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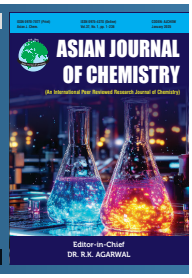

Newer Coumarin-linked Heterocyclic Hybrids: Design, Synthesis and Biological Assessment as Possible Anti-Alzheimer Drugs

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As newer acetyl cholinesterase antagonists which could be effective in Alzheimer's disease management, derivatives of 4-hydroxycoumarin were prepared. The 2-((2-oxo-2*H*-chromen-4-yl)oxy)-*N*-(pyridin-3-yl)acetamide derivative (**4c**) and 2-((2-oxo-2*H*-chromen-4-yl)oxy)-*N*-(pyridin-4-yl)acetamide (**4d**) showed the maximum AChE inhibition effect (IC_{50} = 0.957 ± 0.014 and 1.377 ± 0.018 mM, respectively) among the 16 coumarin-derived compounds evaluated against human acetylcholinesterase (hAChE). PHE 338 and HID 447 are responsible for ligand identification and trafficking by creating a polar π-π interaction with the pyridine ring of *m*-aminopyridine moiety according to the docking research of the most potent molecule **4c**. Furthermore, the stabilization of the ligand in the active site may result in a stronger interdict of the enzyme by the development of a second hydrophobic π-π interaction between the phenyl ring of coumarin moiety and Trp 286 of the peripheral anionic site. In compound **4d**, the coumarin moiety exhibited π-π stacking with amino acids (TYR 341 and TRP 286), hydrophobic interaction with TYR 72, pyridine ring of *p*-aminopyridine showed π-π stacking with TYR 124. The C=O group of coumarin ring formed a hydrogen bond with PHE 295 and the coumarin moiety also formed a hydrophobic interaction with PHE 297 at acyl binding pocket. The findings could be applied to the logical development of formidable and selective AChE inhibitors.

Keywords: Acetylcholinesterase, Coumarins, 4-Hydroxy coumarin, Alzheimer disease.

INTRODUCTION

Several heteroaromatic compounds were synthesized in order to assess their likelihood for managing this escalating disease, especially those that contained nitrogen atoms [1-3] including pyridine, phenothiazine, carbazole, aniline, *etc.* [4-7]. Natural and synthetic coumarin derivatives are reported to exhibited the various potential therapeutics [8], including neuroprotective, antiviral, antimicrobial, antidepressant, antioxidant, antidiabetic, *etc*. [9-13]. It is reported that coumarin showed the potential AChE inhibition activities. The introduction of several synthesized coumarin compounds including ensaculine was caused by the structural alteration of AChE antagonist moieties [14,15] as effective substances to halt the cognitive and progression of Alzheimer's disease (AD) in patients [16]. The fact that coumarins can undergo chemical substitutions at a wide variety of places in this nucleus makes them potentially intriguing building blocks for the synthesis of various physiologically active compounds [17-19]. Moreover, the donepezil-hAChE complex utilizing X-ray crystallography showed that donepezil moieties interact with the two binding sites of AChE, respectively. Possible agents For managing AD, agents who are able to interact with both the binding sites of AChE would be a good choice [20-22].

Memory loss and cognitive impairment are linked to a several variables, including aging, exposure to chemicals, brain injuries and the emergence of neurodegenerative diseases namely Alzheimer, depressive symptoms and delusion [23-25]. The most frequent reason of memory loss and cognitive deterioration associated with dementia, in millions of aged people [26], is thought to be AD, a gradual corticodecadence disorder of the brain [27]. Acetylcholine, dopamine, serotonin and glutamate are neurotransmitters that control cognitive function [28,29]. AD involves the loss neurons in cortex region of the brain, which is responsible for memory and learning process. The US FDA has currently licenced three AChE inhibitors

currently used to counteract AD, *viz.* donepezil [30], galantamine and rivastigmine as well as NMDA antagonist, memantine [31-33]. AChE's enzyme cavity is shaped as about 20 Å deep, thin groove with two binding sites CAS and PAS. At the base of the binding pocket is the catalytic active site (CAS) [34]. Ensaculine (coumarin derivative) is one of them and slows or halt the progression of AD and neurodegeneration. Additionally, Piazzi *et al.* [35] synthesized AP2238, a coumarin analogue, as an inhibitor of acetyl cholinesterase. Despite intensive research in this field, only few drugs are available in the market, which are approved and currently licenced AChE inhibitors to manage manifestations of AD, although these medicaments also do not actively halt the advancement of the illness. The side effects of these medications, including unrestraint urination, weakness and muscle spasms, prevent their usage in more severe phases of the illness. Thus, it is crucial to prepare novel neurotherapeutics that inhibit AChE, prevent Aβ aggregation, hinder the metabolism of ACh and have antioxidant action to slow the progression of disease [35].

We focused our endeavors on synthesizing novel 4-hydroxy coumarin heterocyclic hybrid compounds that might bind with both AChE sites in an attempt to find novel AChEI. To generate the novel compounds that might block AChE, the 4-hydroxy coumarin moiety was connected to a substituted heterocyclic nucleus *via* an alkoxy linkage. The design and synthesis of 4-hydroxy coumarin derivatives (**4a-p**) as novel AChE blockers, as well as their molecular docking investigations and biological assessment, are thus described in the present work.

EXPERIMENTAL

The progress of reaction was monitored by TLC and the melting values were measured. The FT-IR spectrum was recorded using an Alpha ECO-ATR spectrophotometer. ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectra were measured with a Bruker FT-NMR spectrophotometer. The elements were studied with an EXETER CE-440 elemental analyzer. Scopolamine hydrobromide was purchased from Sigma-Aldrich, India. Two-compartment passive avoidance equipment and a Y-Maze test were used in the studies.

General method for synthesis of ethyl 2-((2-oxo-2*H*-chromen-4-yl)oxy)acetate (2): Potassium carbonate (5 mmol) and 4-hydroxycoumarin (5 mmol) were mixed in 5 mL of DMF. The aforementioned solution was agitated for few minutes at room temperature before ethyl 2-bromoacetate (5.2 mmol) was added gradually and heated for 5 h at 90 ºC. The TLC method was done to check the progress of reaction. After filtration, the white solid precipitate was obtained having m.p.: 200-204 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1720 (C=O), 1620 (C=O); ^{1}H NMR (CDCl$_3$-d_6, 500 MHz) δ ppm: 7.72 (d, *J* = 7.5 Hz, 1H), 7.55 (t, *J* = 7.5 Hz, 1H), 7.32-7.23 (m, 2H), 5.85 (s, 1H), 4.86 (s, 2H), 4.12 (m, 2H), 1.29 (s, 3H). ^{13}C NMR (125 MHz, CDCl$_3$-d_6) δ ppm: 87.5, 162.4, 152.5, 116.4, 128.3, 125.4, 123.3, 169.9, 116.2, 152.2, 169.2, 61.3, 14.1 *m/z*: 248 (M+H)$^+$. Anal. calcd. (found) % for $C_{13}H_{12}O_5$ (*m.w.* 248.23): C, 62.90 (61.10); H, 4.87 (4.91); O, 32.23 (33.11).

