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The anti-Alzheimer potential of some novel Tacrine-Coumarin Derivatives as a Cholinesterase inhibitor

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***Abstract*---**A series of Tacrine-coumarylthiazole derivatives linked through urea were synthesized, and their inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were evaluated for the treatment of Alzheimer's disease. The result revealed that all the synthesized compounds exhibited a moderate inhibitory effect on both cholinesterases. Amongst them, 1-(7-methoxy-1,2,3,4-tetrahydroacridin-9-yl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (SC4, IC50= 37.09 μM) was found to be the most active compound against AChE, and 11-(6,8-dichloro-1,2,3,4-tetrahydroacridin-9-yl)-3-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (SC10, IC50= 5.89 μM) exhibited the strongest inhibition against BuChE. The selectivity of SC4 and SC10 were 0.85 and 0.04, respectively.

***Keywords*---**acetylcholinesterase, butyrylcholinesterase, coumarin, tacrine, urea, thiazole.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia worldwide, with symptoms of memory loss, cognition defect, and behavioral impairment [1]–[5]. The cause of AD is still unclear and has been described for the several reasons, such as genetic factors [6]–[8], amyloid-β peptides aggregation [9], neurofibrillary tangles (NFTs) [10], cholinergic hypofunction, oxidative stress [11], metal ion dyshomeostasis [12], and inflammation [13], etc. Among them, cholinergic hypofunction has been known as one of the significant causes of AD [14]. Acetylcholine (ACh) plays an important role in attention, learning, memory, and motivation. One of the main ways to increase the ACh level in the brain is the inhibition of cholinesterase enzymes

(ChEs). Two ChEs, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), catalyze the hydrolysis of ACh. In the healthy brain, AChE has predominant activity in terminating ACh-mediated neurotransmission, whereas BuChE acts as the secondary enzyme [15], [16]. In patients with AD, a misbalance occurs between AChE and BChE so that the activity of AChE does not change or decrease, whereas BChE activity significantly increases. Therefore, it seems that both enzymes are involved in regulating the ACh level and are considered valuable therapeutic targets against AD. As a result, dual inhibition of AChE and BuChE enzymes would be more useful for AD patients in the late stages of disease than AChE selective inhibitors.

Regarding the X-ray crystallographic structure of AChE from Torpedo California, three main binding sites have been determined: (a) a deep and narrow gorge at the bottom of the catalytic triad of the active site, including Ser200, His440, and Glu327; (b) the catalytic anionic site (CAS) at the vicinity of the catalytic triad consisting of Trp84, Tyr130, Gly199, His441, and His444; (c) peripheral anionic site (PAS) at the gorge rim comprising Tyr70, Asp72, Tyr121, Trp279 and Tyr334 [17]–[25]. AChE inhibitors may inhibit AChE via a competitive mechanism, by interacting with the catalytic active site (CAS) of the enzyme, via a non-competitive mechanism, by binding with the peripheral anionic site (PAS), or via both mechanisms, by exerting a dual binding AChE inhibition [21]. The main difference between them is associated with the acyl binding site (ABS) in such a manner that BChE is significantly bigger than AChE. It seems that stimulatory inhibition of CAS and PAS affords more potent inhibitors.

Inhibition of AChE can be accomplished in three different ways depending on the interaction of the inhibitor with the enzyme binding site Irreversible inhibitors, Pseudo-irreversible inhibitors & Reversible inhibitors from which the last one give a transient non-covalent binding through electrostatic interactions with the active and peripheral sites. The reversible inhibitors may be classified as (a) active-site inhibitors directed toward the catalytic anionic subsite at the bottom of the gorge, (b) peripheral anionic site inhibitors which bind at the entrance to the gorge, or (c) elongated gorge-spanning inhibitors which bridge the two sites [23]. In addition, recent evidence suggested that the PAS of AChE plays a key role in Aβ-aggregation [24], [25]. Also, BChE knockout animal model showed diminished fibrillar Aβ plaque deposition suggesting that the lack of BChE reduces the deposition of fibrillar Aβ in AD [26]. Consequently, a great deal of attention has been paid to developing dual AChE and BChE inhibitors to increase inhibitory activity via increasing the ligand-target interaction.

