Med Chem Res (2016) 25:1831–1841
DOI 10.1007/s00044-016-1626-7

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Hetero-annulated coumarins as new AChE/BuChE inhibitors: synthesis and biological evaluation

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Received: 10 January 2015 / Accepted: 27 June 2016 / Published online: 8 July 2016

Abstract A series of chromene-fused coumarins known as 10,11-dihydrochromeno[4,3-*b*]chromene-6,8(7*H*,9*H*)-diones **4a–o** were synthesized through one-pot reaction of appropriate benzaldehydes, dimedone, and 4-hydroxycoumarin in the presence of nano-silica sulfuric acid under solvent-free condition in good yields. The in vitro anticholinesterase assay revealed that the 3-hydroxyphenyl analog **4e** showed the highest inhibitory activity against both acetylcholinesterase and butyrylcholinesterase, possessing IC_{50} values of 3.28 and 2.19 μM, respectively. The structure-activity relationships study demonstrated that the selectivity for acetylcholinesterase over butyrylcholinesterase could be modulated by introducing second hydroxyl or methoxy substituent on the *para*-position of the 3-hydroxyphenyl pendent group. The docking study of compound **4e** with acetylcholinesterase confirmed π–π stacking interaction between the coumarin moiety and Trp279 as well as the formation of hydrogen bonding between hydroxyl group and Asn85.

Keywords Alzheimer's disease · Acetylcholinesterase · Coumarin-fused derivatives · One-pot synthesis · Multicomponent reactions

Electronic supplementary material The online version of this article (doi:10.1007/s00044-016-1626-7) contains supplementary material, which is available to authorized users.

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Introduction

Acetylcholinesterase (AChE) is a serine hydrolase enzyme mainly found in peripheral and central nervous system. It catalyzes the hydrolysis of the ACh to choline in order to terminate nerve impulses (Čolović et al., 2013). Hence, cholinergic neurotransmission is involved in a variety of pathophysiological conditions; AChE is a known pharmacological target for the treatment of several pathologies particularly Alzheimer's disease (AD) (Orhan et al., 2011). The pathogenesis of AD is related to the deficiency of ACh in the brain and in this respect, AChE inhibitors may be used to treat some symptoms of AD (Hong-Qi et al., 2012). Moreover, AChE inhibitors have been used in the treatment of glaucoma (McKinnon et al., 2008), myasthenia gravis, and neuromuscular blockade (Meriggioli and Sanders, 2009; Bevan et al., 1992).

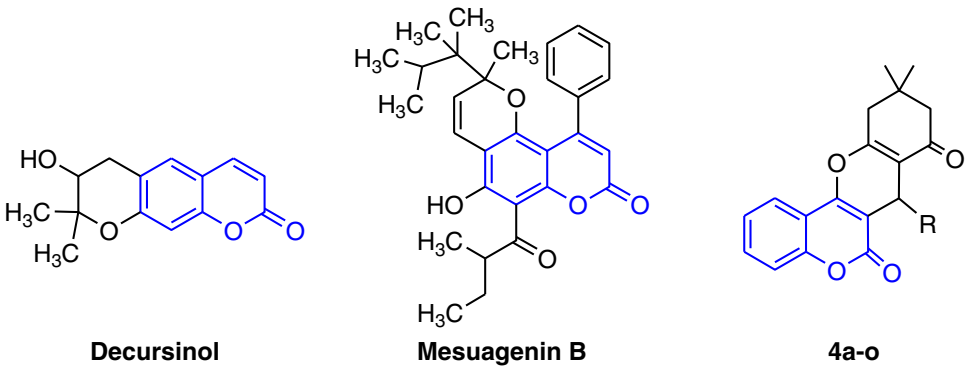


Fig. 1 Structures of hetero-annulated coumarins (decursinol and mesuagenin B) with AChE inhibitory activity and new designed compounds **4a–o**

Besides AChE, butyrylcholinesterase (BuChE) plays a crucial role in ACh hydrolysis, especially when selective anticholinesterases inhibit AChE. However, the neurobiological role of BuChE has not yet been fully understood (Alves-Amaral et al., 2010).

The X-ray crystallographic structures of AChE–ligand complexes revealed that there are two main binding sites in the enzyme: (i) the catalytic binding site, comprising the Ser-His-Glu catalytic triad, and (ii) the peripheral anionic site (PAS), connected by a deep, hydrophobic gorge (Mishra and Sasmal, 2013). Previous studies demonstrated that AChE could also play an important role in increasing amyloid β-peptide (Aβ) plaques deposition in the brain (Hardy and Selkoe, 2002). It seems that AChE binds to Aβ via a pool of amino acids located in the proximity of PAS and promotes amyloid fibril formation (De Ferrari et al., 2001). It has been shown that compounds interacting with PAS or both PAS and catalytic anionic site (CAS) can prevent pro-aggregation activity of AChE toward Aβ (Inestrosa et al., 1996).

Coumarin derivatives are chemically synthesized compounds or naturally occurring phytochemicals with a broad spectrum of bioactivities such as antimicrobial, antifungal, antiviral, anti-HIV-1, anti-cancer, anti-inflammatory, anti-allergic, antiasthmatic, antioxidant, antinociceptive, and hepatoprotective effects (Anand et al., 2012). Previous studies have demonstrated that coumarin derivatives have shown potent cholinesterase inhibitory activity. Moreover, anti-amnestic activity and memory restorative effect of coumarins have been documented in the literature (Wu et al., 2007). The importance of coumarin moiety from both chemical and biological points of view have made coumarins useful and interesting scaffold for drug discovery research (Riveiro et al., 2010). Youkwan et al. (2010) reported that furanocoumarin derivatives found in *Citrus hystrix* fruits acted as AChE inhibitors. Decursinol (Fig. 1), a dihydropyranocoumarin isolated from *Angelica gigas* (Umbelliferae), exhibited potent AChE inhibition ($IC_{50} = 0.28$ μM) (Kang et al., 2001). Also, mesuagenin B (Fig. 1), derived from pyranocoumarin was found to be a potent AChE inhibitor possessing $IC_{50} = 0.7$ μM (Awang et al., 2010).

Recently, we designed and synthesized different series of hetero-annulated comarins namely tetrahydrochromeno[3′,4′:5,6]pyrano[2,3-*b*]quinolin-6(7*H*)-ones and 5-oxo-4,5-dihydropyrano[3,2-*c*]chromene derivatives as AChE and BuChE inhibitors (Khoobi *et al.*, 2013a, b). Herein, in continuation of our research program on the synthesis of bioactive compounds (Mahdavi et al., 2015; Mohammadi-Khanaposhtani et al., 2015a, b; Rahmani-Nezhad et al., 2015; Rayatzadeh et al., 2015), we describe synthesis, anti-cholinesterase activity, and docking study of 10,11-dihydrochromeno[4,3-*b*]chromene-6,8(7*H*,9*H*)-diones **4a–o** (Fig. 1).

Materials and methods

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker FT-400 MHz, using TMS as an internal standard. The infrared spectra were obtained on a Nicolet Magna FTIR 550 spectrophotometer (in KBr). Mass spectra were determined on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. The elemental analysis was performed on an Elementar Analysensystem GmbH VarioEL.