General method for synthesis of 2-(2-oxo-2*H*-chromen-4-yloxy)acetic acid (3): Compound **2** (2 mmol) was mixed in 5 mL of 1,4-dioxane and then 2 mL of NaOH (5%) was added to the solution dropwise. This was followed by a 2 h reflux. Ethyl acetate was employed to remove the mixture when it had cooled. After the aforementioned reaction mixture was acidified (with 6% HCl), a white solid was obtained having m.p.: 200-210 ºC (Lit. [36] m.p.: 219-220); FT-IR (KBr, ν_{max}, cm^{-1}): 1720 (C=O), 1620 (C=O); ^{1}H NMR (CDCl$_3$-d_6, 500 MHz) δ ppm: 7.72 (d, *J* = 7.5 Hz, 1H, 5H, 7.55 (t, *J* = 7.5 Hz, 1H, 7H), 7.32-7.23 (m, 2H, 6,8H), 5.85 (s, 1H, 3H), 4.86 (s, 2H, CH_2O). *m/z*: 220.04 (100.0%), 221.04 (11.9%), 222.04 (1.0%). Anal. calcd. (found) % for $C_{11}H_8O_5$ (*m.w.* 220.18): C, 60.11; (60.11); H, 3.59 (3.61); O, 36.33 (36.31).

General method for the synthesis of 4-hydroxy coumarin derivatives (4a-p): Compound **3** (1 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 1 mmol) and 1-hydroxybenzotriazole (HBT, 1 mmol) were dissolved in 5 mL of dry acetonitrile. After stirring the mixture for approximately 1 h, substituted amine (1 mmol) was added. A magnetic stirrer was then used to agitate the solution for 30 h. After the completion of reaction, which was TLC-monitored, little water was added in the solution and the precipitate was obtained before being rinsed with a saturated solution of Na_2CO_3. Compounds **4a-p** were obtained by refining the generated compounds using column chromatography.

4-(2-(9*H*-Carbazol-9-yl)-2-oxoethoxy)-2*H*-chromen-2-one (4a): Yield: 85%; m.p.: 405-407 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1700 (C=O), 1650 (C=O); ^{1}H NMR (CDCl$_3$-d_6, 500 MHz) δ ppm: 7.84 (d, *J* = 7.8 Hz, 1H), 7.65 (t, *J* = 7.8 Hz, 1H), 7.25-7.42 (m, 6H), 7.63-8.56 (m, 4H), 5.38 (s, 1H), 5.07 (s, 2H). ^{13}C NMR (CDCl$_3$-d_6, 125 MHz) δ ppm: 169.9, 168.3, 162.4, 152.5, 138.0, 128.3, 125.4, 125.1, 124.3, 123.3, 121.4, 119.8, 116.4, 116.2, 115.6, 87.5, 68.3. Mass: *m/z* 370 (M+H)$^+$. Anal. calcd. (found) % for $C_{23}H_{15}NO_4$ (*m.w.* 369.38): C, 74.79 (74.89); H, 4.09 (4.14); N, 3.81 (3.71); O, 17.33 (17.28).

2-((2-Oxo-2*H*-chromen-4-yl)oxy)-*N*-(pyridin-2-yl) acetamide (4b): Yield: 83%; m.p.: 400-402 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1633 (C=O), 1614 (C=O), 3267 (N-H); ^{1}H NMR (CDCl$_3$-d_6, 500 MHz) δ ppm: 7.84 (d, *J* = 8.0 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.20-7.42 (m, 3H), 8.02-8.45 (m, 3H), 5.38 (s, 1H), 4.77 (s, 2H), 9.15 (s, 1H). ^{13}C NMR (CDCl$_3$-d_6, 125 MHz) δ ppm: 169.9, 169.3, 162.4, 152.5, 151.8, 146.7, 138.7, 128.3, 125.4, 124.4, 123.3, 116.4, 116.2, 115.8, 65.9. Mass: *m/z* 297 (M+H)$^+$. Anal. calcd. (found) % for $C_{16}H_{12}N_2O_4$ (*m.w.* 296.90): C, 64.82 (59.80); H, 3.90 (3.14); N, 9.45 (9.38); O, 21.62 (21.66).

2-((2-Oxo-2*H*-chromen-4-yl)oxy)-*N*-(pyridin-3-yl) acetamide (4c): Yield: 85%; m.p.: 400-402 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1718 (C=O), 1664 (C=O), 3299 (N-H); ^{1}H NMR (CDCl$_3$-d_6, 500 MHz) δ ppm: 7.84 (d, *J* = 7.5 Hz, 1H), 7.65 (t, *J* = 7.5 Hz, 1H), 7.40-7.42 (m, 2H), 7.97-9.36 (m, 4H), 6.25 (s, 1H), 4.42 (s, 2H), 7.23 (s, 1H). ^{13}C NMR (CDCl$_3$-d_6, 125 MHz) δ ppm: 169.9, 169.3, 162.4, 146.2, 143.6, 140.4, 128.3, 125.4, 123.3, 117.8, 116.4, 116.2, 87.5, 65.9. Mass: *m/z* 297 (M+H)$^+$. Anal. calcd. (found) % for $C_{16}H_{12}N_2O_4$ (*m.w.* 296.98): C, 64.90 (64.80); H, 4.11 (4.14); N, 9.48 (9.38); O, 21.60 (21.66).

2-((2-Oxo-2*H*-chromen-4-yl)oxy)-*N*-(pyridin-4-yl) acetamide (4d): Yield: 85%; m.p.: 400-402 ºC; FT-IR (KBr,

ν_{max}, cm^{-1}): 1700 (C=O), 1590 (C=O), 3310 (N-H); ^1H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.84 (d, *J* = 7.7 Hz, 1H), 7.65 (t, *J* = 7.7 Hz, 1H), 7.40-7.42 (m, 2H), 8.41-8.56 (m, 4H), 5.38 (s, 1H), 4.77 (s, 2H), 7.23 (s, 1H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 169.9, 169.3, 162.4, 155.3, 152.5, 150.2, 128.3, 125.4, 123.3, 116.4, 116.2, 109.0, 87.5, 65.9. Mass: *m/z*: 297 $(M+H)^+$. Anal. calcd. (found) % for $C_{16}H_{12}N_2O_4$ (*m.w.* 296.28): C, 64.79 (64.76); H, 4.04 (4.18); N, 9.50 (9.42); O, 21.60 (21.62).

