Mohammad Mahdavi,[a] Mina Saeedi,[b,c] Laleh Gholamnia,[a] Seyed Amir Behzad Jeddi,[d] Reyhaneh Sabourian,[c] Abbas Shafiee,[a] Alireza Foroumadi,[a] and Tahmineh Akbarzadeh[c,d]*

[a]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran
[b]Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[c]Persian Medicine and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran, Iran
[d]Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
*E-mail: akbarzad@tums.ac.ir

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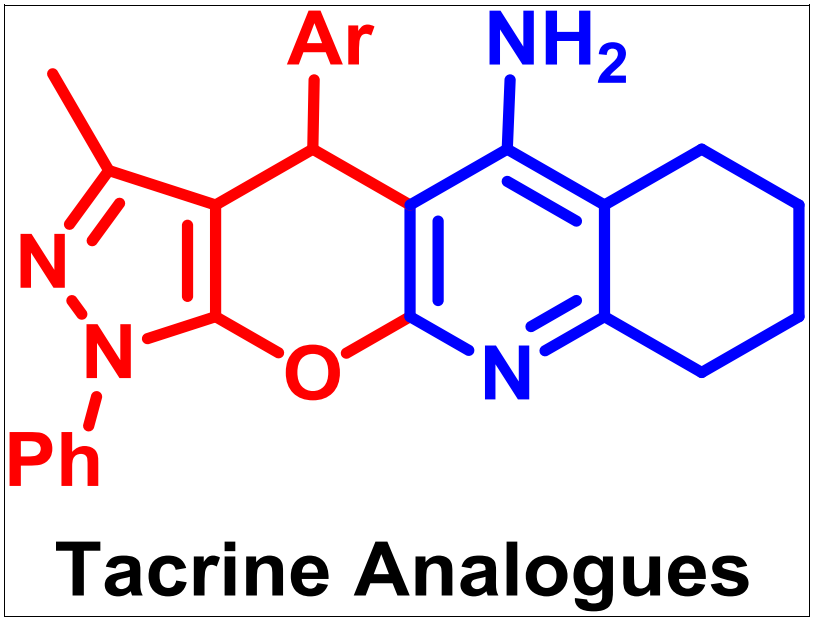

In this work, a wide range of novel pyrazolo[4′,3′:5,6]pyrano[2,3-*b*]quinolin-5-amines were synthesized as tacrine analogs. At first, reaction of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one, aromatic aldehydes, and malononitrile gave 6-amino-4-aryl-3-methyl-1-phenyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitriles. Then, reaction of the latter compounds with cyclohexanone led to the formation of the title compounds. Also, they were evaluated for their *in vitro* acetylcholinesterase and butyrylcholinesterase inhibitory activities. Interestingly, most of them showed good inhibitory activity comparing with rivastigmine as the reference drug.

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INTRODUCTION

O-Heterocycles are of significant interest as they possess versatile biological activities, and among various oxygen containing heterocyclic compounds, pyran derivatives have attracted lots of attention because of their important biological activities including antimycobacterial [1], antibacterial and antifungal [2], cytotoxic [3], quorum sensing inhibitory [4], and antitumor activities [5]. Besides, pyrazole and its derivatives are brilliant among *N*-heterocyclic compounds as they possess outstanding biological activities and have been widely applied in pharmaceutical industry [6]. In this respect, synthesis and biological properties of fused pyrano–pyrazole derivatives have attracted lots of attention because anti-inflammatory [7], antibacterial, cytotoxic [8], and acetylcholinesterase (AChE) inhibitory [9] activities have been reported in the literature.

Acetylcholine and butyrylcholine are neurotransmitters that play significant role in various cognitive abilities, neuronal and non-neuronal signaling. AChE and butyrylcholinesterase (BChE) are enzymes that degrade choline-based esters in numerous cholinergic pathways in the central and peripheral nervous systems [10]. It has been revealed that loss of cholinergic transmission is one the most important cause of Alzheimer's disease (AD). In this respect, much research has been devoted to the enhancement of cholinergic system through several ways and anticholinesterase drugs have attracted lots of attention to reduce symptoms of AD [11].

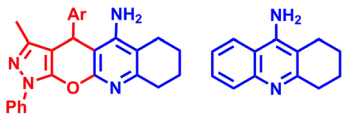


Figure 1. The structure of tacrine and synthesized analogs **6**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

9-Amino-1,2,3,4-tetrahydroacridine known as tacrine (Fig. 1) [12] was found to be an efficient drug inhibiting both AChE and BChE [13]. However, extensive use of tacrine was limited as it showed various side effects [14]. Hence, recent reports have been attributed to the design and synthesis of novel and efficient tacrine-based AChE inhibitors.