General procedure for the synthesis of chromeno[4,3-*b*] chromenederivatives 4a–o

A mixture of appropriate aldehyde **1** (1 mmol), dimedone **2** (1 mmol, 0.140 g), 4-hydroxycoumarine **3** (1 mmol, 0.162 g), and nano-silica sulfuric acid (nano-SSA) (0.015 g) was heated under solvent free conditions at 100 °C for 2–3 h. After completion of the reaction (monitored by thinb layer chromatography), the mixture was dissolved in hot dichloromethane and was filtered to remove the catalyst. The solvent was evaporated under vacuum and the pure product **4** was obtained by recrystallization from ethanol.

*10,10-Dimethyl-7-phenyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4a**)*

Yellow solid (This compound was prepared by the reaction of benzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as yellow solid); mp 215–216 °C; IR (KBr) ν_{max}2960, 1718, 1666 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.89$ (1H, dd, $J = 8.0$, 1.6 Hz, H_1), 7.58 (1H, td, $J = 8.0$, 1.6 Hz, H_3), 7.41–7.37 (3H, m, Ph), 7.34 (1H, dd, $J = 8.0$, 1.6 Hz, H_4), 7.29–7.25 (2H, m, Ph), 7.16 (1H, td, $J = 8.0$, 1.6 Hz, H_2), 4.99 (1H, s, CH, H_7), 2.74 (1H, d, $J = 18.0$ Hz, $CH_{2b'}$), 2.67 (1H, d, $J = 18.0$ Hz, CH_{2b}), 2.35 (1H, d, $J = 16.0$ Hz, $CH_{2a'}$), 2.28 (1H, d, $J = 16.0$ Hz, CH_{2a}), 1.19 (3H, s, Me), 1.11 (s, 3H, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta =$ 196.0 (C=O, C-8), 161.9 (C=O, C-6), 160.6 (C-O, C-O12), 153.9 (C-O, C11-C-O12), 152.6 (C-O, C-O5), 142.5 (C-1′), 132.2 (C-3′, C-5′), 128.6 (C-3), 128.3 (C-2′, C-6′), 127.1 (C-2), 124.2 (C-4′), 122.4 (C-1), 116.9 (C-4), 115.1 (C, C-C1), 113.7 (C, C7-C-C8), 106.8 (C, C6-C-C7), 50.7 (CH_2), 40.8 (CH_2), 33.4 (C-7), 32.4 (C-10), 29.2 (Me), 27.6 (Me); anal. calcd. for $C_{24}H_{20}O_4$: C, 77.40; H,5.41. Found: C, 77.63; H, 5.57.

*7-(3-Bromophenyl)-10,10-dimethyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4b**)*

Pale yellow solid (This compound was prepared by the reaction of 3-bromobenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 233–235 °C; IR (KB) ν_{max}3063, 2977, 1729, 1663 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.90$ (1H, dd, $J = 8.0$, 1.2 Hz, H_1), 7.61 (1H, t, $J = 8.0$ Hz, H_3), 7.43–7.41 (3H, m, $H_{4'}$, $H_{5'}$, $H_{6'}$), 7.39 (1H, d, $J = 4.0$ Hz, $H_{2'}$), 7.33 (1H, d, $J = 8.0$ Hz, H_4), 7.16 (1H, t, $J = 8.0$ Hz, H_2), 4.95 (1H, s, CH, H_7), 2.71 (1H, d, $J = 17.0$ Hz, $CH_{2b'}$), 2.68 (1H, d, $J = 17.0$ Hz, CH_{2b}), 2.35 (1H, d, $J = 16.5$ Hz, $CH_{2a'}$), 2.30 (1H, d, $J = 16.5$ Hz, CH_{2a}), 1.19 (3H, s, Me), 1.13 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 195.9$ (C=O, C-8), 162.3 (C=O, C-6), 160.5 (C-O, C-O12), 154.1 (C-O, C11-C-O12), 149.9 (C-O, C-O5), 144.7 (C-1′), 132.5 (C-2′), 131.3 (C-5′), 130.3 (C-4′), 129.8 (C-6′), 127.9 (C-2), 124.4 (C-1), 122.5 (C-3), 122.4 (C-3′), 116.9 (C-4), 114.6 (C, C-C1), 113.6 (C, C7-C-C8), 106.1 (C, C6-C-C7), 60.7 (CH_2), 40.8 (CH_2), 33.3 (C-7), 32.4 (C-10), 29.1 (Me), 27.6 (Me); anal. calcd. for $C_{24}H_{19}BrO_4$: C, 63.87; H, 4.24. Found: C, 64.01; H, 4.38.

*7-(3-Fluorophenyl)-10,10-dimethyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4c**)*

Pale yellow solid (This compound was prepared by the reaction of 3-fluorobenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 203-205 °C; IR (KBr) ν_{max}2980, 2870, 1724, 1665 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.87$ (1H, dd, $J = 8.0$, 1.2 Hz, H_1), 7.58 (1H, td, $J = 8.0$, 1.2 Hz, H_3), 7.38–7.33 (2H, m, H_4, $H_{5'}$), 7.23–7.19 (2H, m, H_2, $H_{2'}$), 7.05–7.02 (1H, m, $H_{6'}$), 6.89–6.83 (1H, m, $H_{4'}$), 4.98 (1H, s, CH, H_7), 2.73 (1H, d, $J = 17.6$ Hz, $CH_{2b'}$), 2.65 (1H, d, $J = 17.6$ Hz, CH_{2b}), 2.34 (1H, d, $J = 16.4$ Hz, $CH_{2a'}$), 2.28 (1H, d, $J =$ 16.4 Hz, CH_{2a}), 1.17 (3H, s, Me), 1.10 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 196.0$ (C=O, C-8), 162.7 (d, $J_{C\text{-}F} = 245.0$ Hz, C-3′), 162.2 (C=O, C-6), 160.6 (C-O, C-O12), 154.2 (C-O, C11-C-O12), 152.6 (C-O, C-O5), 145.0 (C-1′), 144.9 (C-5′), 132.5 (C-3), 129.9 (C-2), 129.7 (C-6′), 124.5 (C-1), 124.4 (C-4), 122.5 (C, C-C1), 116.9 (C, C7-C-C8), 115.5 (d, $J_{C\text{-}F} = 21.7$ Hz, C-2′), 114.1 (d, $J_{C\text{-}F} = 20.9$ Hz, C-4′), 106.2 (C, C6-C-C7), 50.7 (CH_2), 40.8 (CH_2), 33.2 (C-7), 32.3 (C-10), 29.1 (Me), 27.6 (Me); anal. calcd. for $C_{24}H_{19}FO_4$: C, 73.84; H, 4.91. Found: C, 74.00; H, 5.09.