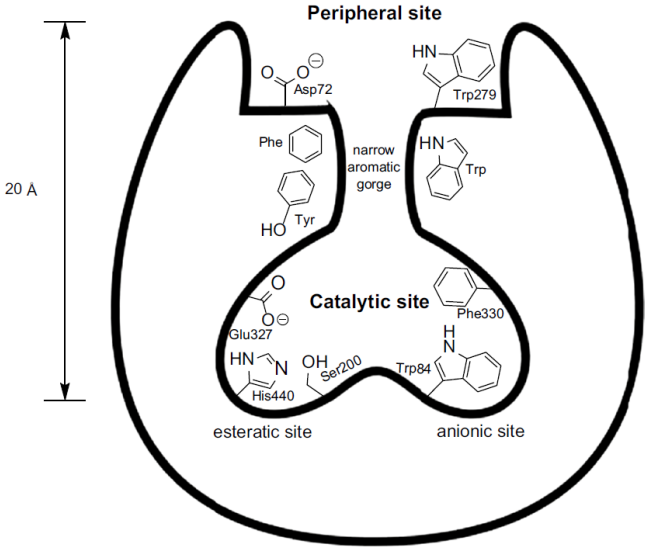


Figure 1. Representation of the active and peripheral site of TcAChE including amino acids residues of catalytic triad, anionic site, narrow aromatic gorge and peripheral site. [27]

Among six drugs that FDA has approved for AD treatment (figure 2), five of them (Donepezil, galantamine, rivastigmine, memantine, and Tacrine) are AChE inhibitors [28]–[33],[34], [35], from which tacrine, was withdrawn from the market in 2013 due to liver toxicity [36]. Moreover, gastrointestinal problems and varied heart rates are common limitations of available therapeutics [37]–[39]. These drugs are aimed to inhibit the acetylcholinesterase at the initial stage of disease, maintaining a balanced acetylcholine level in CNS [40]. With the gradual progression of the disease, the routinely used drugs may not be effective. Therefore, the limitation of the available effective therapeutic agents in the market has attracted life science researchers to investigate the synthesis and development of novel drugs for AD. However, these medicines only confer modest beneficial effects on mild to moderate AD patients. Considering the multifactorial pathogenesis of AD, the design and synthesis of dual-site/multi-site AChE inhibitors simultaneously interact with multiple subsites of the gorge-like pocket. Multifunctional AChE inhibitors targeting AChE, butyrylcholinesterase (BuChE), Aβ aggregation, or β-secretase may provide more effective candidates for AD drug discovery [41]–[44].

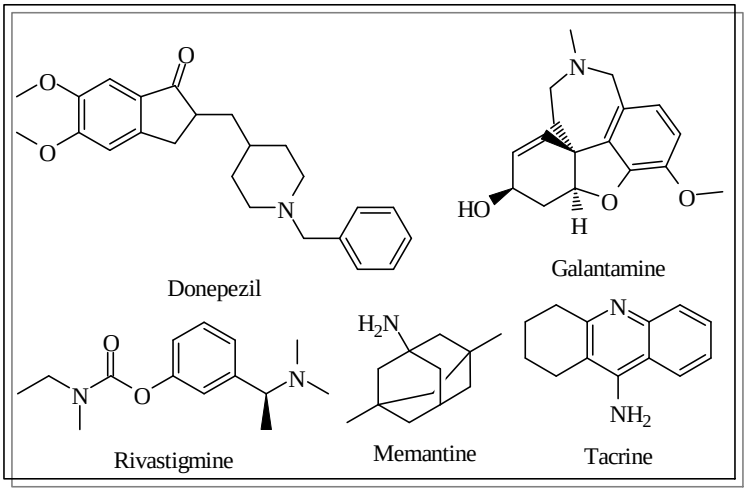


Figure 2. FDA approved drugs

Tacrine is the first AChE inhibitor permitted by the FDA to enter the medical market. After a long period of practically using, Tacrine is still of interest due to its classical pharmacophore for potent AChE inhibition and well-known action mode, except for its toxicity issue. To discover new tacrine derivatives with higher activity, low toxicity, and multiple functions, a variety of hybrids have been synthesized [45], such as Quinolotacrine hybrids [46], tacrine-arylisoxazolyl hybrids [47], Vilazodone-tacrine hybrid [48], tacrine-dipicolylamine dimers [49], tacrine-carbamate derivatives [50], triazole grafted tacrine-chalcone conjugates [51], Tacrine(10)-hupyridone dimer [52], Tacrine-Pyrimidone Hybrids [53], Phenothiazine-Tacrine Heterodimers [54] and many more. Thus, the development of tacrine-based dimers and hybrids with improved pharmacological properties and decreased side effects has been the focus of a great deal of research in recent years [55]–[57].

