anal. Calcd. for $C_{12}H_{12}ClNS$: C, 60.62; H, 5.09; N, 5.89. Found: C, 60.91; H, 5.17; N, 6.02.

10-Chloro-2,3,6,7,8,9-hexahydro-1*H*-[1]benzothieno[2,3-*b*]cyclopenta[*e*]pyridine (2b): Yield: 75%; methanol; mp 200°C; IR cm^{-1}: 2933-2856 (CH aliphatic), 1683 (C=N); ^{1}HNMR ($CDCl_3$) δ: 1.61 – 2.25 (m, 8H, $4CH_2$), 2.76 – 3.11 (m, 6H, $3CH_2$); ^{13}CNMR (75 MHz, $CDCl_3$) δ: 22.21, 22.67, 24.34, 25.59, 31.19, 124.26, 132.80, 133.02, 139.79, 154.33; MS: m/z (% abundance) 263 (M^+) (0.13); Anal. Calcd. for $C_{14}H_{14}ClNS$: C, 63.74; H, 5.35; N, 5.31. Found: C, 63.97; H, 5.40; N, 5.42.

7-Chloro-8,9-dimethyl-5,6-dihydrobenzo[*h*]thieno[2,3-*b*]quinoline (3a) (14)**:** Yield: 70%; mp 210°C.

7-Chloro-5,6,8,9,10,11-hexahydrobenzo[*h*][1]benzothieno[2,3-*b*]quinoline (3b): Yield: 70%; n-hexane; mp 140°C; IR cm^{-1}: 3055-3012 (CH aromatic), 2929-2854 (CH aliphatic), 1683 (C=N);^{1}HNMR ($CDCl_3$) δ: 1.26 – 1.47 (m, 2H, CH_2), 1.81 – 2.17 (m, 4H, $2CH_2$), 2.56 – 3.22 (m, 6H, $3CH_2$), 7.25 – 7.48 (m, 4H, CH aromatic); ^{13}CNMR (75 MHz, $CDCl_3$) δ: 22.26, 23.24, 24.58, 25.57, 26.15, 26.96, 29.70, 116.35, 120.71, 125.84, 126.40, 127.14, 127.86, 128.77, 132.75, 133.39, 134.09, 138.69, 144.50; MS: m/z (% abundance) 325 (M^+) (100); Anal. Calcd. for $C_{19}H_{16}ClNS$: C, 70.03; H, 4.95; N, 4.30. Found: C, 70.24; H, 4.99; N, 4.38.

General procedure for preparation of 4a,b:

The desired chloropyridine derivatives 2a or 2b (23.1 mmol.) was dissolved in 1-pentanol (30 ml). 1,5-diaminopentane (11.5 mmol.) was added and the reaction was heated under reflux for 72 h. The reaction mixture was cooled to room temperature and diluted with methylene chloride (50 ml). The organic layer was washed with

10% NaOH (1 × 50 ml) and water (2 × 40 ml). The solid produced from the concentration of the organic layer was then boiled in acetic acid as a method of purification.

***N,N`*(pentane-1,5-diyl)bis(2,3-dimethyl-6,7-dihydro-5*H*-cyclopenta[*b*]thieno[3,2-*e*]pyridine-4-amine)(4a):**
Yield: 43%; mp > 300°C; IR cm^{-1}: 3446-3367 (2 NH), 2929, 2864 (CH aliphatic), 1660 (C=N); ^{1}HNMR (DMSO-d_6, TFA-H) δ: 1.11 – 1.24 (m, 12H, $6CH_2$), 1.60 – 1.82 (m, 12H, $6CH_2$), 2.44 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.82 – 2.91 (m, 2H, CH_2); MS: m/z (% abundance) 504 (M^+) (77.36); Anal. Calcd. for $C_{29}H_{36}N_4S_2$: C, 69.01; H, 7.19; N, 11.10. Found: C, 69.17; H, 7.128; N, 11.32.