*10,10-Dimethyl-7-(3-nitrophenyl)-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4d**)*

White solid (This compound was prepared by the reaction of 3-nitrobenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as white solid); mp 228–230 °C; IR (KBr) ν_{max}2959, 1740, 1682, 1545, 1341 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 8.08$–8.03 (2H, m, $H_{2'}$, $H_{4'}$), 7.94–7.90 (2H, m, H_1, $H_{6'}$), 7.61 (1H, t, $J = 8.0$ Hz, H_3), 7.47 (1H, t, $J = 8.0$ Hz, $H_{5'}$), 7.38 (1H, t, $J = 8.0$ Hz, H_2), 7.35 (1H, d, $J = 8.0$ Hz, H_4), 5.06 (1H, s, CH, H_7), 2.78 (1H, d, $J = 17.6$ Hz, $CH_{2b'}$), 2.70 (1H, d, $J = 17.6$ Hz, CH_{2b}), 2.34 (1H, d, $J = 16.4$ Hz, $CH_{2a'}$), 2.27 (1H, d, $J =$ 16.4 Hz, CH_{2a}), 1.19 (3H, s, Me), 1.10 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 195.0$ (C=O, C-8), 162.8 (C=O, C-6), 160.5 (C-O, C-O12), 154.5 (C-O, C11-C-O12), 152.7 (C-O, C-O5), 148.4 (C-1′), 144.6 (C-3′), 136.7 (2′), 132.8 (C-3), 129.1 (C-2), 124.6 (C-1), 122.8 (C-5′), 122.7 (C6′), 122.3 (C-4′), 117.0 (C-4), 114.1 (C, C-C1), 113.3 (C, C7-C-C8), 105.4 (C, C6-C-C7), 50.6 (CH_2), 40.8 (CH_2), 33.6 (C-7), 32.4 (C-10), 29.1 (Me), 27.5 (Me); EIMS *m/z* 417 $[M]^+$ (33), 400 (100), 370 (27), 295 (98), 239 (50), 211 (14), 165 (5), 139 (15), 92 (8), 76 (8); anal. calcd. for $C_{24}H_{19}NO_6$: C, 69.06; H,4.59; N, 3.36. Found: C, 69.30; H, 4.40; N, 3.22.

*7-(3-Hydroxyphenyl)-10,10-dimethyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4e**)*

Pale yellow solid (This compound was prepared by the reaction of 3-hydroxybenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 202–204 °C; IR (KBr) ν_{max}3441, 2990, 1715, 1665 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 9.29$ (1H, s, OH), 7.93

(1H, d, $J = 8.0$ Hz, H_1), 7.69 (1H, t, $J = 8.0$ Hz, H_3), 7.46–7.43 (2H, m, H_2, H_4), 7.03 (1H, t, $J = 8.0$ Hz, $H_{5'}$), 6.72 (1H, s, $H_{2'}$), 6.66 (1H, d, $J = 8.0$ Hz, $H_{6'}$), 6.55 (1H, d, $J = 8.0$ Hz, $H_{4'}$), 4.63 (1H, s, CH, H_7), 2.75–2.80 (2H, m, CH_2), 2.32 (1H, d, $J = 16.4$ Hz, $CH_{2a'}$), 2.26 (1H, d, $J = 16.4$ Hz, CH_{2a}), 1.10 (3H, s, Me), 1.01 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 196.7$ (C=O, C-8), 163.4 (C=O, C-6), 160.1 (C-O, C-O12), 157.9 (C-O, C11-C-O12), 154.5 (C-3′), 152.8 (C-O, C-O5), 144.9 (C-1′), 133.6 (C-5′), 129.9 (C-3), 125.6 (C-2), 123.4 (C-1), 119.7 (C-6′), 117.4 (C-2′), 116.4 (C-4), 114.8 (C-4′), 114.7 (C, C-C1), 113.9 (C, C7-C-C8), 106.8 (C, C6-C-C7), 50.9 (CH_2), 39.9 (CH_2), 33.6 (C-7), 32.9 (C-10), 29.4 (Me), 27.6 (Me); anal. calcd. for $C_{24}H_{20}O_5$: C, 74.21; H, 5.19. Found: C, 74.48; H, 5.31.

*7-(4-Hydroxyphenyl)-10,10-dimethyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4f**)*

Pale yellow solid (This compound was prepared by the reaction of 4-hydroxybenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 203–204 °C; IR (KBr) ν_{max}3440, 2990, 1715, 1665, 1606, 1362, 1189 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta =$ 7.87 (1H, dd, $J = 8.0$, 1.6 Hz, H_1), 7.56 (1H, t, $J = 8.0$ Hz, H_3), 7.31–7.37 (2H, m, H_2, H_4), 7.19 (2H, d, $J = 8.5$ Hz, $H_{2'}$, $H_{6'}$), 6.63 (2H, d, $J = 8.5$ Hz, $H_{3'}$, $H_{5'}$), 4.88 (1H, s, CH, H_7), 2.72 (1H, d, $J = 18.0$ Hz, $CH_{2b'}$), 2.64 (1H, d, $J =$ 18.0 Hz, CH_{2b}), 2.33 (1H, d, $J = 16.4$ Hz, $CH_{2a'}$), 2.27 (1H, d, $J = 16.4$ Hz, CH_{2a}), 1.16 (3H, s, Me), 1.10 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 196.8$ (C=O, C-8), 162.1 (C=O, C-6), 160.9 (C-O, C-O12), 155.0 (C-O, C11-C-O12), 153.8 (C-4′), 152.5 (C-O, C-O5), 134.4 (C-1′), 132.2 (C-2′, C-6′), 129.7 (C-3), 124.3 (C-2), 122.4 (C-1), 116.9 (C-4), 115.4 (C-3′, C-5′), 115.3 (C, C-C1), 113.7 (C, C7-C-C8), 106.5 (C, C6-C-C7), 50.7 (CH_2), 40.8 (CH_2), 32.5 (C-7), 29.7 (C-10), 29.1 (Me), 27.6 (Me); EIMS *m/z* 388 $[M]^+$ (6), 295 (6), 256 (69), 239 (5), 211 (2), 192 (17), 160 (36), 128 (48), 96 (26), 64 (98); anal. calcd. for $C_{24}H_{20}O_5$: C, 74.21; H, 5.19. Found: C, 74.08; H, 5.05.

*7-(4-Methoxyphenyl)-10,10-dimethyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4g**)*

Pale yellow solid (This compound was prepared by the reaction of 4-methoxybenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 188–190 °C; IR (KBr) ν_{max}3447, 2989, 1728, 1658 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.86$ (1H, dd, $J =$ 8.0, 1.2 Hz, H_1), 7.56 (1H, td, $J = 8.0$, 1.2 Hz, H_3), 7.31–7.31 (2H, m, H_2, H_4), 7.29 (2H, d, $J = 8.5$ Hz, $H_{2'}$, $H_{6'}$), 6.78 (2H, d, $J = 8.5$ Hz, $H_{3'}$, $H_{5'}$), 4.91 (1H, s, CH, H_7), 3.74 (3H, s, OMe), 2.71 (1H, d, $J = 17.6$ Hz, $CH_{2b'}$), 2.64 (1H, d, $J = 17.6$ Hz, CH_{2b}), 2.32 (1H, d, $J = 16.0$ Hz, $CH_{2a'}$), 2.26 (1H, d, $J = 16.0$ Hz, CH_{2a}), 1.16 (3H, s, Me), 1.09 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 196.2$ (C=O, C-8), 161.8 (C=O, C-6), 160.7 (C-O, C-O12), 158.5 (C-4′), 153.7(C-O, C11-C-O12), 152.6 (C-O, C-O5), 134.9 (C-1′), 132.2 (C-2′, C-6′), 129.6 (C-3), 129.3 (C-2), 124.3 (C-1), 122.4 (C-4), 116.9 (C, C-C1), 115.3 (C-4′), 113.7 (C, C7-C-C8), 106.9 (C, C6-C-C7), 55.2 (OCH_3), 50.7 (CH_2), 40.8 (CH_2), 32.5 (C-7), 29.7 (C-10), 29.2 (Me), 27.6 (Me); anal. calcd. for $C_{25}H_{22}O_5$: C, 74.61; H, 5.51. Found: C, 74.48; H, 5.62.