As a fragrant compound, coumarin widely distributed in nature and displayed extensive biological activities, such as anti-bacterial [58], anti-diabetic and antidepressant [59], antioxidant [60], [61], antimicrobial [62], antifungal [63], anticoagulant [64], anti-inflammatory [65]–[67], anticancer [68], antinociceptive [69], anti-proliferative [70], antitubercular [71], hepatoprotective, anti-allergic, anti-HIV-1, antiviral, antimicrobial and antiasthmatic [72] activities. Moreover, coumarin derivatives are usually easy to synthesize and possess good solubility, low cytotoxicity, and excellent cell permeability. Recent studies have also shown that coumarin analogs exhibit potent AChE activity, which has led to these compounds being seen as potential drugs for treating AD [73], [74]. Accordingly, the coumarin scaffold has been considered to design new AChE inhibitors [75]–[77], and many efforts were addressed to synthesize dual binding site inhibitors of AChE by hybridizing a catalytic site interacting moiety with the coumarin scaffold through an appropriate spacer [78]. Amidic or imidic substituents are key functionalities acting as hydrogen bond donors at the catalytic triad (Ser203-Glu334-His447) of the active site residues of the human acetylcholinesterase by the unpaired electron of N and O atoms [79]–[82].

Structural modifications of the currently used clinical AChE inhibitors resulted in the exploration of the importance of the incorporation of thiazole moieties as essential building blocks for the optimization of AChE inhibitory activity, e.g., acridine−thiazole hybrid [83], benzofuranylthiazole derivatives [84], Coumarylthiazole derivatives [85], benzylpiperidine-linked diarylthiazole [86]. Thiazolo-triazine derivatives are considered efficient acetylcholinesterase inhibitors for the ability to form a hydrogen bond with Tyr124 and T-stacking interaction with Trp286 [87]. Benzofuran and coumarin derivatives bearing thiazole ring and aryl urea/thiourea moieties were synthesized as ChE inhibitors; however, they exhibited moderate inhibitory activities against AChE in our previous studies [84], [85]. Several studies have presented benzofuran or coumarin molecules that interacted with CAS and PAS of AChE via the steric and T-stacking interactions; the H-bonding and T-stacking interactions were determined between thiazole ring or amide moiety and PAS; the phenyl ring of urea moiety interacted with CAS by T-stacking [57], [88]. It has also been reported that the cation–p interactions were observed between N-alkyl chains or heterocyclic moieties and the mid-gorge site of AChE [89], [90]. However, some docking studies showed that the nitrogen atom of the thiazole ring might form a hydrogen bond and interact with active sites of AChE [84], [91].

Based on a literature survey, it has been found that tricyclic and heterocyclic compounds, such as Tacrine, quinolizidine, and coumarin derivatives, can bind both PAS, catalytic anionic site (CAS) of the enzyme by hydrophobic interactions, T-stacking with the aromatic residues of the enzyme gorge in AChE, and incorporation of thiazole moieties as essential building blocks for the optimization of AChE inhibitory activity [22], [23], [92]–[94]. According to the literature, Tacrine has been found as a potent inhibitor of the catalytic anionic site (CAS) of CS, whereas coumarin has an affinity for the PAS of ChEs [75], [95]. Docking into the BuChE gorge, which is larger than that of AChE, showed that heterocyclic rings give (i) a cation-π interaction with Trp82 and Trp430 and also (i) hydrophobic and/or π-π (pi-pi) stacking interactions with Phe118 and Trp231 [81], [96]. The various research groups have synthesized amidic- or imidic-based AChE inhibitors. These studies have reported that a hydrogen bond has been formed between amide moieties of the inhibitors and anionic sites of the enzyme [77], [78]. The urea group was chosen based on its potency of H-bonding, probability of complexation, and wide biological activity [80]–[82], [97]. Even though the tacrine and coumarin functionalities have separately shown considerable efficacy in the treatment of Alzheimer's disease, only a little effort was made to design tacrine-coumarin hybrids as novel multifunctional cholinesterase inhibitors[55], [90], [98]–[105].

