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Synthesis of 4-[(diethylamino)methyl]-phenol derivatives as novel cholinesterase inhibitors with selectivity towards butyrylcholinesterase

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ABSTRACT

A series of novel cholinesterase inhibitors, being composed of 4-[(diethylamino)methyl]-phenoxy and secondary amine which were linked with a different length alkyl chain, were designed and synthesized from the starting material *p*-hydroxybenzaldehyde. These compounds were evaluated as acetylcholinesterase and butyrylcholinesterase (AChE/BChE) inhibitors. Compounds **25–31** having a secondary amine moiety connected to the phenyl ring via eight CH_2 units spacer were found to be the most potent inhibitors with IC_{50} value lower than 220 nM and 48 nM against AChE and BChE, respectively. Interestingly, these inhibitors showed a surprising selectively toward BChE, and compounds **26**, **27**, and **30** displayed 12.5, 18.6, and 18.8-fold higher affinity to BChE. The inhibition kinetics analyzed by Linewear–Burk plots revealed that such compounds were mix-type inhibitors.

Alzheimer's disease (AD), the most common form of dementia accounting for about 50–60% of the overall cases of dementia among persons over 65 years of age,[1] is a neurodegenerative alteration characterized by a low acetylcholine (ACh) in hippocampus and cortex.[2] ACh is a neurotransmitter that is hydrolyzed by acetylcholinesterase (AChE, E.C. 3.1.1.7) and butyrylcholinesterase (BChE, E.C. 3.1.1.8).[3,4] Recently, the genesis of amyloid protein plaques was associated with some alterations of both ChEs (AChE and BChE), given that by using ChE inhibitors (ChEI) such plaques decreased considerably in patients with AD.[5,6] Beside this, AChE activity decreases progressively in certain brain regions from mild to severe stages of AD to reach 10–15% of normal values, while BChE activity is unchanged or even increased by 20%, therefore, a large pool of BChE is available in glia neurons and neuritic plaques. It may not be an advantage for a ChEI to be selective for AChE; on the contrary a good balance between AChE and BChE even with selectivity towards BChE may result in a higher efficacy.[7]

The crystallographic structure of AChE from Torpedo california (TcAChE) has been established recently, enabling a close look at its three-dimensional structure and a better understanding of its mechanism of action.[8,9] Within the structure, it exists (1) a catalytic triad active center (Ser 200, His440, Glu327) in the bottom of a deep and narrow gorge; (2) 14 aromatic residues lined a substantial portion of the surface of the gorge; (3) a peripheral anionic binding site (PAS) of the enzyme at the gorge mouth.

On the basis of the crystallographic structure, recently, a series of *p*-hydroxybenzaldehyde derivatives[10,11] and 4-[(diethylamino)-methyl]phenoxy compounds[12] were designed and evaluated as potent AChE and BChE inhibitors. Further study indicated that these compounds exhibited the potent activity mainly due to its strong interaction with the active site gorge. In addition, lots of investigations also showed that the introduction of amine cation moiety remarkably improved the AChE inhibitory by the electrostatic interaction with the PAS of enzyme.[13–15]

Taking advantage of above information, we focused on the development of more active compounds which are able to interact with the active site gorge and bind the PAS of enzyme (i.e., a dual-site inhibitor). In the present investigation, a series of novel ChE inhibitors was designed by linking a 4-[(diethylamino)methyl]phenoxy (DEAMP) group and secondary amine moiety with a different length alkyl chain using *p*-hydroxybenzaldehyde as starting material. In addition, the size of secondary amine moiety and the length of the alkyl chain were explored systematically.

The synthetic routes of compounds **3–38** were outlined in Scheme 1. The *p*-hydroxybenzaldehyde was treated with ω-dibromoalkanes in the presence of K_2CO_3 to afford ω-bromoalkoxy derivatives **1a–f**.[16,17] Compounds **1a–f** reacted with diethyl amine in 1,2-dichloroethane (DCE), the resulting product was reduced with $NaBH(OAc)_3$ to obtain the DEAMP ω-bromoalkyl derivatives **2a–f**.[18,19] Compounds **2a–f** reacted with the selected secondary amines to give the final compounds **3–38**.[20] The final compounds were treated with 3 N HCl/Et_2OH to obtain their hydrochloride salts. All compounds synthesized were characterized by chemical and spectral methods.