Herein, in continuation of our research on the synthesis of novel heterocycles [15] and bioactive heterocyclic compounds [16], we focused on the synthesis of novel pyrazolo[4′,3′:5,6]pyrano[2,3-*b*]quinolin-5-amines **6** as anti-AChE and BChE agents (Fig. 1).

RESULTS AND DISCUSSION

Chemistry. The preparation of pyrazolo[4′,3′:5,6]pyrano[2,3-*b*]quinolin-5-amines **6** is shown in Scheme 1. For this purpose, various 6-amino-4-aryl-3-methyl-1-phenyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitriles **4** were prepared through the reaction of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**1**), aromatic aldehydes **2**, and malononitrile (**3**). To obtain the best reaction conditions, reaction of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**1**), benzaldehyde (**2a**), and malononitrile (**3**) was conducted as a model reaction under various conditions. Accordingly, the model reaction was achieved in the presence of different basic reagents such as DABCO, NEt_3, K_2CO_3, and piperidine. Also, solvent screening was performed in EtOH, MeOH, CH_3CN, $PhCH_3$, and CH_2Cl_2. It was found that using DABCO in EtOH led to the formation of the corresponding product **4a** in good yield (85%). It should be noted that the previously mentioned reaction was successfully performed at room temperature for 12 h, and increasing temperature did not lead to higher yield. The scope of the reaction was investigated by varying aromatic aldehydes **2**. It was clear that all aldehydes possessing different electronic substituents participated in the reaction to afford product **4**. In the next step, reaction of compounds **4** and cyclohexanone (**5**) was investigated. In this regard, reaction of 6-amino-3-methyl-1,4-diphenyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (**4a**) and cyclohexanone (**5**) was conducted in the presence of different Lewis acids such as $ZnCl_2$ and $AlCl_3$ as well as different solvents like CH_2Cl_2, $ClCH_2CH_2Cl$ (DCE), and $CHCl_3$. It was found that the best result was obtained in the presence of $AlCl_3$ in DCE at reflux for 12–18 h. Then, reaction of all substrates **4** and cyclohexanone (**5**) was performed under the optimized conditions to give the corresponding products **6** in good yields (Table 1). Also, the structure of all compounds was confirmed using IR, ^{1}H-, and ^{13}C-NMR spectroscopy as well as chemical analysis.

Biological investigation. The *in vitro* anti-AChE activity of the all compounds **6** was evaluated using Ellman's method [17] and compared with rivastigmine as the reference drug (Table 1). Our results depicted that compound **6a** having phenyl substituent at the 4-position of the compound possessed the best inhibitory activity ($IC_{50} = 2.77\ \mu M$) in comparison to rivastigmine as the reference drug ($IC_{50} = 11.07\ \mu M$). Compound **6b** having 4-MeO-phenyl group at the 4-position showed activity with $IC_{50} = 2.84\ \mu M$ slightly different from compound **6a**. However, increasing the number of methoxy groups on the phenyl ring at different positions (compounds **6c** and **6d**) led to the lack of activity ($IC_{50} > 100$). Replacing the 4-MeO-phenyl group by 4-Me-phenyl (compound **6e**) reduced the activity ($IC_{50} = 13.04\ \mu M$). It should be noted that changing the position of methyl group did not improve the inhibitory activity ($IC_{50} = 26.01$ and >100 for compounds **6f** and **6g**, respectively). However, the presence of methyl group at *ortho* position deleted the anti-AChE activity (compound **6g**). Compound **6j** having 3-F-phenyl group at the 4-position showed $IC_{50} = 4.04\ \mu M$. Changing the position of fluorine on the phenyl ring gave lower activity because compounds **6i** and **6k** exhibited $IC_{50} = 9.00$ and $15.71\ \mu M$, respectively. It was found that introduction of chlorine at different positions reduced AChE inhibitory activity. Compounds **6l** and **6m** possessing 4-Cl-phenyl and 2-Cl-phenyl, respectively, at the 4-position of the synthesized compounds showed $IC_{50} = 24.24$ and $29.57\ \mu M$, respectively. Also, increasing the number of chlorine at 2,3-, 2,4-, 3,4- (compounds **6n–p**) deleted the inhibitory activity. Nevertheless, 2,6-diCl showed moderate activity with $IC_{50} = 16.78\ \mu M$. Introduction of electron-withdrawing NO_2 group into the discussing position (compound **6h**) led to

Scheme 1. Synthesis of pyrazolo[4′,3′:5,6]pyrano[2,3-*b*]quinolin-5-amines **6**.