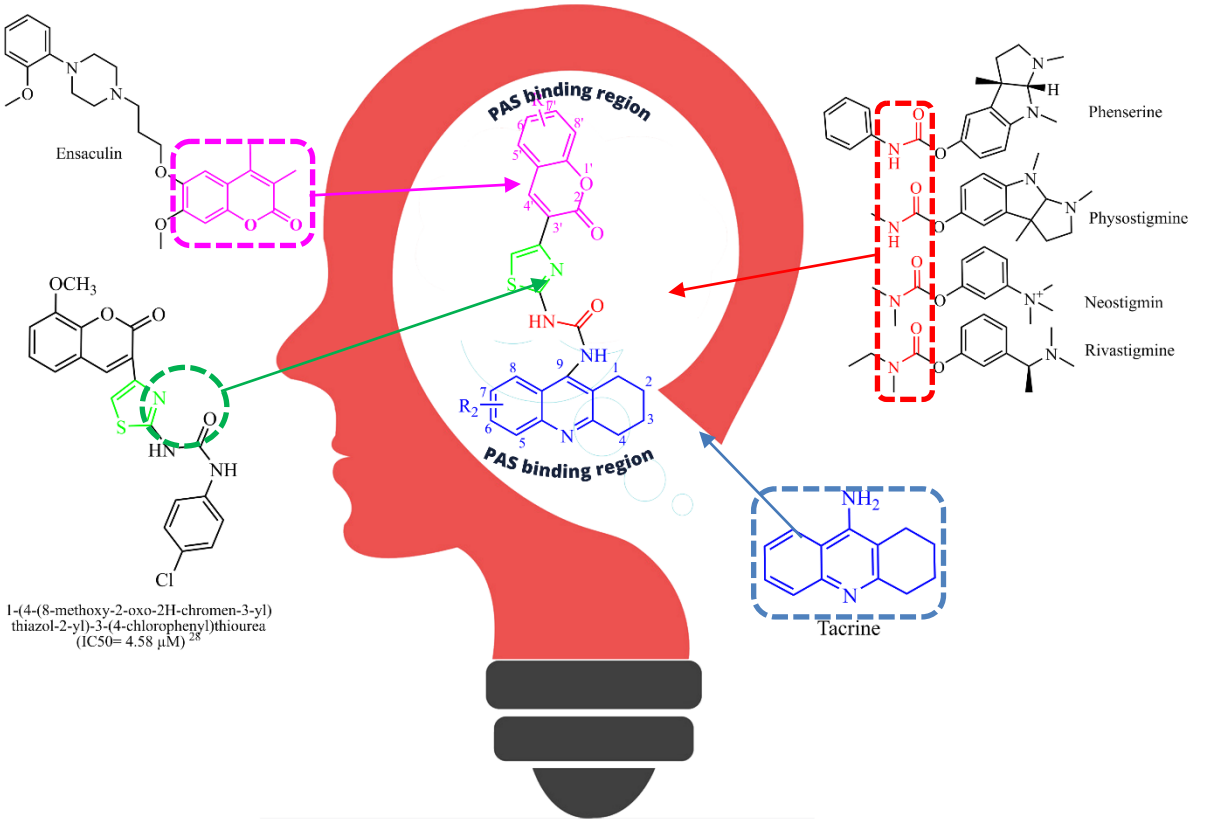


Figure 3. Rational behind designed molecule

Based on the above consideration, we hypothesized (figure 3) a new hybrid molecule based on frameworks of the coumarin modified by adding a thiazole ring. In the design of this new molecule, the coumarylthiazole moiety was substituted with a urea group-containing tacrine ring. We hypothesized that these heterocyclic and aromatic rings might show strong parallel π-π stacking against the CAS of the enzyme. On the other hand, the presence of urea moiety contributes to inhibitor activity by interacting with active sites. This study synthesized 12 novel urea substituted coumarylthiazole derivatives, and their inhibitory effects on AChE and BuChE were evaluated.