***N,N`*(pentane-1,5-diyl)bis(2,3,6,7,8,9-hexahyro-1*H*-[1]benzothieno[2,3-*b*]cyclopenta[*e*]pyridine-10-amine) (4b):** Yield: 50%; mp > 300°C; IR cm^{-1}: 3446-3369 (2 NH), 2927, 2854 (CH aliphatic), 1610 (C=N); ^{1}HNMR (DMSO-d_6, TFA-H) δ: 1.6 – 1.92 (2m, 12H, $6CH_2$), 2.45 – 2.66 (2m, 24H, $12CH_2$); MS: m/z (% abundance) 556 (M^+) (82.08); Anal. Calcd. for $C_{33}H_{40}N_4S_2$: C, 71.18; H, 7.24; N, 10.06. Found: C, 71.39; H, 7.29; N, 10.31.

General procedure for preparation of compounds 5a,b and 6a,b:
The selected chlorothienopyridines 2a,b or 3a,b (0.01 mol) were dissolved in ethylene glycol (10 ml). 1,5-diaminopentane (0.01 mol) was then added in the presence of catalytic amounts of cuprous oxide and potassium carbonate. The mixture was refluxed for 24h and filtered while hot. The filtrate was left to cool down and then poured on ice-cold water mixture. The solid formed was filtered, dried, and purified using the suitable solvent.

4-[(5-Aminopentyl)amino]-2,3-dimethyl-6,7-dihydro-5*H*-cyclopenta[*b*]thieno-[3,2-*e*]pyridine (5a): Yield: 58%; ethanol (95%);.mp > 300°C; IR cm^{-1}: 3408-3238 (NH_2, NH), 2926, 2860 (CH aliphatic), 1629 (C=N); ^{1}HNMR (DMSO-d_6, TFA-H) δ: 1.21 – 1.55 (m, 4H, $2CH_2$), 1.79 – 1.89 (2m, 4H, $2CH_2$), 2.06 (s, 3H, CH_3), 2.26 – 2.44 (2m, 4H, $2CH_2$), 2.49 – 2.54 (m, 2H, CH_2), 2.72 – 2.79 (m, 2H, CH_2), 2.98 (s, 3H, CH_3); MS: m/z (% abundance) 303 (M^+) (25.82); Anal. Calcd. for $C_{17}H_{25}N_3S$: C, 67.28; H, 8.30; N, 13.85. Found: C, 67.42; H, 8.39; N, 14.02.

10-[(5-Aminopentyl)amino]-2,3,6,7,8,9-hexahydro-1*H*-[1]benzothieno[2,3-*b*]cyclopenta[*e*]pyridine (5b): Yield: 60%; boiled in acetic acid; mp > 300°C; IR cm^{-1}: 3566-3446 (NH_2, NH), 2931-2839 (CH aliphatic), 1683 (C=N); ^{1}HNMR (DMSO-d_6, TFA-H) δ: 1.21 – 1.37 (m, 2H, CH_2), 1.79 – 1.96 (m, 2H, CH_2), 2.01 – 2.27 (2m, 4H, $2CH_2$), 2.41 – 2.57 (2m, 4H, $2CH_2$), 2.72 – 2.39 (2m, 4H, $2CH_2$), 3.52 – 3.84 (2m, 4H, $2CH_2$), 3.85 – 4.17 (2m, 4H, $2CH_2$); MS: m/z (% abundance) 329 (M^+) (7.91); Anal. Calcd. for $C_{19}H_{27}N_3S$: C, 69.25; H, 8.26; N, 12.75. Found: C, 69.44; H, 8.31; N, 12.97.

7-[(5-Aminopentyl)amino]-8,9-dimethyl-5,6-dihydrobenzo[*h*]thieno[2,3-*b*]quinoline (6a): Yield: 55%; acetic acid; mp 250°C; IR cm^{-1}: 3385-3248 (NH_2, NH), 3050 (CH aromatic), 2929, 2858 (CH aliphatic), 1660 (C=N); ^{1}HNMR (DMSO-d_6, TFA-H) δ: 1.19 – 2.05 (2m, 8H, $4CH_2$), 2.38 – 2.50 (m, 4H, $2CH_2$), 2.73 – 2.86 (m, 4H, $2CH_2$), 2.90 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 3.38 – 3.48 (m, 2H, CH_2), 7.30 – 7.69 (m, 4H, CH aromatic); MS: m/z (% abundance) 367 (M^+) (21.69); Anal. Calcd. for $C_{22}H_{27}N_3S$: C, 72.29; H, 7.45; N, 11.50. Found: C, 72.58; H, 7.52; N, 11.68.