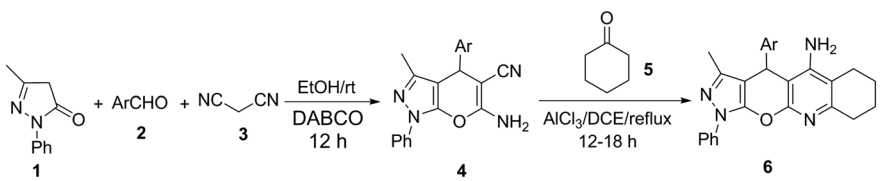
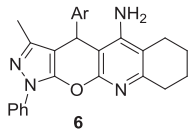


Table 1

The IC_{50} values of the compounds **6** against AChE and BChE.



6

Entry	Compound **6**	Ar	AChE inhibition [IC_{50} (μM)]	BChE inhibition [IC_{50} (μM)]
1	**6a**	C_6H_5	2.77 ± 0.25	2.21 ± 0.02
2	**6b**	4-$MeOC_6H_4$	2.84 ± 0.42	1.02 ± 0.03
3	**6c**	2,4-di$MeOC_6H_3$	>100	0.06 ± 0.01
4	**6d**	2,3,4-tri$MeOC_6H_2$	>100	>100
5	**6e**	4-MeC_6H_4	13.04 ± 0.34	>100
6	**6f**	3-MeC_6H_4	26.01 ± 0.76	5.92 ± 0.09
7	**6g**	2-MeC_6H_4	>100	>100
8	**6h**	3-$NO_2C_6H_4$	6.24 ± 1.18	>100
9	**6i**	4-FC_6H_4	15.71 ± 1.04	1.57 ± 0.05
10	**6j**	3-FC_6H_4	4.04 ± 0.14	>100
11	**6k**	2-FC_6H_4	9.00 ± 0.17	10.00 ± 0.06
12	**6l**	4-ClC_6H_4	24.24 ± 0.59	3.84 ± 0.50
13	**6m**	2-ClC_6H_4	29.57 ± 0.085	>100
14	**6n**	2,3-diClC_6H_3	>100	>100
15	**6o**	2,4-diClC_6H_3	>100	>100
16	**6p**	3,4-diClC_6H_3	>100	>100
17	**6q**	2,6-diClC_6H_3	16.78 ± 1.29	>100
18	**6r**	2-Thiophenyl	>100	4.16 ± 1.01
21	Rivastigmine		11.07 ± 0.01	7.72 ± 0.02

Data are expressed as mean ± SE (three independent experiments).

$IC_{50} = 6.24\ \mu M$. Clearly, the efficacy of nitro group is higher than chlorine and methyl groups. Another instructive result is related to the introduction of heterocyclic substitution, 2-thiophenyl at the 4-position of compound **6r** that led to the lack of activity.

Results related to the anti-BChE activity revealed that compounds **6a–c**, **6f**, **6i**, **6k**, **6l**, and **6r** showed good to high activity ($IC_{50} = 0.06–10.00\ \mu M$) toward BChE comparing with rivastigmine ($IC_{50} = 7.72\ \mu M$). Other compounds exhibited no anti-BChE activity ($IC_{50} > 100$). The best activity was related to compound **6c** having 2,4-diMeO-phenyl group, which showed no anti-AChE activity ($IC_{50} = 0.06\ \mu M$), 128 times more potent than the reference drug. Compounds **6b**, **6i**, and **6a** depicted good activity with IC_{50}s = 1.02, 1.57, and 2.21 μM. Also, compound **6l** having 4-Cl-phenyl group at the 4-position showed good activity ($IC_{50} = 3.48\ \mu M$), and the introduction of heterocyclic substituent, 2-thiophenyl at the 4-position of compound **6r** gave $IC_{50} = 4.16\ \mu M$. Compound **6f** possessing 3-Me-phenyl group inhibited BChE with $IC_{50} = 5.92\ \mu M$. These findings indicated that compounds **6a–c**, **6f**, **6i**, **6l**, and **6r** were 1.8–128 times more potent than rivastigmine. It should be noted that compound **6k** showed moderate anti-BChE activity ($IC_{50} = 10.00\ \mu M$). According to our results, both compounds **6a** and **6b** showed high anti-AChE and anti-BChE activity.

CONCLUSIONS

In conclusion, novel pyrazolo[4′,3′:5,6]pyrano[2,3-*b*] quinolin-5-amines were synthesized as tacrine analogs. It was found that the prepared compounds possessed anticholinesterase activity. In this respect, 3-methyl-1,4-diphenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-*b*] quinolin-5-amine and 4-(2,4-dimethoxyphenyl)-3-methyl-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-*b*] quinolin-5-amine were the most potent anti-AChE and anti-BChE compounds.