To determine the potential interest of compounds **3–38** for the treatment of AD, their anticholinesterase activities were assayed

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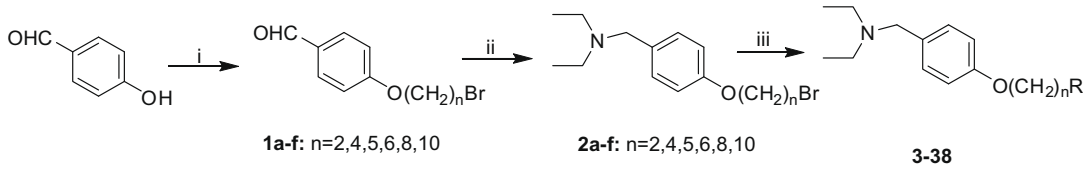
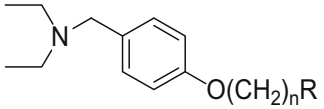


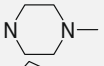
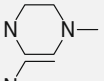
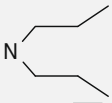
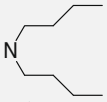
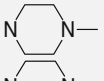
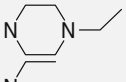
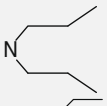
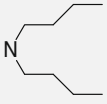
Scheme 1. Synthesis of compounds **3**–**38**. Reagents and conditions: (i) $Br(CH_2)_nBr$, K_2CO_3 reflux, 24 h; (ii) diethyl amine, $NaBH(OAc)_3$, DCE, rt 1.5–4 h; (iii) the corresponding secondary amine, KI, anhydrous ethanol, reflux 5–15 h.

according to Ellmann et al.[21] against freshly prepared AChE from *Electrophorus electricus* and horse BChE from plasma using galanthamine-HBr as reference compounds.[22] Table 1 summaries the data comparing AChE and BChE inhibition as well as the selectivity for BChE inhibitory activities from IC_{50} values for the new 4-[(diethylamino)methyl]phenol derivatives.

As shown in Table 1, all the compounds were active for both AChE and BChE inhibition. The variations of the alkyl chain length (*n* in the general formula) significantly affected ChE inhibitory potency and the selectivity. Remarkably, the behavior of these compounds is rather similar for AChE and BChE inhibition up to a chain length of six methylene units, while it dramatically diverges

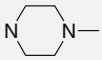
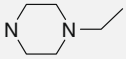
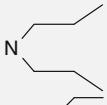
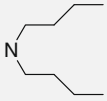
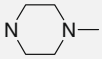
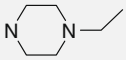
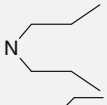
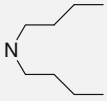
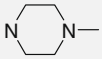
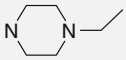
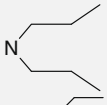
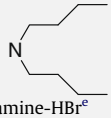
Table 1
Chemical structure and inhibitory activity against isolated AChE[a] and BChE[b] of compounds **3**–**38**, respectively, and resulting selectivities expressed as the ratio of IC_{50} values



Compd	*n*	R	Yield[d] (%)	ChE inhibition IC_{50} (μM)		Selectivity (AChE/BchE)
				AChE[c]	BChE[c]	
3	2		91	13.68	10.10	1.35
4	2		85	11.45	20.27	0.56
5	4		82	3.42	1.40	2.44
6	4		92	6.74	0.71	9.40
7	4		88	5.58	9.73	0.57
8	4		86	10.63	2.74	3.87
9	4		83	13.68	11.69	1.17
10	4		82	10.26	2.52	4.01
11	5		80	0.50	1.54	0.32
12	5		92	4.31	0.67	6.44
13	5		86	2.48	9.35	0.27
14	5		83	1.76	3.08	0.57
15	5		85	8.37	4.42	1.89
16	5		83	5.59	1.78	3.13
17	5		84	2.46	1.90	1.29