7-[(5-Aminopentyl)amino]-5,6,8,9,10,11-hexahydrobenzo[*h*][1]benzothieno[2,3-*b*]quinoline (6b): Yield: 65%; acetic acid; mp > 300°C; IR cm^{-1}: 3385-3273 (NH_2, NH), 3057 (CH aromatic), 2929-2841 (CH aliphatic), 1620 (C=N); ^{1}HNMR (DMSO-d_6, TFA-H) δ: 1.21 – 1.51 (m, 2H, CH_2), 1.74 – 2.10 (2m, 10H, $5CH_2$), 2.27 – 2.56 (m, 4H, $2CH_2$), 2.66 – 3.08 (2m, 6H, $3CH_2$), 7.33 – 7.65 (m, 4H, CH aromatic); MS: m/z (% abundance) 391 (M^+) (23.48); Anal. Calcd. for $C_{24}H_{29}N_3S$: C, 73.61; H, 7.47; N, 10.73. Found: C, 73.93; H, 7.56; N, 11.02

General procedure for preparation of 7a,b and 8a,b:
Phthalic anhydride (0.1 mol.) was added to the glacial acetic acid solution (10 ml) of the desired aminopyridine derivatives (5a,b / 6a,b) (0.1 mol.). The reaction mixture was heated under reflux for 18 h. The separated solid was filtered, washed with water, air dried and recrystallized from ethanol to afford the appropriate pyridine derivatives.

4-{[5-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)pentyl]amino}-2,3-dimethyl-6,7-dihydro-5*H*-cyclopenta[*b*]thieno[3,2-*e*]pyridine (7a): Yield: 80%; mp > 300°C; IR cm^{-1}: 3446-3398 (NH), 3099 (CH aromatic), 2924, 2852 (CH aliphatic), 1714, 1699 (2 C=O), 1620 (C=N); ^{1}HNMR (DMSO-d_6) δ: 1.23 – 1.60 (2m, 6H, $3CH_3$), 2.02 (s, 3H, CH_3), 2.49 – 2.73 (2m, 10H, $5CH_2$), 4.19 (s, 3H, CH_3), 7.55 – 7.81 (m, 4H, CH aromatic), 7.71 (s, 1H,

NH, D_2O exchangeable); MS: m/z (% abundance) 433 (M^+) (82.98); Anal. Calcd. for $C_{25}H_{27}N_3O_2S$: C, 69.25; H, 6.28; N, 9.69. Found: C, 69.48; H, 6.39; N, 10.01.

10-{[5-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)pentyl]amino}-2,3,6,7,8,9-hexahydro-1*H*-[1]benzothieno[2,3-*b*]cyclopenta[*e*]pyridine (7b): Yield: 82%; mp > 300°C; IR cm^{-1}: 3446-3367 (NH), 3060 (CH aromatic), 2933, 2858 (CH aliphatic), 1716, 1701 (2 C=O), 1622 (C=N);^{1}HNMR (DMSO-d_6) δ: 1.91 – 2.10 (2m, 8H, $4CH_2$), 2.49 – 2.60 (2m, 16H, $8CH_2$), 7.55 – 7.68 (m, 4H, CH aromatic), 7.99 (s, 1H, NH, D_2O exchangeable); MS: m/z (% abundance) 459 (M^+) (28.79); Anal. Calcd. for $C_{27}H_{29}N_3O_2S$: C, 70.56; H, 6.36; N, 9.14. Found: C, 70.89; H, 6.44; N, 9.35.