EXPERIMENTAL

Chemistry. Melting points were taken on a Kofler hot stage apparatus (England) and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker FT-500 (Germany), using TMS as an internal standard. The IR spectra were obtained on a Nicolet Magna FTIR 550 spectrometer (KBr disks) (USA). The elemental analysis was performed with an Elementar Analysensystem GmbH *VarioEL* CHNS mode (Germany).

Procedure for the synthesis of 6-amino-3-methyl-1-phenyl-4-aryl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile 4. A mixture of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one **1**

(1 mmol), appropriate aldehyde **2** (1 mmol), malononitrile **3** (1 mmol), and DABCO (20 mol%) in EtOH (10 mL) was stirred at room temperature for 12 h. After completion of the reaction, the mixture was filtered off, washed with cold EtOH, and dried at 50°C to give pure compounds **4**.

Procedure for the synthesis of 4-aryl-3-methyl-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-*c*]quinolin-5-amine 6. A mixture of compound **4** (1 mmol) and cyclohexanone **5** (2 mmol) was added to the suspension of $AlCl_3$ in dry DCE (2 mmol in 30 mL) and heated at reflux for 12–18 h. After completion of the reaction (checked by TLC), a mixture of H_2O/THF (1:1, 100 mL) was added to the mixture, and it was basicified with NaOH (10%). The mixture was stirred at room temperature for 30 min, and the crude product was extracted with CH_2Cl_2 (2 × 50) and washed with brine (2 × 50). The organic phase was dried over Na_2SO_4, and the solvent was evaporated under vacuum. All compounds were recrystallized from EtOH to afford pure products **6**.

3-Methyl-1,4-diphenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-c]quinolin-5-amine (6a). Yield 85%; mp 217–219°C; IR (KBr): 3490, 3375, 2909, 2850, 2195, 1634, 1597, 1517 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.70–1.72 (m, 4H, $2CH_2$), 2.00 (s, 3H, CH_3), 2.18–2.35 (m, 2H, CH_2), 2.60–2.62 (m, 2H, CH_2), 5.29 (s, 1H, CH), 5.55 (s, 2H, NH_2), 7.17 (t, J = 7.2 Hz, 1H, Ph), 7.26–7.36 (m, 5H, Ph), 7.53 (t, J = 8.2 Hz, 2H, Ph), 7.80 (d, J = 8.2 Hz, 2H, Ph); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 12.7, 22.0, 22.2, 23.0, 31.9, 34.7, 98.0, 100.2, 113.3, 119.9, 125.9, 126.5, 127.8, 128.5, 129.4, 137.9, 144.2, 145.2, 145.9, 152.5, 152.7, 154.5. *Anal*. Calcd for $C_{26}H_{24}N_4O$: C, 76.45; H, 5.92; N, 13.72. Found: C, 76.31; H, 6.14; N, 13.58.

4-(2,4-Dimethoxyphenyl)-3-methyl-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6c). Yield 75%; mp >250°C; IR (KBr): 3463, 3376, 2929, 2835, 1632, 1597, 1515 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.70–1.72 (m, 4H, $2CH_2$), 2.02 (s, 3H, CH_3), 2.30–2.81 (m, 4H, CH_2), 3.67 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 5.17 (s, 1H, CH), 5.50 (s, 2H, NH_2), 6.67 (d, J = 8.5 Hz, 1H, H6′), 6.83 (dd, J = 8.5, 2.0 Hz, 1H, H5′), 7.14 (s, 1H, H3′), 7.31 (t, J = 7.5 Hz, 1H, Ph), 7.53 (t, J = 7.5 Hz, 2H, Ph), 7.79 (d, J = 7.5 Hz, 2H, Ph). *Anal*. Calcd for $C_{28}H_{28}N_4O_3$: C, 71.78; H, 6.02; N, 11.96. Found: C, 71.89; H, 6.18; N, 12.14.