***N*-(3-Chlorophenyl)-2-((2-oxo-2*H*-chromen-4-yl)oxy)-acetamide (4e):** Yield: 82%; m.p.: 382-384 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1700 (C=O), 1600 (C=O), 3302 (N-H); ^1H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.84 (d, *J* = 7.4 Hz, 1H), 7.65 (t, *J* = 7.4 Hz, 1H), 7.99 (m, 1H), 7.23-7.49 (m, 5H), 5.38 (s, 1H), 4.82 (s, 2H), 7.7(1s,1H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 169.9, 169.3, 162.4, 152.5, 139.9, 134.5, 130.0, 128.3, 127.9, 125.4, 123.3, 122.0, 119.7, 116.4, 116.2, 87.5, 65.9. Mass: *m/z* 330 $(M+H)^+$. Anal. calcd. (found) % for $C_{17}H_{12}ClNO_4$ (329.74): C, 60.92 (61.82); H, 3.70 (3.59); Cl, 10.75 (10.85); N, 4.30 (4.29); O, 19.41 (19.47).

4-(2-Oxo-2-(10*H*-phenothiazin-10-yl)ethoxy)-2*H*-chromen-2-one (4f): Yield: 92%; m.p.: 488-490 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1700 (C=O), 1600 (C=O); ^1H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.84 (d, *J* = 7.2 Hz and 1.5 Hz, 1H), 7.65 (t, *J* = 7.2 Hz and 1.5 Hz 1H), 7.60-7.67 (m, 2H), 6.97-7.51 (m, 8H), 5.38 (s, 1H), 4.82 (s, 2H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 169.9, 163.7, 162.4, 152.5, 138.9, 132.9, 128.3, 128.1, 127.2, 126.7, 123.3, 122.2, 116.4, 116.2, 87.5, 63.3. Mass: *m/z* 401 $(M+H)^+$. Anal. calcd. (found) % for $C_{23}H_{15}NO_4S$ (*m.w.* 401.44): C, 68.82 (70.01); H, 3.82 (3.87); N, 3.49 (3.42); O, 15.94 (15.98); S, 7.99 (7.94).

1-(2-((2-Oxo-2*H*-chromen-4-yl)oxy)acetyl)indoline-2,3-dione (4g): Yield: 78%; m.p.: 503-505 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O), 1600 (C=O); ^1H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.84 (d, *J* = 7.7 Hz 1H), 7.65 (t, *J* = 7.2 Hz 1H), 7.38-7.42 (m, 3H), 7.99-8.49 (m, 4H), 5.38 (s, 1H), 4.82 (s, 2H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 184.3, 169.7, 166.2, 162.4, 155.2, 152.5, 148.8, 134.7, 130.0, 129.9, 128.3, 125.7, 125.4, 123.3, 117.7, 116.4, 116.2, 87.5, 63.2. Mass: *m/z* 350 $(M+H)^+$. Anal. calcd. (found) % for $C_{19}H_{11}NO_6$ (*m.w.* 349.30): C, 65.33 (65.33); H, 3.17 (3.16); N, 4.01 (4.00); O, 27.48 (27.50).

***N*-(2,5-Dichlorophenyl)-2-((2-oxo-2*H*-chromen-4-yl)-oxy)acetamide (4h):** Yield: 82%, m.p.: 425-427 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1680(C=O), 1610 (C=O), 3300 (N-H); ^1H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.84 (d, *J* = 7.8 Hz and 1.5 Hz, 1H), 7.65 (t, *J* = 7.8 Hz and 1.5 Hz 1H), 7.42-7.54 (m, 4H), 7.93 (m, 1H), 5.38 (s, 1H), 4.82 (s, 2H), 7.23 (s, 1H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 169.9, 169.3, 162.4, 152.5, 138.7, 132.6, 128.5, 128.3, 125.8, 125.4, 123.4, 123.3, 116.4, 116.2, 87.5, 65.9. Mass: *m/z* 365 $(M+H)^+$. Anal. calcd. (found) % for $C_{17}H_{11}Cl_2NO_4$ (*m.w.* 364.18): C, 56.07 (56.17); H, 3.04 (3.08); Cl, 19.47 (19.59); N, 3.85 (3.79); O, 17.57 (17.52).

***N*-(2,3-Dichlorophenyl)-2-((2-oxo-2*H*-chromen-4-yl)-oxy)acetamide (4i):** Yield: 80%, m.p.: 422-424 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1710 0(C=O), 1600 (C=O), 3299 (N-H); ^1H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.84 (d, *J* = 7.7 Hz, 1H), 7.65 (t, *J* = 7.7 Hz, 1H), 7.25-7.53 (m, 3H), 7.84-7.92 (m, 2H), 5.38 (s, 1H), 4.82 (s, 2H), 7.23 (1s,1H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 169.9, 169.3, 162.4, 152.5, 138.2, 132.6, 131.2, 130.0, 128.4, 128.3, 127.1, 125.4, 123.3, 116.4, 116.2, 87.5, 65.9. Mass: *m/z* 364 $(M+H)^+$. Anal. calcd. (found) % for $C_{17}H_{11}Cl_2NO_4$ (*m.w.* 364.18): C, 56.07 (56.17); H, 2.96 (3.08); Cl, 20.11 (19.58); N, 3.85 (3.79); O, 17.57 (17.51).

***N*-Acetyl-2-((2-oxo-2*H*-chromen-4-yl)oxy)-*N*-phenyl-acetamide (4j):** Yield: 86%, m.p.: 375-379 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1710 (C=O), 1600 (C=O); ^1H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.84 (d, *J* = 8.2 Hz, 1H), 7.65 (t, *J* = 8.2 Hz, 1H), 7.19-7.43 (m, 6H), 7.97-8.21 (m, 2H), 5.48 (s, 1H), 4.47 (s, 2H), 6.25 (s, 1H), 0.23 (s, 1H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 172.1,169.9, 166.2, 152.5, 131.9, 128.9, 128.3, 128.1, 128.0, 125.4, 123.3, 116.4, 116.2, 87.5, 63.2, 26.1. Mass: *m/z* 342 $(M+H)^+$. Anal. calcd. (found) % for $C_{19}H_{15}NO_5$ (*m.w.* 341.33): C, 75.65 (70.60); H, 4.61 (4.56); N, 4.21 (4.13); O, 23.71 (23.73).

Ethyl 1-(2-((2-oxo-2*H*-chromen-4-yl)oxy)acetyl)piperidine-3-carboxylate (4k): Yield: 90%, m.p.: 302-304 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1591 (C=O), 1400 (C=O); ^1H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.55 (d, *J* = 7.5 Hz and 1.5 Hz, 1H), 7.75 (t, *J* = 7.5 Hz and 1.5 Hz 1H), 7.46-7.48 (m, 2H), 2.33-2.86 (m, 5H), 5.37 (s, 1H), 4.21-4.52 (s, 4H), 3.24-3.72 (s, 4H), 1.29 (s, 3H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 173.0, 170.1, 169.9, 162.4, 152.5, 128.3, 125.4, 123.3, 116.4, 116.2, 87.5, 64.1, 61.6, 48.9, 45.2, 43.3, 23.4, 20.2, 14.1. Mass: *m/z* 360 $(M+H)^+$. Anal. calcd. (found) % for $C_{19}H_{21}NO_6$ (*m.w.* 359.14): C, 70.50 (70.40); H, 5.79 (6.10); N, 3.98 (3.92); O, 26.71 (26.74).