7-{[5-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)pentyl]amino}-8,9-dimethyl-5,6-dihydrobenzo[*h*]thieno[2,3-*b*]quinoline (8a): Yield: 75%; mp > 300°C; IR cm^{-1}: 3446-3421 (NH), 3080 (CH aromatic), 2924, 2854 (CH aliphatic), 1714, 1683 (2 C=O), 1635 (C=N);^{1}HNMR (DMSO-d_6) δ: 1.23 – 1.91 (m, 4H, $2CH_2$), 2.49 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.29 – 3.39 (m, 10H, $5CH_2$), 7.57 – 7.81 (2m, 8H, CH aromatic); ^{13}CNMR (75 MHz, DMSO-d_6) δ: 21.41,21.52, 25.74, 123.44, 127.54, 128.89, 129.93, 131.13, 132.06, 134.81, 168.43; MS: m/z (% abundance) 495 (M^+) (88.68); Anal. Calcd. for $C_{30}H_{29}N_3O_2S$: C, 72.70; H, 5.90; N, 8.48. Found: C, 72.88; H, 5.96; N, 8.63.

7-{[5-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)pentyl]amino}-5,6,8,9,10,11-hexahydrobenzo[*h*][1]benzothieno[2,3-*b*]quinoline (8b): Yield: 85%; mp 288°C; IR cm^{-1}: 3523-3327 (NH), 3080 (CH aromatic), 2931, 2854 (CH aliphatic), 1712, 1701 (2 C=O), 1624 (C=N); ^{1}HNMR (DMSO-d_6) δ: 1.23 – 2.02 (2m, 8H, $4CH_2$), 2.49 – 2.50 (m, 2H, CH_2), 2.68 – 2.95 (2m, 4H, $2CH_2$), 3.11 – 3.30 (2m, 8H, $4CH_2$), 7.10 – 7.81 (2m, 8H, CH aromatic); MS: m/z (% abundance) 521 (M^+) (100); Anal. Calcd. for $C_{32}H_{31}N_3O_2S$: C, 73.67; H, 5.99; N, 8.06. Found: C, 73.90; H, 6.13; N, 8.19.

5-(1,2-Dithiolan-3-yl)-*N*-{5-[(2,3,6,7,8,9-hexahyro-1*H*-[1]benzothieno[2,3-*b*]cyclopenta[*e*]pyridin-10-yl)amino]pentyl}pentanamide (9): Lipoic acid (0.15 mol.) was added to a mixture of compound **5b** (0.1 mol.) and carbonyl diimidazole (0.12 mol.) in DMF (10 ml). The reaction mixture was stirred at room temperature under nitrogen for 24 h. The mixture was poured into ice – cold water. The separated solid was filtered and washed with cold ethanol. Yield: 26%; mp > 300°C; IR cm^{-1}: 3406-3375 (2 NH), 2935-2856 (CH aliphatic), 1650 (C=O), 1627 (C=N); ^{1}HNMR (DMSO-d_6) δ: 1.39 – 1.55 (2m, 10H, $5CH_2$), 1.56 – 1.77 (m, 12H, $6CH_2$), 2.20 – 2.31 (m, 2H, CH_2), 2.49 – 2.50 (m, 1H, CH), 2.74 – 3.33 (m, 12H, $6CH_2$), 4.22 (m, 2H, 2NH, D_2O exchangeable); MS: m/z (% abundance) 517 (M^+) (71.31); Anal. Calcd. for $C_{27}H_{39}N_3OS_3$: C, 62.63; H, 7.59; N, 8.12. Found: C, 62.81; H, 7.68; N, 8.31.

Biological screening

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*. [22]. 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 acetylthioncholine iodide as substrate of the enzymatic reaction. Briefly, the method used was as follows: AChE of rat homogenates either drug-free (control group), or containing-compounds (AChE-inhibitors) was mixed with cholinesterase kit assay solution (without the substrate). Each mixture was then preincubated with the enzyme for 10 minutes at 37°C. Following the preincubation, the substrate (0.5 mM acetylthioncholine solution) was added. The absorbance changes at 405 nm were recorded for 5 minutes tested with a microplate reader GENios F129004 (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 (protocol serial number: OC (1063) in 31/ 3/ 2014).

Statistical analysis

Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparison test for comparisons 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).