*10,10-Dimethyl-7-(p-tolyl)-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4h**)*

Pale yellow solid (This compound was prepared by the reaction of 4-methylbenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 174–176 °C; IR (KBr) ν_{max}2982, 1730, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.86$ (1H, dd, $J = 8.0$, 1.6 Hz, H_1), 7.56 (1H, t, $J = 8.0$ Hz, H_3), 7.36–7.31 (2H, m, H_2, H_4), 7.25 (2H, d, $J = 8.0$ Hz, $H_{3'}$, $H_{5'}$), 7.05 (2H, d, $J = 8.0$ Hz, $H_{3'}$, $H_{5'}$), 4.92 (1H, s, CH, H_7), 2.72 (1H, d, $J = 17.6$ Hz, $CH_{2b'}$), 2.65 (1H, d, $J = 17.6$ Hz, CH_{2b}), 2.32 (1H, d, J = 16.4 Hz, $CH_{2a'}$), 2.26 (3H, s, Me), 2.22 (1H, d, $J = 16.4$ Hz, CH_{2a}), 1.16 (3H, s, Me), 1.09 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 196.1$ (C=O, C-8), 161.8 (C=O, C-6), 160.6 (C-O, C-O12), 152.6 (C-O, C11-C-O12), 139.6 (C-O, C-O5), 136.6 (C-1′), 132.1 (C-4′), 129.0 (C-2′, C-6′), 128.5 (C-3′, C-5′), 128.2 (C-3), 124.2 (C-2), 122.4 (C-1), 116.9 (C-4), 115.2 (C, C-C1), 113.7 (C, C7-C-C8), 106.9 (C, C6-C-C7), 50.7 (CH_2), 40.8 (CH_2), 32.9 (C-7), 31.4 (Me), 29.1 (C-10), 27.5 (Me), 21.1 (Me); anal. calcd. for $C_{25}H_{22}O_4$: C, 77.70; H, 5.74. Found: C, 77.61; H, 5.55.

*10,10-Dimethyl-7-(4-nitrophenyl)-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4i**)*

Pale yellow solid (This compound was prepared by the reaction of 4-nitrobenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 233–235 °C; IR (KBr) ν_{max}3100, 2957, 1731, 1665, 1657, 1550, 1345 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta =$ 8.12 (2H, d, $J = 8.8$ Hz, $H_{3'}$, $H_{5'}$), 7.91 (1H, dd, $J = 8.0$, 1.6 Hz, H_1), 7.62 (1H, td, $J = 8.0$, 1.6 Hz, H_3), 7.57 (2H, d, $J =$ 8.8 Hz, $H_{2'}$, $H_{6'}$), 7.41 (1H, t, $J = 8.0$ Hz, H_2), 7.35 (1H, dd, $J = 8.0$, 0.8 Hz, H_4), 5.06 (1H, s, CH, H_7), 2.79–2.69 (2H, m, CH_2), 2.36 (1H, d, $J = 16.0$ Hz, $CH_{2a'}$), 2.28 (1H, d, $J =$ 16.0 Hz, CH_{2a}), 1.20 (3H, s, Me), 1.10 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 195.9$ (C=O, C-8), 162.6 (C=O, C-6), 160.4 (C-O, C-O12), 154.5 (C-O, C11-C-O12), 152.7 (C-O, C-O5), 149.7 (C-1′), 146.8 (C-4′), 132.8 (C-3), 129.7 (C-2′, C-6′), 124.6 (C-2), 123.6 (C-3′, C-5′),

122.6 (C-1), 117.3 (C-4), 114.1 (C, C-C1), 113.2 (C, C7-C-C8), 105.3 (C, C6-C-C7), 50.5 (CH_2), 40.8 (CH_2), 33.7 (C-7), 32.4 (C-10), 29.1 (Me), 27.4 (Me); anal. calcd. for $C_{24}H_{19}NO_6$: C, 69.06; H,4.59; N, 3.36. Found: C, 68.87; H, 4.41; N, 3.22.

*7-(4-Fluorophenyl)-10,10-dimethyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4j**)*

Pale yellow solid (This compound was prepared by the reaction of 4-fluorobenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 250–252 °C; IR (KBr) ν_{max}3020, 2921, 1730, 1674 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.87$ (1H, dd, $J =$ 8.0, 1.6 Hz, H_1), 7.58 (1H, td, $J = 8.0$, 1.6 Hz, H_3), 7.38–7.32 (4H, m, H_2, H_4, $H_{2'}$, $H_{6'}$), 6.93 (2H, t, $J = 8.5$ Hz, $H_{3'}$, $H_{5'}$), 4.94 (1H, s, CH, H_7), 2.72 (1H, d, $J = 17.6$ Hz, $CH_{2b'}$), 2.66 (1H, d, $J = 17.6$ Hz, CH_{2b}), 2.33 (1H, d, $J =$ 16.4 Hz, $CH_{2a'}$), 2.26 (1H, d, $J = 16.4$ Hz, CH_{2a}), 1.17 (3H, s, Me), 1.09 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta =$ 196.0 (C=O, C-8), 162.9 (d, $J_{C\text{-}F} = 237.2$ Hz, C-4′), 161.9 (C=O, C-6), 153.9 (C-O, C-O12), 152.6 (C-O, C11-C-O12), 138.4 (C-O, C-O5), 132.3 (C-1′), 129.8 (C-3), 130.2 (d, $J_{C\text{-}F} = 8.2$ Hz, C2′, C-6′), 124.3 (C-2), 122.4 (C-1), 116.9 (C-4), 114.9 (C, C-C1), 115.1 (d, $J_{C\text{-}F} = 21.3$ Hz, C-3′, C-5′), 113.5(C, C7-C-C8), 106.5 (C, C6-C-C7), 50.7 (CH_2), 40.8 (CH_2), 32.7 (C-7), 32.4 (C-10), 29.1 (Me), 27.5 (Me); EIMS *m/z* 390 $[M]^{+\cdot}$ (27), 368 (28), 295 (37), 273 (38), 256 (88), 239 (16), 192 (26), 160 (52), 128 (68), 96 (36), 64 (100); anal. calcd. for $C_{24}H_{19}FO_4$: C, 73.84; H,4.91. Found: C, 73.70; H, 5.12.