1-(2-((2-Oxo-2*H*-chromen-4-yl)oxy)acetyl) piperidine-4-carboxylic acid (4l): Yield: 90%, m.p.: 400-403 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1705 (C=O), 1710 (C=O), 3320 (O-H); ^1H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.58 (d, *J* = 7.5 Hz and 1.5 Hz, 1H), 7.76 (t, *J* = 7.5 Hz and 1.5 Hz 1H), 7.46-7.48 (m, 2H), 1.81-3.83 (m, 9H), 5.36 (s, 1H), 4.44 (s, 2H), 11.0 (s, 1H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 175.0, 170.1, 169.9, 162.4, 152.5, 128.3, 125.4, 123.3, 116.4, 116.2, 64.1, 44.4, 39.9, 28.7. Mass: *m/z* 332 $(M+H)^+$. Anal. calcd. (found) % for $C_{17}H_{17}NO_6$ (*m.w.* 331.32): C, 60.63 (61.54); H, 6.17 (6.27); N, 4.23 (4.10); O, 28.97 (28.91).

4-(2-(3-Methyl-3a,7a-dihydro-1*H*-indol-1-yl)-2-oxoethoxy)-2*H*-chromen-2-one (4m): Yield: 88%, m.p.: 482-485 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1718 (C=O), 1672 (C=O); ^1H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.74 (d, *J* = 7.7 Hz 1H), 7.78 (t, *J* = 7.2 Hz 1H), 7.47-7.52 (m, 2H), 3.45-6.78 (m, 7H), 5.38 (s, 1H), 4.42 (s, 2H), 1.82 (s, 3H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 170.1, 169.9, 162.4, 152.5, 136.1, 134.1, 128.3, 125.4, 123.3, 122.5, 122.4, 116.4, 116.2, 87.5, 64.5, 61.3, 47.2, 34.4, 30.8. Mass: *m/z* 324 $(M+H)^+$. Anal. calcd. (found) % for $C_{19}H_{17}NO_4$ (*m.w.* 323.35): C, 70.58 (69.68); H, 5.30 (5.19); N, 4.33 (4.30); O, 19.79 (19.82).

4-(2-Oxo-2-(2,3,3a,7a-tetrahydro-1*H*-indol-1-yl)-ethoxy)-2*H*-chromen-2-one (4n): Yield: 88%, m.p.: 308-310 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1718 (C=O), 1672 (C=O); ^1H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.74 (d, *J* = 7.7 Hz 1H), 7.78 (t, *J* = 7.2 Hz 1H), 7.47-7.52 (m, 2H), 3.55-6.44 (m, 7H), 5.38 (s, 1H), 4.42 (s, 2H), 1.85 (s, 3H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 169.9, 162.4, 160.4, 152.5, 134.8, 129.8, 128.3,

125.4, 124.1, 123.3, 122.9, 120.9, 116.4, 116.2, 115.7, 87.5, 64.6, 53.7, 46.7, 18.5. Mass *m/z*: 336 $(M+H)^+$. Anal. calcd. (found) % for $C_{20}H_{17}NO_4$ (*m.w.* 335.36): C, 71.63 (69.50); H, 5.11 (5.01); N, 4.18 (4.11); O, 19.08 (19.16).

***N*-(1-Benzylpiperidin-4-yl)-2-((2-oxo-2*H*-chromen-4-yl)oxy)acetamide (4o):** Yield: 88%, m.p.: 450-452 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1724 (C=O), 1710 (C=O), 3308 (N-H); ^{1}H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.54 (d, *J* = 8.4 Hz 1H), 7.61 (t, *J* = 8.4 Hz 1H), 7.47-7.52 (m, 2H), 7.84-7.86 (m, 5H), 5.34 (s, 1H), 4.49 (s, 4H), 7.77 (s, 1H), 3.24-3.72 (s, 5H), 3.75-3.88 (s, 4H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 169.9, 168.3, 162.4, 152.5, 138.6, 128.4, 128.3, 127.2, 125.5, 123.3, 116.4, 116.2, 87.5, 66.6, 64.7, 51.6, 47.9, 30.2. Mass: *m/z* 393 $(M+H)^+$. Anal. calcd. (found) % for $C_{23}H_{24}N_2O_4$ (*m.w.* 392.46): C, 71.10 (69.32); H, 6.20 (5.90); N, 7.20 (6.96); O, 16.31 (16.38).

6-(2-((2-Oxo-2*H*-chromen-4-yl)oxy)acetamido) nicotinic acid (4p): Yield: 82%, m.p.: 585-590 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 1718 (C=O), 1672 (C=O), 3300 (N-H), 3318 (O-H); ^{1}H NMR ($CDCl_3$-d_6, 500 MHz) δ ppm: 7.58 (d, *J* = 8.4 Hz 1H), 7.66 (t, *J* = 8.4 Hz 1H), 7.46-7.52 (m, 2H), 7.84-7.88 (m, 4H), 5.48 (s, 1H), 5.21 (s, 2H), 11.0 (s, 1H). ^{13}C NMR ($CDCl_3$-d_6, 125 MHz) δ ppm: 169.9, 169.3, 166.3, 162.4, 154.8, 152.5, 148.7, 139.9, 128.3, 125.4, 123.3, 118.9, 116.4, 116.2, 115.8, 87.5, 65.9 *m/z*: 341 $(M+H)^+$. Anal. calcd. (found) % for $C_{17}H_{12}N_2O_6$ (*m.w.* 340.29): C, 60.00 (61.10); H, 3.55 (3.50); N, 8.23 (8.33); O, 28.21 (28.14).

Biological activity

ChE inhibition: The inhibitory activity of synthesized compounds on acetylcholinesterase (AChE) was evaluated using a modified Ellman's method [36,37]. In brief, the stock solution of human AChE was dissolved using a 20 mM HEPES buffer solution with a pH of 8 followed by the addition of 0.1% v/v of Triton X-100. Five inhibitor dosages that resulted in inhibition of at least 20-80% were prepared from a 1% v/v DMSO solution. After 10 min of pre-incubation, 340 µM of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and 550 µM of AChE were added to a mixture containing 25 L of either hAChE (0.25 U/mL) and 10 L of test chemical. The purpose of the blank measurements was to take into consideration substrate hydrolysis that occurs without the use of enzymes. A spectrophotometer was used to measure the absorbance at λ = 412 nm for 6 min at 37 ºC. The inhibition percentage was calculated using the formula $[(V_o - V_i)/V_o] \times 100$ to compare response rates with and without inhibitors. where the response rate in the presence and absence of an inhibitor is represented by V_i and V_o [38,39]. The non-linear variable slopes of log (inhibitor) *vs.* normalized response in Graph Pad Prism 5.0 were used to get the IC_{50} value for each test compound. In three separate trials, the assay was performed three times. Using a UV double-beam spectrophotometer, the absorbance change was measured. The graph of log inhibitor concentration *versus* percentage inhibition was used to obtain the IC_{50} values [40-42].