(*continued on next page*)

Table 1 (*continued*)

Compd	*n*	R	Yield[d] (%)	ChE inhibition IC_{50} (μM)		Selectivity (AChE/BchE)
				AChE[c]	BChE[c]	
18	6		83	0.44	1.28	0.34
19	6		96	0.89	1.37	0.65
20	6		88	1.82	3.76	0.48
21	6		85	1.62	2.20	0.74
22	6		89	0.96	1.66	0.58
23	6		83	2.16	1.34	1.61
24	6		84	0.67	0.23	2.92
25	8		82	0.084	0.015	5.42
26	8		94	0.092	0.0073	12.50
27	8		89	0.21	0.011	18.60
28	8		84	0.077	0.014	5.38
29	8		90	0.14	0.048	2.99
30	8		87	0.17	0.0091	18.82
31	8		85	0.14	0.017	8.45
32	10		88	0.30	0.068	4.41
33	10		95	0.60	0.078	7.66
34	10		91	0.54	0.033	16.48
35	10		89	0.58	0.19	3.10
36	10		90	0.31	0.027	11.29
37	10		89	0.34	0.026	13.07
38	10		86	0.26	0.015	16.54
		Galanthamine-HBr[e]		0.67	1.52	0.044

[a] AChE, E.C. 3.1.1.7, from electric eel.
[b] BChE, E.C. 3.1.1.8, from horse serum.
[c] IC_{50} values are means of three different experiments.
[d] The yields of the final step.
[e] IC_{50} values of AChE reported in the literature: 0.3–0.8 μM.

from eight to ten carbon units. Compounds **25–31** having an secondary amine moiety connected to the phenyl ring via eight CH_2 unit spacer were found to be the most potent inhibitors with IC_{50} value lower than 0.21 μM and 0.048 μM against AChE and BChE, respectively, suggesting that the optimal distance between 4-[(diethylamino)methyl]-phenoxy function and secondary amine moiety is eight CH_2 units.

We further investigated the inhibitory effect and the selectivity of various secondary amine moieties on AChE and BChE. As shown in Table 1, compounds **25**, **26**, and **28**, bearing the cyclic amine

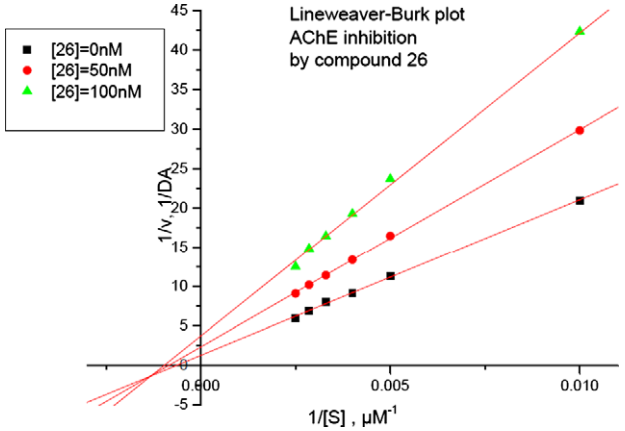


Figure 1. Lineweaver–Burk plots resulting from substrate–velocity curves of AChE activity with different substrate concentrations (100–400 μM) in the absence and presence of compound **26** with concentration of 50 and 100 nM.

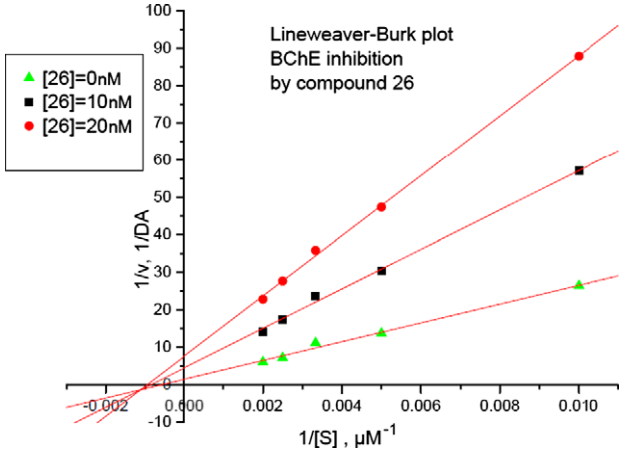


Figure 2. Lineweaver–Burk plots resulting from substrate–velocity curves of BChE activity with different substrate concentrations (100–500 μM) in the absence and presence of compound **26** with concentration of 10 and 20 nM.