*7-(2-Chlorophenyl)-10,10-dimethyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4k**)*

Pale yellow solid (This compound was prepared by the reaction of 2-chlorobenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 230–232 °C; IR (KBr) ν_{max}3062, 2978, 1728, 1663 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.89$ (1H, dd, $J =$ 8.0, 1.6 Hz, H_1), 7.59 (1H, td, $J = 8.0$, 1.6 Hz, H_3), 7.55 (1H, dd, $J = 7.0$, 1.6 Hz, $H_{3'}$), 7.39–7.34 (2H, m, H_2, H_4), 7.26 (1H, dd, $J = 7.0$, 1.6 Hz, $H_{6'}$), 7.22 (1H, td, $J = 7.0$, 1.6 Hz, $H_{4'}$), 7.15 (1H, td, $J = 7.0$, 1.6 Hz, $H_{5'}$), 5.24 (1H, s, CH, H_7), 2.71 (1H, d, $J = 17.6$ Hz, $CH_{2b'}$), 2.66 (1H, d, $J =$ 17.6 Hz, CH_{2b}), 2.33 (1H, d, $J = 16.4$ Hz, $CH_{2a'}$), 2.27 (1H, d, $J = 16.4$ Hz, CH_{2a}), 1.19 (3H, s, Me), 1.12 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 196.2$ (C=O, C-8), 162.7 (C=O, C-6), 160.4 (C-O, C-O12), 154.6 (C-O, C11-C-O12), 152.7 (C-O, C-O5), 139.5 (C-1′), 138.4 (C-2′), 138.3 (C-3′), 133.4 (C-3), 132.4 (C-4′), 130.3 (C-5′), 128.5 (C6′), 126.6 (C-2), 124.3 (C1), 122.6 (C-4), 116.9 (C, C-C1), 113.4 (C, C7-C-C8), 104.6 (C, C6-C-C7), 50.7 (CH_2), 40.8 (CH_2), 33.3 (C-7), 32.2 (C-10), 29.2 (Me), 27.5 (Me); EIMS *m/z* 408 $[M+2]^+$ (2), 406 $[M]^+$ (6), 388 (52), 371 (100), 349 (16), 295 (76), 239 (45), 211 (15), 139 (13), 121 (13), 65 (11); anal. calcd. for $C_{24}H_{19}ClO_4$: C, 70.85; H,4.71. Found: C, 70.72; H, 4.58.

*7-(2,4-Dichlorophenyl)-10,10-dimethyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4l**)*

Pale yellow solid (This compound was prepared by the reaction of 2,4-dichlorobenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 207–208 °C; IR (KBr) ν_{max}2983, 1729, 1668 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.89$ (1H, dd, $J = 8.0$, 1.2 Hz, H_1), 7.84 (1H, d, $J = 8.0$ Hz, $H_{5'}$), 7.60 (1H, td, $J = 8.0$, 1.2 Hz, H_3), 7.46 (1H, d, $J = 4.4$ Hz, $H_{3'}$), 7.39–7.29 (3H, m, H_2, H_4, $H_{6'}$), 5.83 (1H, s, CH, H_7), 2.71 (1H, d, $J = 17.6$ Hz, $CH_{2b'}$), 2.64 (1H, d, $J = 17.6$ Hz, CH_{2b}), 2.30 (1H, d, $J =$ 16.0 Hz, $CH_{2a'}$), 2.23 (1H, d, $J = 16.0$ Hz, CH_{2a}), 1.16 (3H, s, Me), 1.07 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 196.2$ (C=O, C-8), 162.9 (C=O, C-6), 160.4 (C-O, C-O12), 154.4 (C-O, C11-C-O12), 152.7 (C-O, C-O5), 147.3 (C-1′), 145.7 (C-2′), 134.7 (C-4′), 134.4 (C-6′), 132.7 (C-3′), 127.1 (C-3), 124.5 (C-5′), 122.6 (C-2), 116.9 (C-1), 114.5 (C-4), 114.1 (C, C-C1), 113.3 (C, C7-C-C8), 105.3 (C, C6-C-C7), 50.6 (CH_2), 40.8 (CH_2), 32.2 (C-7), 31.7 (C-10), 29.1 (Me), 27.4 (Me); anal. calcd. for $C_{24}H_{18}Cl_2O_4$: C, 65.32; H,4.11. Found: C, 65.10; H, 4.28.

*7-(3,4-Dihydroxyphenyl)-10,10-dimethyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione (**4m**)*

Pale yellow solid (This compound was prepared by the reaction of 3,4-dihydroxybenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 232–234 °C; IR (KBr) ν_{max}3443, 2989, 1715, 1665 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 8.84$ (1H, s, OH), 8.74 (1H, s, OH), 7.93 (1H, d, $J = 8.0$ Hz, H_1), 7.69 (1H, t, $J =$ 8.0 Hz, H_3), 7.45–7.49 (2H, m, H_2, H_4), 6.68 (1H, d, $J =$ 1.6 Hz, $H_{2'}$), 6.57 (1H, d, $J = 8.4$ Hz, $H_{5'}$), 6.47 (1H, dd, $J =$ 8.4, 1.6 Hz, $H_{6'}$), 4.54 (1H, s, CH, H_7), 2.74 (2H, s, CH_2), 2.34 (1H, d, $J = 16.4$ Hz, $CH_{2a'}$), 2.19 (1H, d, $J = 16.4$ Hz, CH_{2a}), 1.10 (3H, s, Me), 1.01 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 195.9$ (C=O, C-8), 162.1 (C=O, C-6), 159.9 (C-O, C-O12), 153.2 (C-O, C11-C-O12), 151.8 (C-O, C-O5), 144.7 (C-3′), 144.1 (C-4′), 133.8 (C-1′), 132.6 (C-3), 124.7 (C-2), 122.4 (C-1), 118.9 (C6′), 116.5 (C2′), 115.9 (C-4), 115.2 (C-5′), 114.2 (C, C-C1), 113.2 (C, C7-C-C8), 106.3 (C, C6-C-C7), 50.1 (CH_2), 40.1 (CH_2), 39.6 (C-7), 31.9 (C-10), 28.5 (Me), 26.7 (Me); anal. calcd. for $C_{24}H_{20}O_6$: C, 71.28; H, 4.98. Found: C, 71.37; H, 5.18.

7-(3-Hydroxy-4-methoxyphenyl)-10,10-dimethyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione ***(4n)***

Pale yellow solid (This compound was prepared by the reaction of 3-hydroxybenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 249–251 °C; IR (KBr) ν_{max}3447, 1713, 1665, 1514 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.86$ (1H, dd, $J =$ 7.8, 1.5 Hz, H_1), 7.59 (1H, td, $J = 7.8$, 1.5 Hz, H_3), 7.37–7.32 (2H, m, H_2, H_4), 7.13 (1H, d, $J = 2.0$ Hz, $H_{2'}$), 6.75 (1H, d, $J = 8.4$ Hz, $H_{5'}$), 6.62 (1H, dd, $J = 8.4$, 2.0 Hz, $H_{6'}$), 4.88 (1H, s, CH, H_7), 3.92 (3H, s, OMe), 2.72 (1H, d, $J = 17.0$ Hz, $CH_{2b'}$), 2.65 (1H, d, $J = 17.0$ Hz, CH_{2b}), 2.33 (1H, d, $J = 16.4$ Hz, $CH_{2a'}$), 2.28 (1H, d, $J = 16.4$ Hz, CH_{2a}), 1.17 (3H, s, Me), 1.11 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 196.2$ (C=O, C-8), 161.8 (C=O, C-6), 160.8 (C-O, C-O12), 153.7 (C-O, C11-C-O12), 152.5 (C-O, C-O5), 150.8 (C-4′), 146.1 (C-3′), 144.6 (C-1′), 134.8 (C-3), 132.1 (C-2), 124.3 (C-1), 122.4 (C-6′), 120.2 (C-4), 116.9 (C, C-C1), 115.2 (C-2′), 114.1 (C, C7-C-C8), 112.5 (C-5′), 106.9 (C, C6-C-C7), 55.9 (OCH_3), 50.7 (CH_2), 40.8 (CH_2), 32.8 (C-7), 32.4 (C-10), 29.2 (Me), 27.5 (Me); EIMS *m/z* 418 $[M]^+$ (100), 387 (20), 295 (80), 239 (70), 211 (28), 165 (13), 139 (28), 108 (25), 77 (54); anal. calcd. for $C_{25}H_{22}O_6$: C, 71.76; H, 5.30. Found: C, 71.88; H, 5.49.