In vivo studies

Animals: The 25-30 g healthy male Swiss albino mice were selected for *in vivo* experiments. They were procured from Animal House at the IAEC of Deshpande Laboratories, Bhopal, India. The mice were housed in packs of six in polyacrylic cages and they were fed a semi-synthetic balanced meal and allowed unlimited access to water. The mice were housed in 12 h light/dark cycles at 23-27 ºC and 55 ± 10% relative humidity. Each behavioural research involved a different animal. The institutional animal ethics committee gave the study protocols its approval (CPCSEA No. 1582/PO/Re/S/11/CPCSEA).

Acute oral toxicity study: According to OECD-423, 2001 recommendations, the oral toxicity of analogues **4c** and **4d** was assessed in adult Swiss albino mice. Animals were given the test substance at different doses of up to 500 mg/kg p.o. and their autonomic and behavioural responses were measured at 0.5 h, 2 h, 4 h and 24 h. Additionally, for 14 days, the animals were watched coma, convulsions, seizures, lacrimation, diarrhoea, lethargy and sleep.

Experimental design: The test compound was dissolved in sodium carboxymethylcellulose (CMC, 0.3%w/v). The behavioural experiments involved seven groups of six mice each, with the following treatments: (a) control; (b) scopolamine hydrobromide (0.5 mg/kg, i.p.); (c) vehicle + scopolamine; (d) donepezil (5 mg/kg, p.o.); (e) test analog **4c/4d** (2.5 mg/kg, p.o.) + scopolamine (f) test analog **4c/4d** (5 mg/kg, p.o.) + scopolamine and (g) test analog **4c** (10 mg/kg, p.o.) + scopolamine. The appropriate group of animals received treatments once every day for 7 days straight. On the 7th day of test, scopolamine hydrobromide was injected intraperitoneally into the mice for 30 min adhering to therapy.

Y-maze test: Instantaneous and short-term working memory in mice are commonly assessed using the three-arm Y-maze equipment. Scopolamine hydrobromide was given to all groups on the 7th day of therapy in an intraperitoneal way, with the exception of the control group, 30 min after the test chemical was given. Each mouse was Permitted exploration of every single arm of the maze while being kept in the middle of it. Over the course of 5 min, the total arm entries and spontaneous changes were recorded. The score of improvement in memory was computed employing the algorithm that follows: % spontaneous alteration rate = [Number of alterations/(total arm entries − 2)] × 100 [43].

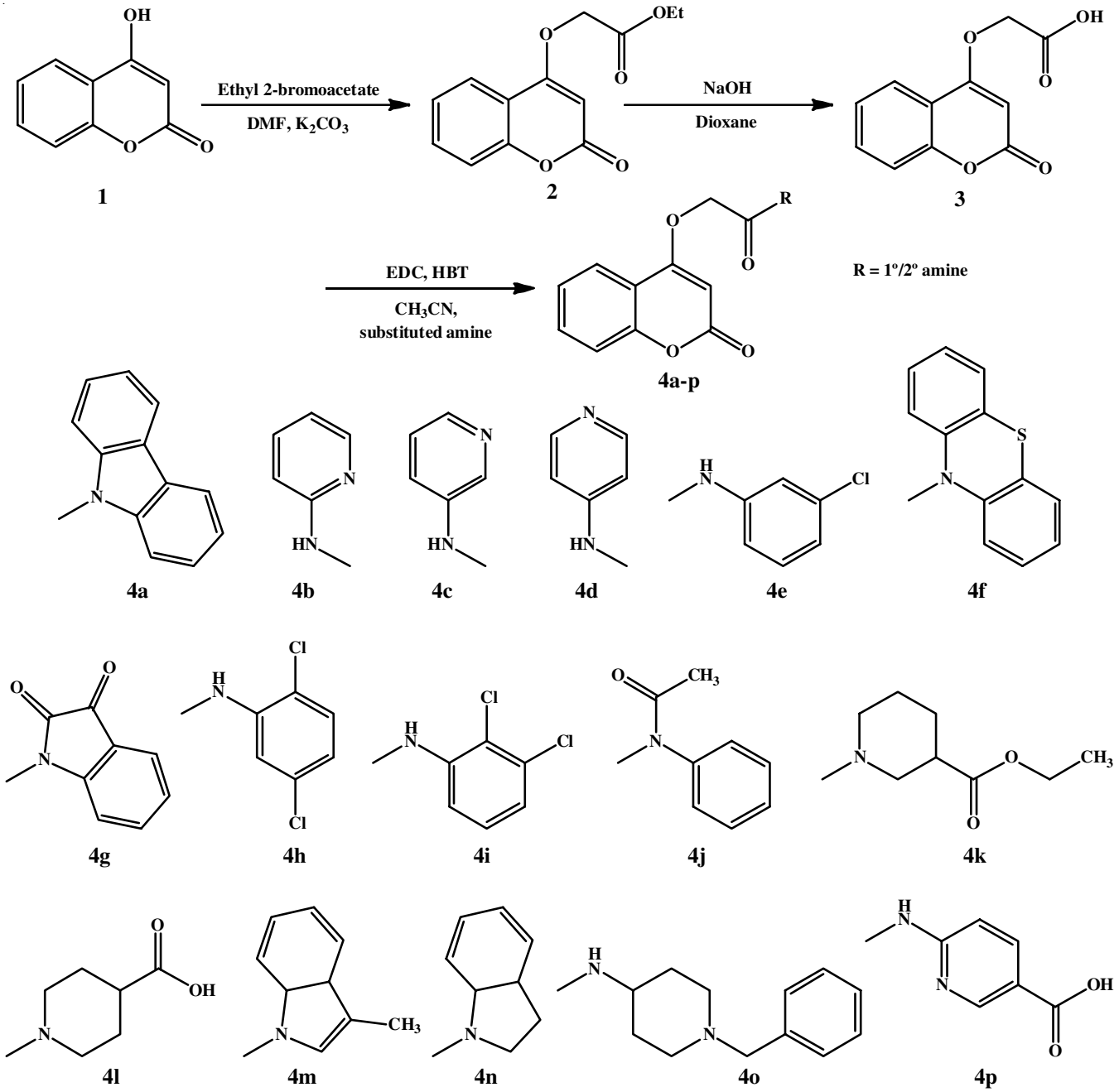
Computational studies

Simulations using *in silico* docking: The interactions between compound **4c/4d** and enzyme active site were studied with *in silico* methods (PDB Code: 4EY7). We generated the crystal structure of the protein using the Schrödinger 2020 module. The side chains and loops that were missing were added using Prime. Following the elimination of all water molecules at neutral pH, protonation states were assigned using Epik. After that the PROPKA approach was used to optimize the design of proteins at neutral, maintaining an RMSD of 0.30 Å for the convergence-heavy atoms during constrained minimization. Using the protein structure that was acquired, a receptor grid was created in order to identify active sites. The stable conformers of donepezil with compounds **4c** and **4d** were generated with the LigPrep module and docked by the Schrödinger

Maestro 2020. The Glide XP Visualizer program was used to analyze the interactions in detail [44].