3-Methyl-1-phenyl-4-(2,4,6-trimethoxyphenyl)-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6d). Yield 70%; mp >250°C; IR (KBr): 3499, 3412, 2940, 2850, 1639, 1592, 1518 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.72–1.74 (m, 4H, $2CH_2$), 2.05 (s, 3H, CH_3), 2.26–2.61 (m, 4H, $2CH_2$), 3.60 (s, 3H, OCH_3), 3.77 (s, 2H, $2OCH_3$), 5.19 (s, 1H, CH), 5.49 (s, 2H, NH_2), 6.67 (s, 2H, H3′, H5′), 7.30 (t, J = 7.5 Hz, 1H, Ph), 7.51 (t, J = 7.5 Hz, 2H, Ph), 7.80 (d, J = 7.5 Hz, 2H, Ph); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 12.7, 21.9, 22.2, 22.8, 31.8, 35.2, 55.9, 59.8, 97.7, 99.8, 105.3, 113.1, 119.9, 125.7, 129.2, 137.9, 139.8, 145.2, 146.1, 150.9, 151.1, 152.6, 152.8, 154.5. *Anal*. Calcd for $C_{29}H_{30}N_4O_4$: C, 69.86; H, 6.06; N, 11.24. Found: C, 69.71; H, 6.24; N, 11.14.

3-Methyl-1-phenyl-4-(p-tolyl)-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6e). Yield 78%; mp >250°C; IR (KBr): 3455, 3370, 3082, 2924, 2801, 1614, 1557, 1404 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.83–1.87 (m, 4H, $2CH_2$), 2.05 (s, 3H, CH_3), 2.27–2.30 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.80–2.81 (m, 2H, CH_2), 4.14 (s, 2H, NH_2), 4.95 (s, 1H, CH), 7.14 (d, J = 7.8 Hz, 1H, H3′, H5′), 7.21–7.26 (m, 3H, H2′, H6′, Ph), 7.44 (t, J = 8.5 Hz, 2H, Ph), 7.53 (dd, J = 8.5, 1.0 Hz, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 13.2, 21.0, 22.3, 22.5, 22.87, 32.5, 37.4, 99.03, 99.2, 113.7, 121.0, 125.8, 127.9, 129.1, 129.8, 137.1, 138.2, 140.3, 145.8, 146.0, 152.2, 154.2, 155.1. *Anal*. Calcd for $C_{27}H_{26}N_4O$: C, 76.75; H, 6.20; N, 13.26. Found: C, 76.58; H, 6.34; N, 13.18.

3-Methyl-1-phenyl-4-(m-tolyl)-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6f). Yield 70%; mp >250°C; IR (KBr): 3517, 3422, 2924, 2850, 1632, 1588, 1515 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.72–1.74 (m, 4H, $2CH_2$), 1.99 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.24–2.35 (m, 2H, CH_2), 2.62–2.64 (m, 2H, CH_2), 5.21 (s, 1H, CH), 5.45 (s, 2H, NH_2), 6.98 (t, J = 7.5 Hz, 1H, H5′), 7.12 (s, 1H, H2′), 7.16–7.17 (m, 2H, H4′, H6′), 7.31 (t, J = 8.0 Hz, 1H, Ph), 7.52 (t, J = 8.0 Hz, 2H, Ph), 7.80 (d, J = 8.0 Hz, 2H, Ph); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 12.6, 21.0, 21.9, 22.2, 22.8, 31.8, 34.8, 99.0, 100.0, 113.2, 119.7, 124.9, 125.7, 127.3, 128.2, 128.4, 129.2, 137.5, 137.9, 144.0, 145.1, 145.8, 152.4, 152.6, 154.4. *Anal*. Calcd for $C_{27}H_{26}N_4O$: C, 76.75; H, 6.20; N, 13.26. Found: C, 76.91; H, 6.11; N, 13.35.

3-Methyl-4-(3-nitrophenyl)-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6h). Yield 75%; mp >250°C; IR (KBr): 3525, 3437, 3017, 2946, 2859, 1634, 1599, 1519, 1373 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.74–1.75 (m, 4H, $2CH_2$), 2.11 (s, 3H, CH_3), 2.25–2.39 (m, 2H, CH_2), 2.61–2.63 (m, 2H, CH_2), 5.73 (s, 1H, CH), 5.78 (s, 2H, NH_2), 6.91 (t, J = 8.0 Hz, 1H, Ph), 7.23 (d, J = 3.4 Hz, 1H, H2′), 7.23–7.34 (m, 3H, H4′, H5′, H6′), 7.44 (t, J = 8.0 Hz, 2H, Ph), 7.53 (dd, J = 8.0, 1.0 Hz, 2H, Ph); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 12.7, 22.0, 22.2, 23.0, 31.9, 34.0, 97.3, 99.4, 113.6, 120.0, 121.8, 122.2, 126.1, 129.4, 130.3, 134.6, 137.9, 145.1, 146.1, 146.5, 147.6, 152.6, 153.2, 154.4. *Anal*. Calcd for $C_{26}H_{23}N_5O_3$: C, 68.86; H, 5.11; N, 15.44. Found: C, 68.69; H, 5.27; N, 15.31.