7-(3,4-Dimethoxyphenyl)-10,10-dimethyl-7,9,10,11-tetrahydro-6H,8H-chromeno[4,3-b]chromene-6,8-dione ***(4o)***

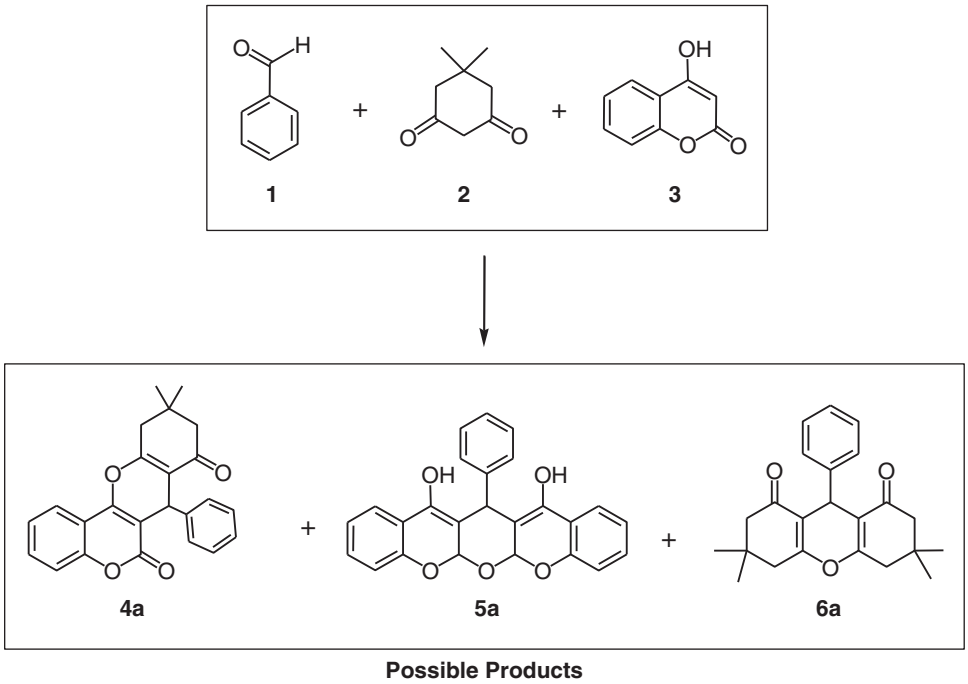
Pale yellow solid (This compound was prepared by the reaction of 3,4-dimethoxybenzaldehyde, dimedone, and 4-hydroxycoumarin. It was obtained as pale yellow solid); mp 181–183 °C; IR (KBr) ν_{max}3448, 2989, 1728, 1658 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.88$ (1H, d, $J = 8.0$ Hz, H_1); 7.59 (1H, t, $J = 8.0$ Hz, H_3), 7.39–7.34 (2H, m, H_2, H_4), 7.06 (1H, s, $H_{2'}$), 6.82 (1H, d, $J = 8.0$ Hz, $H_{5'}$), 6.75 (1H, d, $J = 8.0$ Hz, $H_{6'}$), 4.94 (1H, s, CH, H_7), 3.90 (3H, s, OMe), 3.81 (3H, s, OMe), 2.74 (1H, d, $J = 17.6$ Hz, $CH_{2b'}$), 2.68 (1H, d, $J = 17.6$ Hz, CH_{2b}), 2.36 (1H, d, $J = 16.0$ Hz, $CH_{2a'}$), 2.30 (1H, d, $J = 16.0$ Hz, CH_{2a}), 1.20 (3H, s, Me), 1.14 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 196.2$ (C=O, C-8), 161.9 (C=O, C-6), 160.8 (C-O, C-O12), 153.7 (C-O, C11-C-O12), 152.6 (C-O, C-O5), 148.6 (C-3′), 148.0 (C-4′), 135.4 (C-1′), 132.2 (C-3), 124.3 (C-2), 122.4 (C-1), 120.2 (C-6′), 116.9 (C-4), 115.2 (C, C-C1), 113.7 (C-2′), 112.6 (C, C7-C-C8), 110.9 (C-5′), 106.9 (C, C6-C-C7), 55.9 (OCH_3), 55.8 (OCH_3), 50.7 (CH_2), 40.8 (CH_2), 32.8 (C-7), 32.3 (C-10), 28.5 (Me), 26.7 (Me); anal. calcd. for $C_{26}H_{24}O_6$: C, 72.21; H, 5.59. Found: C, 72.46; H, 5.71.

In vitro inhibition assay

Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from *electric eel*, 1000 unit), butyrylcholinesterase (BuChE, E.C. 3.1.1.8, from equine serum), and butyrylthiocholine iodide were purchased from Sigma-Aldrich. 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide were obtained from Fluka. Briefly, five different concentrations of the compounds were analyzed to obtain 20 to 80 % enzyme inhibition. The assay solution was comprised of 3 mL phosphate buffer (0.1 M, pH = 8), 100 µL DTNB, 50 µL test compound and 50 µL of 5 IU/mL acetylcholinesterase solution. The above mixture was pre-incubated for 10 min accompanied by the addition of enzyme substrate. Blank assays were also carried out comprising all ingredients excluding enzyme to consider the non-enzymatic reaction. The absorbance changes were scored at 412 nm for 6 min and the IC_{50} values were calculated. All experiments were performed in triplicate at 25 °C using a Unico UV spectrophotometer. The same method was used for BuChE inhibition assay (Khoobi *et al.*, 2013a, b).

Docking simulation

Autodock Vina 1.1.2 was used for docking studies (Trott and Olson, 2010). The AChE enzyme complexed with donepezil (1EVE, RCSB Protein Data Bank, http://www.rcsb.org/pdb/home/home.do, 2014) was chosen for docking study. The receptor was prepared according to the following procedure. Initially, the PDB heteroatom including cofactors and bound ligands were manually removed from the coordinate file and then the missing atom types were corrected by Swiss pdb Viewer. Afterwards, the receptor was protonated and the Kollman charge was added using Autodock tools (Sanner, 1999). The two-dimension coordinate of the ligand was prepared by MarvinSketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and then converted to three-dimension using Openbabel (ver. 2.3.1) (O'Boyle et al., 2011). The geometries of the ligands were optimized for 1000 steepest descent steps using Hyperchem 7.5 (Hypercube Inc.). Hydrogen atoms were added to ligand and Gasteiger charges were assigned using Autodock tools and then saved as pdbqt format. Acceptable docking results were obtained by setting the following parameters for Autodock Vina. The grids were centered at the aromatic gorge of the enzyme, with dimensions of 25 Å × 25 Å × 25 Å and a grid spacing of 0.5 Å. The number of calculated models and exhaustiveness were 15 and 80, respectively. Finally, the best conformations in terms of the lowest free energy of binding were chosen for analyzing the interactions with the enzyme in details. All molecular



Scheme 1 One-pot multicomponent reaction of benzaldehyde (1), dimedone (2), and 4-hydroxycoumarin (3)

visualizations were carried out in DS Viewer Pro (Accelrys, Inc., San Diego, CA).