RESULTS AND DISCUSSION

Scheme-I demonstrates the conversion 4-hydroxycoumarin (**1**) into the desired coumarin derivatives (**4a-p**). Ethyl 2-bromoacetate was O-alkylated with compound **1** using DMF and K_2CO_3 to provide an ethyl ester derivative (**2**). In presence of dioxane and an aqueous NaOH, the resultant ester was then hydrolyzed to provide the corresponding acid **3**. The obtained acid **3** was condensed with substituted amines in the presence of good coupling agents such as EDC and HBT to form the coumarin derivatives **4a-p**. By performing spectroscopic and elemental investigations, the structures of the compounds were verified. The formation of compound **4c** was confirmed as indicated by the emergence of a secondary amide proton peak at δ 7.23 ppm in the ^{1}H NMR spectra. The assessment peaks of the methylene group of spacers (–NHCOCH$_2$-) appeared at δ 4.42 ppm. C-H adjacent to the carbonyl group of α pyrone ring observed a peak at δ 6.25 ppm. The diagnostic peaks of coumarin's benzene ring appeared at δ 7.4-8.2 ppm and the pyridine ring appeared at δ 7.4-9.3 ppm. Compound **4d** shows the methylene group peak at δ 4.77 ppm, C-H adjacent to the carbonyl group of α pyrone ring at δ 5.38 ppm and the pyridine ring at δ 8.4-8.5 ppm. Furthermore, the spectra of compounds **4a-o** indicated the presence of a distinctive methyl group at δ 1.2-2.2 ppm, piperidine ring protons at δ 1.38-3.39, carboxylic group peak at δ 11.0, hydroxyl group peak at δ 11.0, cyclo-



Scheme-I: Synthesis of coumarin-linked heterocyclic hybrids

hexane ring at δ 2.20, acetyl group ring peak at 2.20 ppm. The characteristic hydroxy proton peak at δ 16.77 ppm and the subsequent emergence of (CH_2-CO-N-) signals in the spectra supported the formation of the 4-substituted moieties in compounds **4a-o**. ^{13}C NMR spectra of compounds **4a-o** show signals of secondary amide, α pyrone and carbonyl (–NH–CO–CH_2–) groups at approximately δ 169, 162 and 65 ppm, respectively. Compounds **4c** and **4d** bearing aminopyridine group exhibited diagnostic signals at δ 105-162 ppm. The piperidine carbons of amino group in compounds **4k** and **4o** appeared as signals at δ 20-51 ppm. Furthermore, the spectra of compounds **4a-o** derivatives indicated the presence of a distinctive methyl group peak at δ 14.1 ppm ($COOCH_2CH_3$ in compound **4k**, piperidine carbons peak at δ 28-44 ppm, carboxylic group at nearly 170, cyclohexane ring peak at δ 25-56 ppm. One extra carbonyl peak was observed in compound **4g** around δ 165-184 ppm. The spectral observation of CH_2-CO-N- signals in the compounds **4a-p** validated the synthesis of 4-hydroxy coumarin analogues. Using the elemental analysis, the purity of each intermediate and target chemical was determined.

***In vitro* AChE inhibition activity:** The colorimetric method provided by Ellman's method (Table-1) on human AChE was used to evaluate all of the generated compounds **4i(a-p)**, using donepezil as a reference [37]. All compounds **4a-p** inhibited hAChE (IC_{50} < 3.5). Compound **4c**, which was obtained by a coupling process between *m*-aminopyridine and 2-oxo-2*H*-chromen-4-yloxy acetic acid, demonstrated excellent hAChE inhibitory activity (IC_{50} = 0.957 ± 0.014). Additionally, all of compounds **4a-p** shown considerable exhibition against hAChE with an IC_{50} value < 3.5 μM. With an IC_{50} value of 0.957 ± 0.014 μM, compound **4c**, a synthetic coumarin derivative, showed the greatest AChE inhibitory action in the present investigation. With IC_{50} values of 1.377 ± 0.018 and 1.551 ± 0.011, respectively, compounds **4d** and **4g** demonstrated strong AChE inhibitory efficacy among the other derivatives. Compound **4c** exhibited a fair level of inhibitory efficacy despite having lower efficacies than the reference drug donepezil. Furthermore, it might be produced using a simple and low-cost synthesis. It could therefore be considered a new lead for optimization in the future.

TABLE-2
INHIBITION OF hAChE ENZYME BY COMPOUNDS **4a-p**

Compd.	IC_{50} (μM)	Compd.	IC_{50} (μM)
4a	2.457 ± 0.023	**4j**	2.344 ± 0.021
4b	3.146 ± 0.031	**4k**	3.246 ± 0.031
4c	0.957 ± 0.014	**4l**	3.149 ± 0.031
4d	1.377 ± 0.018	**4m**	3.327 ± 0.019
4e	2.101 ± 0.022	**4n**	3.377 ± 0.019
4f	1.779 ± 0.016	**4o**	3.382 ± 0.019
4g	1.551 ± 0.011	**4p**	3.299 ± 0.019
4h	1.972 ± 0.021	Donepezil	0.014 ± 0.033
4i	2.327 ± 0.019		

Acute oral toxicity study: According to OECD 423 recommendations, 25-30 g Swiss albino mice in good health were used for the toxicity studies of compounds **4c** and **4d**. Monitoring included convulsions, seizures, salivation, diarrhoea, sleep, lacrimal gland secretion and dietary behaviour, among other behavioural changes, cholinergic effects and toxic reactions. After the test compound **4c/4d** was administered, there were no indications of any toxicity, adverse effects or mortality. According to the study, derivative **4c/4d** has a substantial safety buffer zone [45].

***In vivo* behavioural studies:** The typical model in the behavioural studies for assessing the potential of AChEI is scopolamine-induced amnesia in mice. A muscarinic antagonist, scopolamine causes cognitive impairment by depleting cholinergic synapses when administered [46].