4-(4-Fluorophenyl)-3-methyl-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6i). Yield 72%; mp >250°C; IR (KBr): 3514, 3415,

3018, 2924, 2850, 1639, 1592, 1518 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.83–1.84 (m, 4H, $2CH_2$), 2.05 (s, 3H, CH_3), 2.28–2.36 (m, 2H, CH_2), 2.80–2.82 (m, 2H, CH_2), 4.10 (s, 2H, NH_2), 4.99 (s, 1H, CH), 7.03 (t, J = 8.5 Hz, 2H, H3′, H5′), 7.25 (t, J = 7.5 Hz, 1H, Ph), 7.31 (t, J = 8.5, 5.2 Hz, 2H, H2′, H6′), 7.44 (t, J = 7.5 Hz, 2H, Ph), 7.87 (dd, J = 7.5, 1.1 Hz, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 13.2, 22.3, 22.5, 22.9, 32.5, 37.1, 98.7, 98.8, 113.9, 116.1 (d, $J_{C\text{-}F}$ = 21.6 Hz), 121.0, 125.9, 129.1, 129.4, 129.5, 138.1, 139.2, 145.8, 152.0, 154.5, 155.0, 161.8 (d, $J_{C\text{-}F}$ = 245.0 Hz). *Anal*. Calcd for $C_{26}H_{23}FN_4O$: C, 73.22; H, 5.44; N, 13.14. Found: C, 73.11; H, 5.52; N, 13.31.

4-(3-Fluorophenyl)-3-methyl-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6j). Yield 70%; mp >250°C; IR (KBr): 3502, 3412, 3015, 2949, 2850, 1639, 1521 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.84–1.85 (m, 4H, $2CH_2$), 2.08 (s, 3H, CH_3), 2.26–2.37 (m, 2H, CH_2), 2.81–2.82 (m, 2H, CH_2), 4.12 (s, 2H, NH_2), 5.01 (s, 1H, CH), 6.94–7.00 (m, 2H, H2′, H4′), 7.18 (d, J = 7.6 Hz, 1H, H6′), 7.26 (t, J = 8.0 Hz, 1H, Ph), 7.32 (td, J = 7.6, 3.0 Hz, 1H, H5′), 7.45 (t, J = 8.0 Hz, 2H, Ph), 7.87 (dd, J = 8.0, 1.0 Hz, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 13.2, 22.2, 22.5, 22.9, 32.5, 37.6, 98.3, 113.8, 114.5 (d, $J_{C\text{-}F}$ = 20.9 Hz), 115.0 (d, $J_{C\text{-}F}$ = 21.5 Hz), 121.0, 123.5, 126.0, 129.1, 130.6, 130.7, 138.1, 145.8, 145.9, 146.1, 152.1, 154.6, 155.0, 163.4 (d, $J_{C\text{-}F}$ = 246.5 Hz). *Anal*. Calcd for $C_{26}H_{23}FN_4O$: C, 73.22; H, 5.44; N, 13.14. Found: C, 73.50; H, 5.28; N, 13.04.

4-(2-Fluorophenyl)-3-methyl-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6k). Yield 65%; mp >250°C; IR (KBr): 3440, 3323, 2950, 2850, 1630, 1588 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.83–1.84 (m, 4H, $2CH_2$), 2.05 (s, 3H, CH_3), 2.25–2.37 (m, 2H, CH_2), 2.79–2.81 (m, 2H, CH_2), 4.24 (s, 2H, NH_2), 5.37 (s, 1H, CH), 7.06–7.14 (m, 3H, H5′, H6′, Ph), 7.21–7.25 (m, 2H, H3′, H4′), 7.44 (t, J = 7.5 Hz, 2H, Ph), 7.88 (dd, J = 7.5, 1.1 Hz, 2H, Ph); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 12.1, 21.8, 22.1, 22.8, 29.9, 31.7, 96.8, 98.1, 113.3, 115.8 (d, $J_{C\text{-}F}$ = 21.2 Hz), 119.8 (d, $J_{C\text{-}F}$ = 11.2 Hz), 124.4, 125.8, 128.8 (d, $J_{C\text{-}F}$ = 8.7 Hz), 129.2, 129.8, 129.9, 130.4, 137.8, 145.0, 152.2, 152.6, 154.4, 159.5 (d, $J_{C\text{-}F}$ = 245.0 Hz). *Anal*. Calcd for $C_{26}H_{23}FN_4O$: C, 73.22; H, 5.44; N, 13.14. Found: C, 73.38; H, 5.31; N, 13.29.