Results and discussion

Chemistry

Currently, the use of reusable heterogeneous catalysts has received considerable attention in organic synthesis due to their environmental, economic, and industrial benefits (Yassaghi et al., 2012). Particularly, the application of silica sulfuric acid (SSA) or nano-silica sulfuric acid (nano-SSA) has been recognized as a stable and efficient heterogeneous catalyst. It has showed excellent activity and selectivity. Also, it can be recovered from a reaction mixture and reused (Emtiazi et al., 2013).

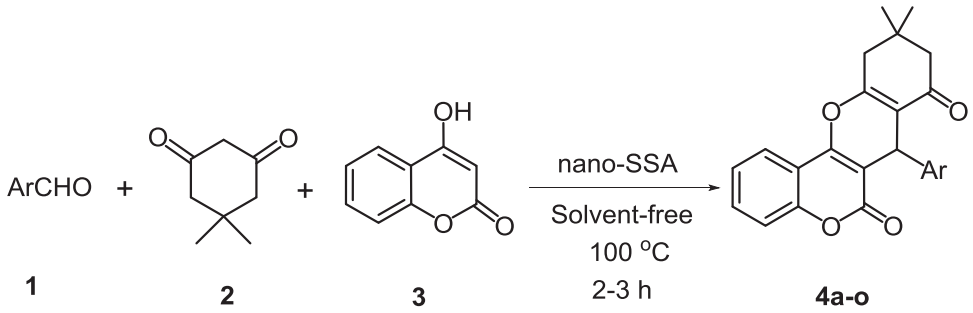
In this work, we report one-pot synthesis of 10,11-dihydrochromeno[4,3-*b*]chromene-6,8(7*H*,9*H*)-diones **4** in the presence of nano-SSA under solvent-free conditions. Primarily, the model reaction was conducted by the reaction of benzaldehyde (**1**), dimedone (**2**), and 4-hydroxycoumarin (**3**) using different solvents such as DMF, THF, H_2O, EtOH, $CHCl_3$, and MeCN as well as solvent-free conditions in the presence of different amounts of nano-SSA (Scheme 1). The best result was obtained using 0.015 g of catalyst under solvent-free conditions at 100 °C. It should be noted that a trace amount of **5a** was produced through the reaction which was removed by recrystallization from ethanol leading to the desired product **4a**. Also, it was found that when the reaction was carried out in a solvent, compound **6a** was formed as the main product. Finally, the generality of this reaction was investigated by preparing a wide variety of substituted chromeno[4,3-*b*]chromene derivatives **4** using various aldehydes, 4-hydroxycoumarin and dimedone under the optimized conditions (Table 1). As shown in Table 1, using aldehydes bearing both electron-donating and electron-withdrawing substituents afforded the corresponding products **4a–o** in good to excellent yields.

Cholinesterase inhibitory activity

The inhibitory activity of synthesized compounds **4a–o** was evaluated against AChE and BuChE, in comparison to tacrine as the reference drug. The IC_{50} values of tested compounds were listed in Table 2. Most compounds showed satisfactory anti-AChE activity with IC_{50} values of 3.28–52.6 μM. Compound **4e** followed by compounds **4m** and **4o** were the most potent compounds against AChE (IC_{50}s ≤ 3.67 μM).

The results obtained from IC_{50} values for compounds **4a** and **4b–e** revealed that substituents at 3-position of aryl ring improved the anti-AChE activity. The comparison of 3-substituted derivatives with the corresponding 4-substituted analogs, demonstrated that 3-regioisomers depicted better AChE inhibitory activity. As observed by compound **4c**, fluorine was found as an effective substituent comparing with the bromine and chlorine. Different *para*-substituted counterpart of compound **4a** showed different anti-AChE effects. Although insertion of methyl and chlorine groups

Table 1 Synthesis of compound **4a–o** under solvent-free conditions



Entry	Ar	Product	Yield (%)	Mp (°C)
1	C_6H_5	**4a**	78	215–216
2	3-Br C_6H_4	**4b**	65	233–235
3	3-F C_6H_4	**4c**	61	203–205
4	3-NO_2 C_6H_4	**4d**	64	228–230
5	3-OH C_6H_4	**4e**	62	202–204
6	4-OH C_6H_4	**4f**	60	203–204
7	4-OMe C_6H_4	**4g**	60	188–190
8	4-Me C_6H_4	**4h**	66	174–176
9	4-NO_2 C_6H_4	**4i**	60	233–235
10	4-F C_6H_4	**4j**	75	250–252
11	2-Cl C_6H_4	**4k**	65	230–232
12	2,4-$Cl_2C_6H_3$	**4l**	62	207–208
13	3,4-$(OH)_2C_6H_3$	**4m**	65	232–234
14	3-OH-4-OMeC_6H_3	**4n**	60	249–251
15	3,4-$(OCH_3)_2C_6H_3$	**4o**	62	181–183

diminished the AChE inhibitory activity, hydroxyl, methoxy, and fluorine substituents increased the anti-AChE activity. Comparing 4-hydroxy and 4-methoxy derivatives (**4f** and **4g**) with compounds **4m** and **4n** showed that the introduction of 3-hydroxy group resulted in more potent compounds, respectively. In general, the compounds **4e** and **4m–o** bearing 3-hydroxy or 3-methoxy group showed superior activity against AChE ($IC_{50} < 4.5$ μM). Thus, anti-AChE activity was directly affected by the oxygenated functionality at 3-position of aryl ring.

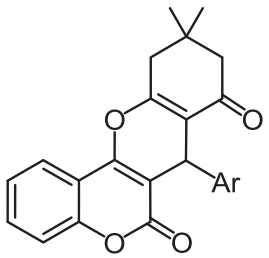
In the terms of anti-BuChE activity, compound **4e** bearing 3-hydroxy group was the most potent compound ($IC_{50} = 2.19$ μM). Besides **4e**, compounds **4b**, **4i**, **4k**, and **4l** with IC_{50} values < 15 μM showed potent activity against BuChE. It is worthwhile mentioning that compounds bearing 3-OH or 3-OMe (**4e** and **4m–o**) showed statistically similar anti-AChE activity whereas their anti-BuChE was very different. Introduction of the second hydroxyl or methoxy group into the *para*-position (compounds **4m** and **4n**) led to the almost same anti-AChE and less anti-BChE activities. It shows that the selectivity against AChE can be regulated through the second substituent on the 3-hydroxyphenyl moiety. On the other hand, compounds **4b**, **4e**, **4f**, **4h**, **4i**, and **4l** showed more selectivity for BuChE over AChE.

Docking study

To determine the plausible enzyme interaction of synthesized compounds, molecular docking studies were performed. All the compounds were docked into the AChE enzyme (1EVE) active site and the analysis of the results showed that they have similar binding modes in the active site. In this regard, the most active compound **4e** was chosen for further analysis. The important residues involved in the interaction of compound **4e** and the type of interactions are summarized in Table 3. Ligand–receptor contacts are based on distance at ≤ 4.5 Å that was calculated by web-based software ContPro (http://procarb.org/contpro/).