Y-maze test: The effectiveness of derivative **4c** in preventing scopolamine-induced cognitive deficits was also analyzed. Scopolamine had a much lower rate (***$p < 0.001$) of spontaneous alternation than the control, according to the results. When compared to scopolamine, treatment with compound **4c** caused a dose-influenced rise (2.5 mg/kg: $^{\#\#}p < 0.01$; 5 and 10 mg/kg: $^{\#\#\#}p < 0.001$) at a spontaneously altered rate (Fig. 1). Moreover, the inconsiderable differences in total arm entries across all groups further demonstrated that the locomotor behaviour of the scopolamine-administered mice remained unchanged (Fig. 2). The effectiveness of derivative **4d** in preventing scopolamine-induced cognitive deficits was tested using the same mamner (Figs. 3 and 4).

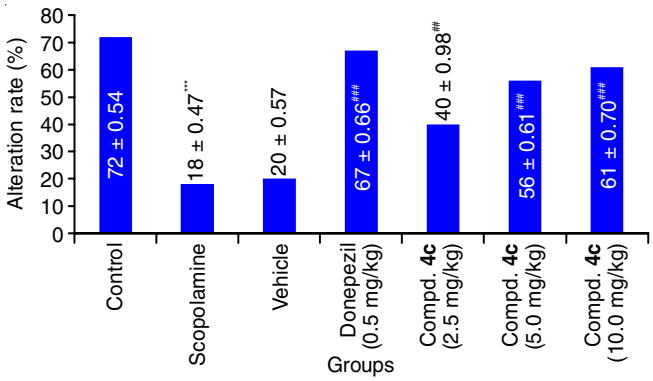


Fig. 1. Compound **4c**, effect on the scopolamine-induced cognitive loss in the Y-maze test: percentage change rate. The bars represent the average ± standard deviation (n = 6); $^{***}p < 0.001$ in comparison to the control; $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$ in comparison to scopolamine

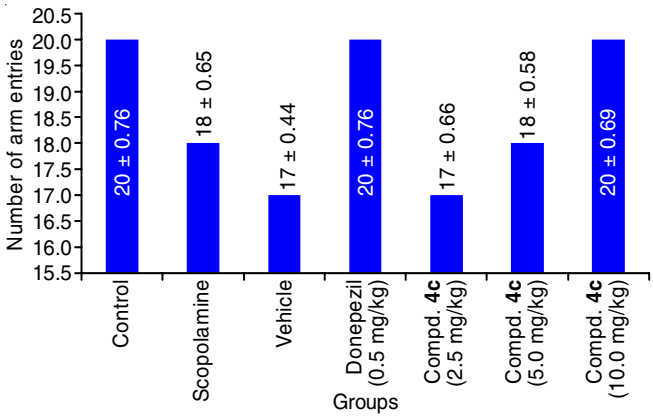


Fig. 2. Compound **4c**, total arm entries and the scopolamine-induced cognitive loss in the Y-maze test. The mean ± standard deviation (n = 6) is represented by bars

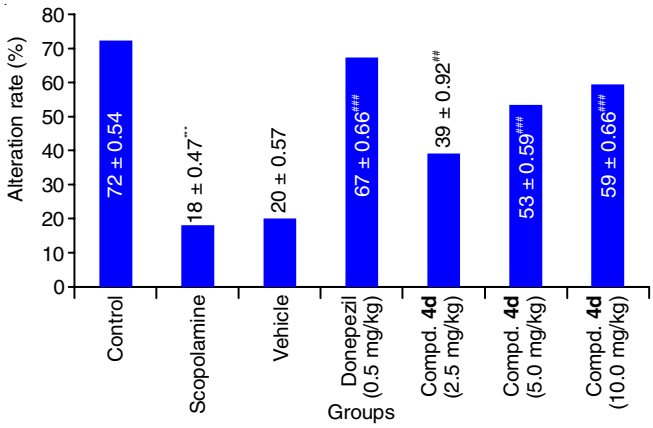


Fig. 3. Compound **4d**, effect on the scopolamine-induced cognitive loss in the Y-maze test: percentage change rate. The bars represent the average ± standard deviation (n = 6); ***$p < 0.001$ in comparison to the control; ###$p < 0.001$, ##$p < 0.01$ in comparison to scopolamine

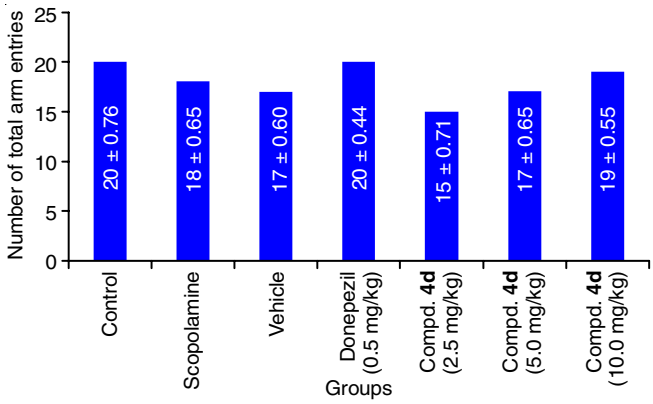


Fig. 4. Compound **4d**, total arm entries and the scopolamine-induced cognitive loss in the Y-maze test. The mean ± standard deviation (n = 6) is represented by bars

Computational studies

Molecular docking study: The compound with good activity, **4c**, was used for study to clarify the inhibitor's mode of interaction. The RCSB PDB was used to obtain human acetylcholinesterase's molecular structure, which was then built using Schrödinger's protein preparation wizard. The molecular docking receptor investigator was selected to be hAChE (PDB code: 4EY7). The co-crystallized aricept ligand was taken out and docked again in the human AChE grid to verify the docking parameters. Docked scores for compound **4c** and donepezil were -10.462 and -12.04, respectively. Fig. 5a-b shows compound **4c** in its ideal docking position and the interactions between the amino acids in the hAchE active site. Over the active site's lower portion, compound **4c** extended to the CAS of hAChE. The pyridine ring formed pi-pi stacking and polar interactions with HID 447; it also showed polar interaction with SER 203 in the CAS. The coumarin moiety exhibited pi-pi stacking with amino acids (TYR 341 and TRP 286), hydrophobic interactions with (TYR 72 and TYR 124) and electrostatic interaction with ASP 74 in the PAS region. The pyridine ring formed pi-pi stacking with PHE 338 and hydrophobic interaction with TYR 337 in anionic subsite. The C=O group of coumarin moiety formed a H-bond with PHE 295 and the coumarin moiety also formed a hydrophobic interaction with PHE 297 at the acyl binding pocket. At the oxyanion site compound **4c** interacted with GLY 121 and GLY 122 and it also formed a hydrophobic interaction with ALA 204 amino acid residue.