groups, showed the highest activities against AChE with IC_{50} value of 0.084, 0.092, and 0.077 μM, respectively. Compounds **26** and **30**, containing piperidine and dipropylamine moiety, respectively, presented the best BChE inhibitory potencies with an IC_{50} value of 0.0073 and 0.0091 μM. It was noted that these inhibitors showed a surprising selectively toward BChE, and compounds **26**, **27**, and **30** displayed 12.50, 18.60, and 18.82-fold higher affinity to BChE.

Compound **26** was selected for kinetic measurements because it showed the highest inhibitory activity against AChE and BChE. The mechanism of inhibition was analyzed by recording substrate–velocity curves in the absence and the presence of compound **26** at different concentrations. Substrate concentration was varied from 100 to 400 μM. For AChE, 50 nM and 100 nM concentrations, respectively, of compound **26** were applied. For BChE, 10 nM and 20 nM concentrations, respectively, of compound **26** were used. Figure 1 showed the Lineweaver–Burk plots, which are reciprocal rates versus reciprocal substrate concentrations for the different inhibitor concentrations resulting from the substrate–velocity curves for AChE. The results showed that the plots of $1/V$ versus 1/[S] gave a family of straight lines with different slopes but they intersected on another in the third quadrant. Similar results were obtained for BChE (Fig. 2). The inhibitory behavior of compound **26**, as deduced from Figure 1, is strictly similar to that of some reported compounds which could bind simultaneously at the catalytic site and at the peripheral anionic site (PAS) of AChE and could be characterized by a linear mixed type of enzyme inhibition.[23] For BChE the results show the same type of inhibition. Therefore, we concluded that compound **26** caused a mixed type of inhibition, that is, compound **26** as a dual-site inhibitor could interact with both active site gorge and PAS of enzyme at the same time.

In conclusion, a series of novel cholinesterase inhibitors, being composed of 4-[(diethylamino)methyl]-phenoxy and secondary amine which were linked with a different length alkyl chain, were reported. These compounds exhibited the expected inhibitory potency against AChE but were additionally found to be very potent inhibitors of BChE. Structure–activity relationships analysis indicated that the optimal distance between 4-[(diethylamino)methyl]-phenoxy function and secondary amine moiety is eight CH_2 units. The inhibition kinetics analyzed by Linewear–Burk plots revealed that such compounds were mix-type inhibitors. All these results suggested that such compounds might be utilized for the development of new candidates for treatment of Alzheimer's disease.

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17. *General procedure for the preparation of compounds* **1a–f**. A stirred suspension of 20 mmol of *p*-hydroxybenzaldehyde, 40 mmol of ω-dibromoalkanes, and 40 mmol of K_2CO_3 in dry acetone was refluxed for 24 h. The reaction was monitored by TLC. The hot reaction mixture was filtered and evaporated to dryness. The residue was purified by chromatography using CH_2Cl_2 as eluent to afford **1a–f** in 60–75% yields. Compound **1e**: yield 70%, colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ, ppm: 9.86 (s, 1H, CHO), 7.82 (d, J = 8.8 Hz, 2H, ArH), 6.99 (d, J = 8.7 Hz, 2H, ArH), 4.04 (t, J = 6.5 Hz, 2H, H1), 3.20 (t, J = 7.0 Hz, 2H, H8), 1.88–1.78 (m, 4H, H7, H2), 1.42–1.30 (m, 8H, H3, H4, H5, H6).
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19. *General procedure for the preparation of compounds* **2a–f**. Compounds **1a–f** (10 mmol) and diethyl amine (10 mmol) were mixed in 1,2-dichloroethane (35 mL) and then treated with sodium triacetoxyborohydride (3.0 g, 14 mmol). The mixture was stirred at rt for 1.5–4 h. The reaction mixture was quenched by adding aqueous saturated $NaHCO_3$, and the product was extracted with EtOAc. The EtOAc extract was dried ($MgSO_4$), and the solvent was evaporated to give **2a–f** in 95–98% yields. Compound **2e**: yield 96%, yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ, ppm: 7.32 (d, J = 8.8 Hz, 2H, ArH), 6.85 (d, J = 8.8 Hz, 2H, ArH), 3.93 (t, J = 6.6 Hz, 2H, H1), 3.72 (s, 2H, CH_2Ph), 3.18 (t, J = 6.6 Hz, 2H, H8), 2.48 (q, J = 7.2 Hz, 4H, NCH_2CH_3), 1.86–1.72 (m, 4H, H7, H2), 1.42–1.30 (m, 8H, H3, H4, H5, H6), 1.01 (t, 6H, J = 7.1 Hz, NCH_2CH_3).
20. *General procedure for the preparation of compounds* **3–38**. A mixture of compounds **2a–f** (2 mmol), KI (3 mmol) and secondary amine (10–20 mmol) in anhydrous ethanol (50 mL) was refluxed for 5–15 h. After completion of the