As can be seen in Fig. 2, compound **4e** has been fitted into the active site covering the space between CAS and PAS. Making hydrogen bonding between Asn85 and

Table 2 The inhibitory activity (IC_{50}, μM) of synthesized compounds 4a–o against AChE and BuChE



4a-o

Compound	Ar	AChE IC_{50} (μM)	BuChE IC_{50} (μM)	Selectivity for AChE
4a	C_6H_5	52.60	272	5.17
4b	3-Br C_6H_4	20.41	11.8	0.58
4c	3-F C_6H_4	10.03	NA[a]	—
4d	3-NO_2 C_6H_4	13.42	53.64	4
4e	3-OH C_6H_4	3.28	2.19	0.66
4f	4-OH C_6H_4	27.80	20.58	0.74
4g	4-OMe C_6H_4	23.50	128	5.44
4h	4-Me C_6H_4	157	78.80	0.5
4i	4-NO_2 C_6H_4	43.50	10.83	0.25
4j	4-F C_6H_4	28.53	508	17.8
4k	2-Cl C_6H_4	NA	10.65	—
4l	2,4-$Cl_2C_6H_3$	37.32	14.07	0.375
4m	3,4-$(OH)_2C_6H_3$	3.67	35.84	9.76
4n	3-OH-4-OMeC_6H_3	4.49	56.58	12.6
4o	3,4-$(OCH_3)_2C_6H_3$	3.38	NA	—
Tacrine	—	0.250	0.40	1.6

[a] No activity

3-hydroxy enabled the compound to anchor the active site at the vicinity of the CAS. In this orientation, cyclohexenone moiety surrounded by Phe330, Phe331, and Trp84 made hydrophobic interactions. Moreover, carbonyl of cyclohexenone participated in π–π interaction with Phe330. Also, the pyran ring showed H–π interaction with Tyr121. In this position, coumarin moiety binds to Trp279 via π–π stacking interaction enabling compound **4e** to interact with the receptor more tightly. It was worth notifying that both *R* and *S* enantiomers were subjected for docking studies however, *S*-enantiomer showed no eminent interactions with the enzyme active site.

In order to compare the mode of interaction of compound **4e** to that of standard drug tacrine, we also docked tacrine in the active site of AChE using the applied parameters for compound **4e**. As illustrated in Fig. 3, while both of them occupied the same area in the active site, they showed different interactions with the enzyme. Indeed, tacrine made π–π interaction with Tyr334 that is different from compound **4e**.

Table 3 Ligand–enzyme interaction summary;key residues, type of interactions and distances are presented

Residue	Position	Heteroatom	Distance (Å)	Type of interaction
Tyr	70	lig	4.10	hydrophobic
Trp	84	lig	3.50	hydrophobic
Asn	85	lig	3.23	Hydrogen bond
Tyr	121	lig	3.86	H–π
Phe	330	lig	3.65	π–π
Trp	279	lig	4.50	π–π

Conclusions

Based on the importance of coumarin moiety as a pharmacophoric scaffold, we designed a series of chromene-fused coumarins known as 10,11-dihydrochromeno[4,3-*b*]chromene-6,8(7*H*,9*H*)-diones as potent cholinesterase inhibitors. The target compounds were synthesized through one-pot reaction of appropriate benzaldehydes, dimedone,

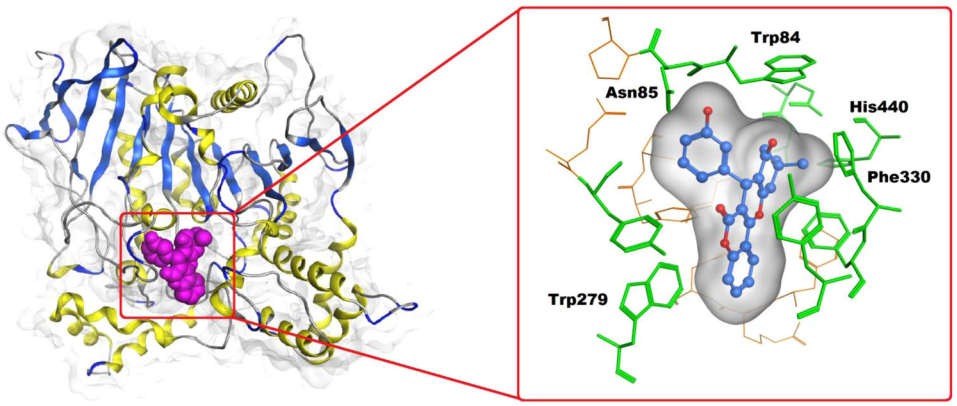


Fig. 2 Binding interaction of best ranked most active compound **4e** in the binding site of AChE

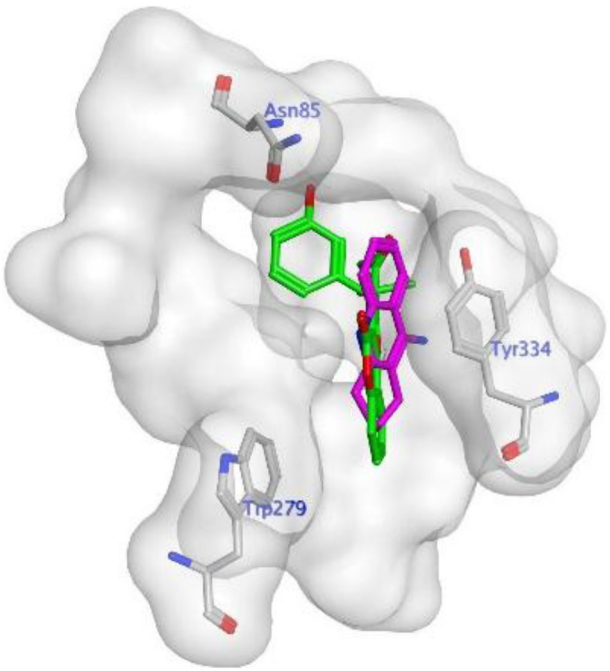


Fig. 3 The binding modes of compound **4e** and tacrine in the active site of AChE. Compound **4e** and tacrine are shown in green and magenta, respectively

and 4-hydroxycoumarin in the presence of nano-silica sulfuric acid under solvent-free conditions in good yields. The in vitro anticholinesterase assay revealed that the 3-hydroxyphenyl analog **4e** had the highest inhibitory activity against both AChE and BuChE. The structure-activity relationships study demonstrated that 3-substituted derivatives were more potent than the corresponding 4-substituted analogs. Furthermore, the results showed that the selectivity for AChE over BuChE could be modulated by introduction of the second hydroxyl or methoxy group into the *para* position of the 3-hydroxyphenyl pendent group in compound **4e**. The docking study of compound **4e** revealed that the coumarin moiety binds to Trp279 via π–π stacking, and a hydrogen bond is formed between hydroxyl group and Asn85. Further studies on the structural requirements and investigation on the other aspects of biological activity of prototype **4e** can introduce new lead compound for anti-AChE activity and potential agent for treating Alzheimer's disease.

Acknowledgments This research was supported by grants from the Research Council of Tehran University of Medical Sciences International Campus (TUMS- IC) and the Iran National Science Foundation (INSF).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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