The docking of compound **4d** was also done using the same procedure as mentioned above. Docked scores for compound **4d** and donepezil were -10.334 and -12.040, respectively. Fig. 6a-b depicts the interaction between compound **4d** in its ideal docking position and the amino acids in the hAchE active site. The coumarin moiety exhibited pi-pi stacking with amino acids (TYR 341 and TRP 286), hydrophobic interaction with TYR 72, pyridine ring of *p*-aminopyridine showed pi-pi stacking with TYR 124, in addition, compound **4d** showed electrostatic interaction with ASP 74 in PAS region. The oxygen of C=O group of coumarin moiety formed a H-bond with PHE 295 and the coumarin moiety also formed a hydrophobic interaction with PHE 297 at acyl binding pocket. Compound **4d** formed hydrophobic interactions with TYR 337, PHE 338 and TRP 86 in anionic subsite of CAS. Compound **4d** interacted with the GLY 121 at the oxoanionic site.

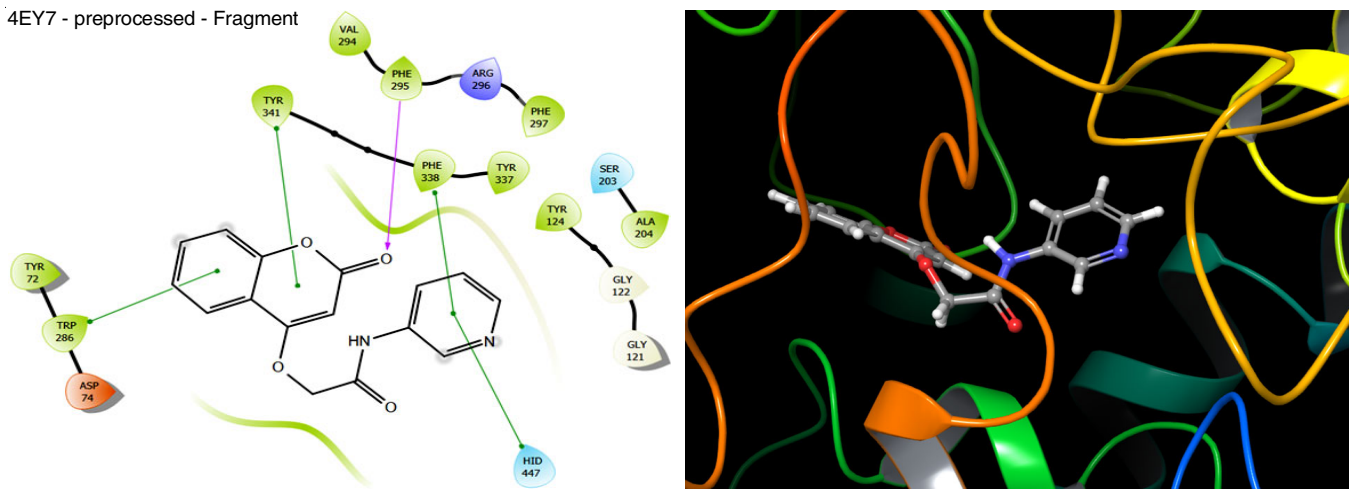


Fig. 5. 2D and 3D image of the active binding site interactions between enzyme and analogue **4c**

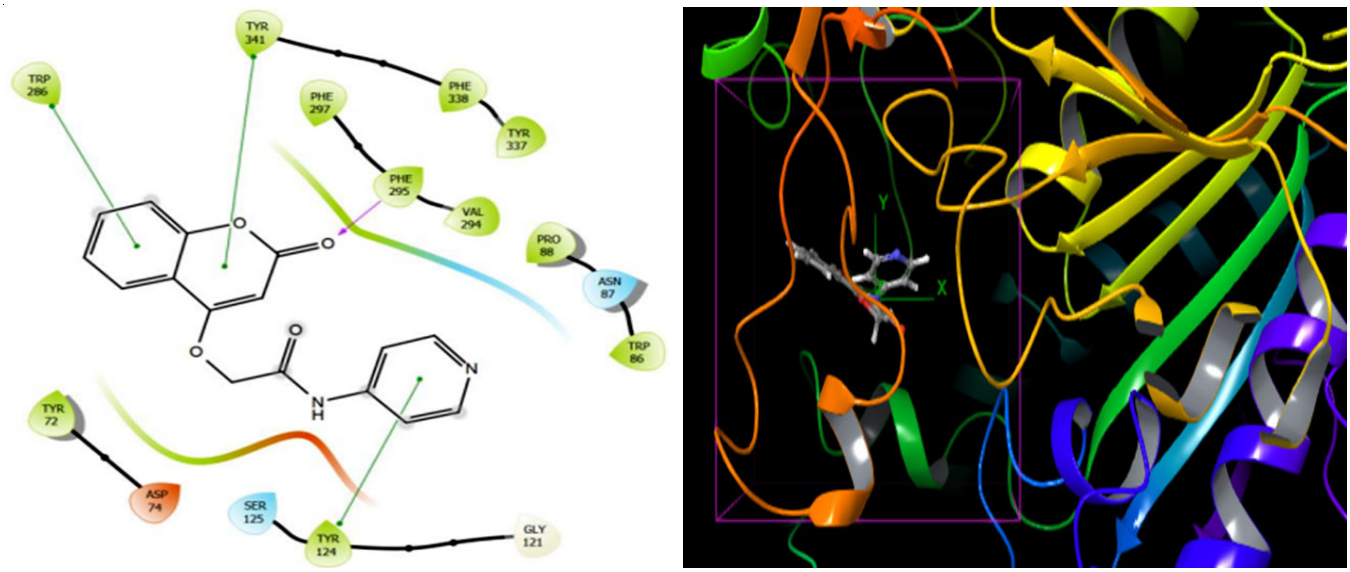


Fig. 6. 2D and 3D image of the active binding site interactions between enzyme and analogue **4d**

Conclusion

In this work, novel 4-hydroxycoumarin amide compounds with possible AChE inhibitory activity were designed and synthesized. We aimed specifically on appropriate amines connected to the coumarin moiety *via* an alkoxy amide bridge in order to interact with the CAS and PAS of hAChE. Among the synthesized derivatives, analog **4c**, which has an attached group of *N*-(pyridin-3-yl)acetamide, exhibited the highest blocking activity ($IC_{50} = 0.957 \pm 0.014$ mM) with a competitive enzyme inhibition. The compound with the highest blocking action ($IC_{50} = 1.377 \pm 0.018$ mM) was compound **4d**, which has an *N*-(pyridine-4-yl)acetamide as the attached group. Research on the SAR, showed that the type of cyclic amine attached to the 2-oxo alkoxy coumarin backbone had an impact on the compounds' anti-AChE activity. *In vivo* behavioural studies employing Y-maze tests revealed that compounds **4c** and **4d** (10 mg/kg) greatly improved the cognitive deficit brought on by scopolamine in mice. Computational studies validated the pharmacological results due to the efficient interaction between compounds **4c**, **4d** and the residues of the PAS active site. Consequently, this study showed that 4-hydroxy coumarin acetamide analogues are promising scaffolds for the treatment of Alzheimer's disease, with compound **4c** being important for future studies focusing on exploring potential Alzheimer's disease therapy possibilities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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