reaction as indicated by TLC, the solution was cooled and filtered, and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2, and then washed with saturated $NaHCO_3$ and brine, dried with anhydrous Na_2SO_4, and solvent removed in vacuum. The crude product was purified by chromatography using CH_2Cl_2/MeOH/aqueous NH_3 (50:1:0.5) as eluent to afford **3**–**38** in 80–96% yields. Compound **26**: yield 94%, yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ, ppm: 7.17 (d, J = 8.8 Hz, 2H, ArH), 6.80 (d, J = 8.8 Hz, 2H, ArH), 3.90 (t, J = 6.6 Hz, 2H, H1), 3.47 (s, 2H, CH_2Ph), 2.48 (q, J = 6.8 Hz, 4H, NCH_2CH_3), 2.34 (m, 4H, H_{pip}-2,6), 2.24 (t, J = 6.4 Hz, 2H, H8), 1.76–1.69 (m, 2H, H2), 1.60–1.52 (m, 4H, H_{pip}-3,5), 1.46–1.42 (m, 6H, H7, H6, H3), 1.37–1.30 (m, 6H, H_{pip}-4, H5, H4), 1.01 (t, 6H, J = 6.8 Hz, NCH_2CH_3). ^{13}C NMR($CDCl_3$, 75 MHz) δ: 158.12(C_{phenyl}-1), 133.99 (C_{phenyl}-4), 130.31 (C_{phenyl}-3,5), 114.26 (C_{phenyl}-2,6), 68.19 (C1), 59.95 (CH_2Ph), 56.99 (C8), 54.93 (C_{pip}-2,6), 46.70 ($2NCH_2CH_3$), 29.65 (C2), 29.43 (C3), 29.24 (C5), 28.05 (C7), 27.22 (C6), 26.69 (C4), 26.27 (C_{pip}-3,5), 24.82 (C_{pip}-4), 11.95 (2 NCH_2CH_3).

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22. *Assay of the AChE and BChE inhibitory activity*. The assay was performed as described in the following procedure. Five different concentration of each compound were measured at 412 nm for 1 min, each concentration in triplicate. For buffer preparation, 0.1 M dipotassium hydrogen phosphate was adjusted to pH 8.0 with 1 M potassium dihydrogen phosphate. Enzyme solutions were prepared to give 2.5 units/mL in 1.5 mL aliquots. Furthermore, 0.01 M DTNB solution, 0.075 M ATC, and BTC solutions, respectively, were used. A cuvette containing 880 μL of phosphate buffer, 10 μL of the respective enzyme, 50 μL of DTNB, and 20 μL of the test compound solution was allowed to pre-incubate for 15 min at 37 °C, and the reaction was started by addition of 40 μL of the substrate solution (ATC/BTC). The reaction was monitored by measuring the absorbance at 412 nm. For the reference value, 20 μL of DMSO replaced the test compound solution. For determining the blank value, additionally 10 μL of buffer replaced the enzyme solution.
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