4-(4-Chlorophenyl)-3-methyl-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6l). Yield 80%; mp >250°C; IR (KBr): 3505, 3409, 3035, 2921, 2859, 1629, 1596, 15717 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.63–1.64 (m, 4H, $2CH_2$), 2.10 (s, 3H, CH_3), 2.25–2.28 (m, 2H, CH_2), 2.42–2.44 (m, 2H, CH_2), 5.25 (s, 1H, CH), 5.52 (s, 2H, NH_2), 7.24–7.29 (m, 5H, H2′, H3′, H5′, H6′, Ph), 7.45 (td, J = 8.5, 1.5 Hz, 2H, Ph), 7.71 (d, J = 8.5 Hz, 2H, Ph); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 12.7, 22.0, 22.2, 23.0, 31.9, 33.9, 97.7, 99.8, 113.4, 119.9, 126.0, 128.4, 129.4, 129.6, 131.1, 137.9, 143.3, 145.2, 145.9, 152.5, 152.8, 154.4. *Anal*. Calcd for $C_{26}H_{23}ClN_4O$: C, 70.50; H, 5.23; N, 12.65. Found: C, 70.38; H, 5.41; N, 12.51.

4-(2,3-Dichlorophenyl)-3-methyl-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6n). Yield 70%; mp >250°C; IR (KBr): 3483, 3406, 3054, 2939, 2856, 1633, 1598, 1517 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.71–1.72 (m, 4H, $2CH_2$), 1.93 (s, 3H, CH_3), 2.22–2.32 (m, 2H, CH_2), 2.55–2.61 (m, 2H, CH_2), 5.26 (s, 2H, NH_2), 5.65 (s, 1H, CH), 7.32–7.33 (m, 3H, H5′, H6′, Ph), 7.53–7.54 (m, 3H, H4′, Ph), 7.80 (d, J = 7.3 Hz, 2H, Ph); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 12.7, 21.9, 22.2, 22.9, 31.9, 39.0, 96.1, 98.9, 113.7, 120.1, 126.1, 128.2, 128.8, 129.4, 130.3, 131.1, 131.8, 132.3, 137.8, 145.2, 147.1, 152.5, 153.2, 154.5. *Anal*. Calcd for $C_{26}H_{22}Cl_2N_4O$: C, 65.41; H, 4.65; N, 11.74. Found: C, 65.28; H, 4.80; N, 11.58.

4-(2,4-Dichlorophenyl)-3-methyl-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6o). Yield 75%; mp >250°C; IR (KBr): 3490, 3412, 2937, 2850, 1639, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.80–1.82 (m, 4H, $2CH_2$), 2.06 (s, 3H, CH_3), 2.23–2.35 (m, 2H, CH_2), 2.78–2.79 (m, 2H, CH_2), 4.22 (s, 2H, NH_2), 5.49 (s, 1H, CH), 7.07 (d, J = 8.0 Hz, 1H, H6′), 7.15 (dd, J = 8.0, 1.5 Hz, 1H, H5′), 7.25 (t, J = 7.5 Hz, 1H, Ph), 7.39 (d, J = 1.5 Hz, 1H, H3′), 7.44 (t, J = 7.5 Hz, 2H, Ph), 7.87 (d, J = 7.5 Hz, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 12.9, 22.3, 22.5, 22.9, 32.5, 36.5, 94.1, 98.5, 113.7, 121.0, 126.0, 128.5, 128.9, 129.2, 130.2, 132.4, 133.5, 138.2, 139.6, 145.8, 146.4, 151.7, 154.6, 155.0. *Anal*. Calcd for $C_{26}H_{22}Cl_2N_4O$: C, 65.41; H, 4.65; N, 11.74. Found: C, 65.31; H, 4.51; N, 11.86.

4-(3,4-Dichlorophenyl)-3-methyl-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6p). Yield 70%; mp >250°C; IR (KBr): 3485, 3375, 2950, 2850, 1640, 1588, 1525 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.71–1.72 (m, 4H, $2CH_2$), 1.94 (s, 3H, CH_3), 2.19–2.36 (m, 2H, CH_2), 2.59–2.61 (m, 2H, CH_2), 5.29 (s, 2H, NH_2), 5.55 (s, 1H, CH), 7.33 (t, J = 8.0 Hz, 1H, Ph), 7.38–7.40 (m, 2H, H5′, H6′), 7.54 (t, J = 8.0 Hz, 2H, Ph), 7.59 (s, 1H, H2′), 7.79 (d, J = 8.0 Hz, 2H, Ph); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 12.6, 21.9, 22.2, 22.9, 31.7, 32.8, 96.9, 97.7, 113.7, 120.1, 126.1, 128.2, 129.2, 129.4, 132.4, 132.5, 132.9, 137.8, 139.3, 145.2, 146.3, 152.5, 153.1, 154.4. *Anal*. Calcd for $C_{26}H_{22}Cl_2N_4O$: C, 65.41; H, 4.65; N, 11.74. Found: C, 65.31; H, 4.74; N, 11.62.