Table 1. AChE inhibition of tacrine and the newly synthesized compounds

Compound (dose)	Cholinesterase content (U / gm wet weight)	Inhibition %
Control (Saline)	1434.65 ± 22.74	0
Tacrine (10 mg / Kg)	422.28 ± 46.90*	70.57
4a (10 mg / Kg)	527.85 ± 45.43$^{*@}$	63.21
4b (10 mg / Kg)	469.20 ± 47.88$^{*@}$	67.30
5a (10 mg / Kg)	997.05 ± 75.72$^{*@}$	30.50

Table 1*(contd.)*. AChE inhibition of tacrine and the newly synthesized compounds

Compound (dose)	Cholinesterase content (U / gm wet weight)	Inhibition %
5b (10 mg / Kg)	1172 ± 47.83$^{*@}$	18.31
6a (10 mg / Kg)	630.49 ± 28.08$^{*@}$	56.05
6b (10 mg / Kg)	791.77 ± 29.33$^{*@}$	44.81
7a (10 mg / Kg)	422.48 ± 46.90$^{*@}$	70.55

Table 1*(contd.)*. AChE inhibition of tacrine and the newly synthesized compounds

Compound (dose)	Cholinesterase content (U / gm wet weight)	Inhibition %
7b (10 mg / Kg)	398.86 ± 28.76$^{*@}$	72.20
8a (10 mg / Kg)	645.15 ± 33.86$^{*@}$	55.03
8b (10 mg / Kg)	835.76 ± 77.12$^{*@}$	41.74
9 (10 mg / Kg)	879.75 ± 33.86$^{*@}$	38.68

$^{@}$ *Significantly different from tacrine group at $p < 0.05$.*
* *Significantly different from normal control group at $p < 0.05$.*

Drug-likeness study

According to Lipinski's rule of five [23], for the best choice in discovering new medications calculated log *P* should be in the range of the values 2.5 – 5. Although compounds 7a,b are not obeying this general rule, they are showing the best percent of inhibition. Nevertheless, upon applying the "Rule of two" [24], which is more concerned with the blood brain barrier passage and absorption, the two compounds are fulfilling the criteria (Tables 2 & 3). The results which can explain the promising activity recorded.

Table 2. Polar surface area (PSA), Lipinski's parameters and drug-likeness model score of the screened compounds

Cpd. #	Molecular weight	Volume (A^3)	PSA (A^2)	NROTB	HBA
Tacrine	198.12	200.65	29.38	0	1
4a	504.24	523.08	40.89	8	4
4b	556.27	580.12	40.73	8	4
5a	303.18	319.78	41.85	6	3
5b	329.19	348.30	41.77	6	3
6a	365.19	376.17	40.58	6	3
6b	391.21	404.69	40.50	6	3
7a	433.18	450.98	49.93	7	4
7b	459.20	479.50	49.85	7	4
8a	495.20	507.38	48.65	7	4
8b	521.21	535.89	48.57	7	4
9	461.16	456.38	45.43	12	5

Table 3. Polar surface area (PSA), Lipinski's parameters and drug-likeness model score of the screened compounds

Cpd. #	HBD	CLog*P*	Drug-likeness model score	Σ(N + O)	CLog*P* - Σ(N + O)
Tacrine	2	3.715	0.86	2	1.715
4a	2	7.263	0.64	4	3.264
4b	2	8.348	0.62	4	4.348
5a	3	3.115	0.65	3	0.115
5b	3	3.692	0.61	3	0.692
6a	3	4.23	0.47	3	1.23
6b	3	4.808	0.39	3	1.808
7a	1	5.523	0.80	5	0.523
7b	1	6.1	0.86	5	1.1
8a	1	6.638	0.19	5	1.638
8b	1	7.215	0.15	5	2.215
9	2	6.69	0.76	4	2.69

Molecular docking study

All molecular modeling calculations and docking studies were carried out using Molecular Operating Environment MOE version 2009.10 [25]. The target compounds were drawn on MOE. The structures were subjected to energy minimization using Hamiltonian-Force Field-MMFF94x. The most stable conformers for each compound were retained and partial charges were calculated. The X-ray crystal structure of AChE enzyme in complex with Tacrine PDB (Code: 1ACJ) was recovered from RSCB protein data bank [26]. The enzyme was prepared for docking as follows: 1) The Co-crystallized ligand and water molecules were removed. 2) The enzyme was 3D protonated, where hydrogen atoms were added at their standard geometry, the partial charges were computed and the system was optimized. Flexible ligand-rigid receptor docking of the most stable conformers was done with MOE-DOCK using triangle matcher as placement method and London dG as a scoring function. The obtained poses were subjected to force field refinement using the same scoring function. Ten of the most stable docking models for each ligand were retained with the best scored conformation. In order to validate the docking procedure, Tacrine was docked into the active site of AChE enzyme. Tacrine binding model suggests being 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 with average distances 3.4 A° and 3.6 A° respectively. Moreover, the two rings showed an interaction with the five-membered ring of indole of Trp 84 with average distances 3.5 A° and 3.55 A° (figure 4).

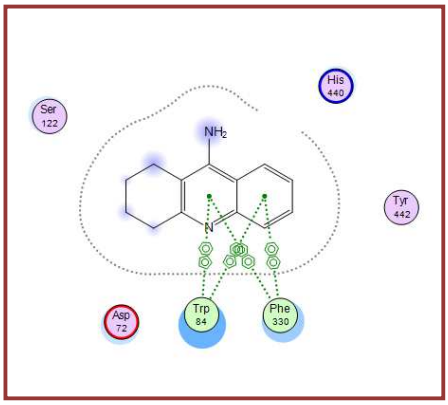


Figure 4. Tacrine binding interaction with the active site gorge of AChE enzyme

The docking results show that the compound 7a and 7b exhibit similar interaction reported in literature with RMSD = 0.957 Å. Moreover, compound 7b shows an extra hydrogen bonding between the phthalic anhydride oxygen moiety and Tyr 121 which may explain its better results as AChE inhibitor (figures 5-7).

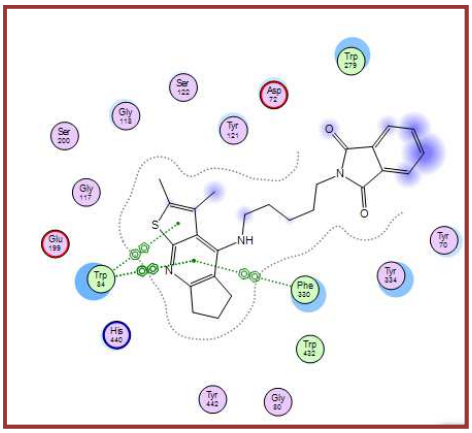


Figure 5. 7a binding interaction with the active site gorge of AChE enzyme

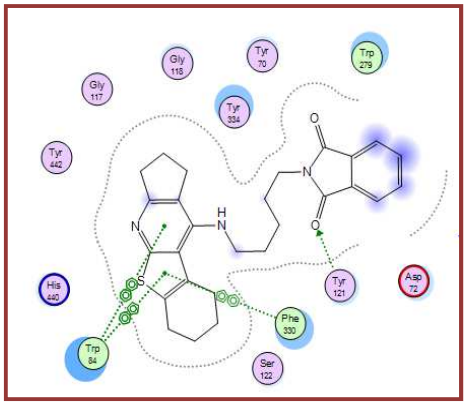


Figure 6. 7b binding interaction with the active site gorge of AChE enzyme

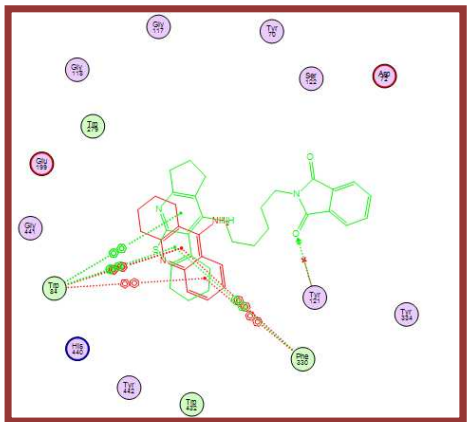


Figure 7. 7b and tacrine binding interactions with the active site gorge of AChE enzyme

The docking results show that the compound 7a and 7b exhibit similar interaction reported in literature with RMSD = 0.957 Å. Moreover, compound 7b shows an extra hydrogen bonding between the phthalic anhydride oxygen moiety and Tyr 121 which may explain its better results as AChE inhibitor.

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