***4-(2,6-Dichlorophenyl)-3-methyl-1-phenyl-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]*quinolin-5-amine (6q).** Yield 70%; mp >250°C; IR (KBr): 3471, 3385, 2937, 2850, 1642, 1592 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.80–1.82 (m, 4H, $2CH_2$), 2.00 (s, 3H, CH_3), 2.25–2.31 (m, 2H, CH_2), 2.75–2.77 (m, 2H, CH_2), 4.19 (s, 2H, NH_2), 6.00 (s, 1H, CH), 7.16 (t, J = 8.0 Hz, 1H, H4′), 7.23 (t, J = 8.0 Hz, 1H, Ph), 7.41–7.45 (m, 4H, Ph, H3′, H5′), 7.86 (d, J = 8.0 Hz, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 12.8, 22.3, 22.5, 22.9, 32.4, 33.6, 95.1, 96.8, 113.5, 121.2, 126.0, 128.0, 129.1, 131.7, 134.3, 135.5, 137.1, 138.2, 145.6, 151.5, 154.2, 156.0. *Anal*. Calcd for $C_{26}H_{22}Cl_2N_4O$: C, 65.41; H, 4.65; N, 11.74. Found: C, 65.29; H, 4.80; N, 11.55.

3-Methyl-1-phenyl-4-(thiophen-2-yl)-1,4,6,7,8,9-hexahydropyrazolo[4′,3′:5,6]pyrano[2,3-b]quinolin-5-amine (6r). Yield 65%; mp >250°C; IR (KBr): 3459, 3323, 3072, 2915,, 1660, 1592, 1514 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.71–1.72 (m, 4H, $2CH_2$), 2.11 (s, 3H, CH_3), 2.24–2.38 (m, 2H, CH_2), 2.60–2.61 (m, 2H, CH_2), 5.72 (s, 1H, CH), 5.78 (s, 2H, NH_2), 6.91 (t, J = 4.0 Hz, 1H, thiophene), 7.22 (d, J = 4.0 Hz, 1H, thiophene), 7.29–7.33 (m, 2H, thiophene, Ph), 7.53 (t, J = 8.0 Hz, 2H, Ph), 7.79 (d, J = 8.0 Hz, 2H, Ph); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 12.6, 22.0, 22.3, 23.0, 29.9, 31.9, 97.9, 100.0, 113.3, 119.9, 124.8, 124.9, 125.0, 126.0, 126.3, 129.4, 137.9, 145.3, 145.8, 149.1, 152.8, 153. 9. *Anal*. Calcd for $C_{24}H_{22}N_4OS$: C, 69.54; H, 5.35; N, 13.52. Found: C, 69.63; H, 5.48; N, 13.68.

Biology. ***Reagents and chemicals.*** Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from electric eel, 1000 unit), BChE (E.C. 3.1.1.8, from equine serum), acetylthiocholine iodide (ATCI), and 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich. Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, and sodium hydrogen carbonate were purchased from Fluka. We followed our previous procedure for the evaluation of AChEI and BChEI activity based on Ellman's method [17]. For this purpose, a solution of compound **6** in a mixture of DMSO (5 mL) and methanol (5 mL) was diluted in 0.1*M* KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to obtain final assay concentrations. All tests were conducted at 25°C, and four different concentrations were evaluated for each compound in triplicate to obtain the range of 20–80% inhibition for AChE. *In vitro* anti-AChE activity was performed using a 96-well plate reader (BioTek ELx808). Each well included 50 μL potassium phosphate buffer (KH_2PO_4/K_2HPO_4, 0.1*M*, pH 8), 25 μL sample dissolved in 50% methanol and 50% DMSO and 25 μL enzyme (final concentration 0.22 U/mL in buffer). They were pre-incubated for 15 min at room temperature, and 125 μL DTNB (3 m*M* in buffer) was added to each plate. Characterization of the hydrolysis of ATCI catalyzed by AChE was performed spectrometrically at 405 nm followed by the addition of substrate (ATCI 3 m*M* in water). The change in absorbance was measured at 405 nm after 15 min. The IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration vs percent of inhibition). A control experiment was performed under the same conditions without inhibitor and the blank contained buffer, water, DTNB, and substrate. The described method was also used for BChE inhibition assay.

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