Methods

Chemistry

All solvents, reagents, and starting materials were obtained from commercial sources unless otherwise indicated. Melting points were taken on a Bio-Medica, LT-BM-115 (Labtronics, India). Tacrine derivatives were synthesized in a Microwave synthesizer by Catalyst Systems (Pune, India), and IR spectra were registered on a JASCO FT/IR-4100 C spectrometer. 1H and 13C NMR spectra were registered on a BRUKER AVANCE III HD spectrometer at 400 MHz and 75 Hz, respectively. 1H and 13C chemical shifts are referenced to the internal

deuterated solvent. Mass spectra were obtained using a Bruker IMPACT HD spectrometer. The elemental analyses were carried out with a CAP RQ ICP-MS instrument (Thermo Scientific). The Acetylcholinesterase from Electrophorus electricus (electric eel) (AChE, Type-VI-S, EC 3.1.1.7, and horse serum butyrylcholinesterase (BuChE, EC 3.1.1.8) were purchased from Sigma. The chemicals and solvents were purchased from Loba Chemie (Mumbai, India) and Sigma-Aldrich Sigma-Aldrich Chemicals Private Limited (Bangalore, India). Reactions were monitored by thin-layer chromatography (TLC) on TLC Silica gel 60 F_{254} from Sigma-Aldrich Chemicals Pvt Ltd (Bangalore, India) and visualized under CAMAG UV Cabinet 4 (Mumbai, India).

Synthesis procedures and spectral data
3-acetylcoumarin derivatives [I-2(a-c)]

A mixture of benzaldehyde derivatives I-1(a-c) (3 mmol), reactive methylene compound, e.g., methyl acetoacetate or ethyl acetoacetate (3 mmol), and a few drops of piperidine were stirred at room temperature without any solvent. Precipitation occurred immediately. The reaction was monitored by TLC. The compound was then recrystallized from ethanol to get pure crystalline I-2(a-c) in 80-90% yields [106]. Spectral data of these compounds were matched with the literature.

3-(bromoacetyl)coumarin derivatives [I-3(a-c)]

To a solution of 1 mol I-2(a-c) in 20 mL chloroform was added 1 mol of bromine in 5 mL chloroform, with intermittent shaking and warming to decompose an addition product. The mixture was heated at 50 °C for 15 min. in a water bath to expel most of the hydrogen bromide, then cooled and filtered. The solid was washed with ether and recrystallized with acetic acid. I-3(a-c) were obtained in 85%, 75%and 80% yields, respectively. Spectral data of these compounds were matched with the literature [107].

3-(2-amino-1,3-thiazol-4-yl)coumarin derivatives [I-4(a-c)]

Thiourea (5 mmol) was added to the solution of I-3(a-c) (5 mmol) in boiling ethanol (20 mL). The mixture was refluxed for 1 hour, then cooled and neutralized with aqueous ammonia. The precipitate was filtered off, washed with ethanol, and used directly without crystallization or other purification. I-4(a-c) were obtained in 80%, 78%, and 75% yields, respectively. Spectral data of these compounds were matched with the literature [108].

General procedure for tacrine derivatives formation [II-2(a-d)]

The appropriately-substituted starting 2-amino-benzonitrile II-1(a-d) (1.0 eq); Zinc Chloride (2.0 eq); and cyclohexanone (2 ml) were challenged by MW irradiation for 10 min at 150^0C. The resulting solid was diluted with 2M NaOH (3 mL) and dichloromethane (DCM) 2 ml and stirred for 30 min. The solution was diluted by another 2M NaOH (20 ml), washed, collected, dried with anhydrous Na_2SO_4, and filtered three times with DCM (3 X 20 ml). The organic layers were filtrate concentrated. The residue was purified by column chromatography to give the

crude product as a base. The base was dissolved in MeOH (10 mL) and HCl (25% in H_2O; 1.5 mL) and stirred overnight. The solution was concentrated and dried to give a crude hydrochloride product [109].

Synthesis of Tacryl Isocyanates [II-3(a-d)]: Substituted Tacrine's II-2(a-d)

(1 g) was dissolved in 20 mL of DCM. For this solution, 20 mL of NaHCO3 solution was stirred at 0^0C. Stirring was stopped momentarily, triphosgene (0.5 equivalents) dissolved in 1 mL of DCM was added with a syringe to the DCM layer, and stirring continued for one h. The DCM layer was separated and passed through a bed of celite® and immediately subjected to the next reaction [110].

Synthesis of coumarylthiazol urea derivatives [SC1-12]

To a solution of I-4(a-c) (1 mmol) and triethyl amine (1 ml) in dry THF was added isocyanate derivatives II-3(a-d) (1 mmol). The mixture was refluxed for 12 hours with stirring, then cooled and evaporated to dryness. The crude product was washed with chloroform and dried under a vacuum. The products were recrystallized from ethanol over 98% purity. SC1-12 were obtained with 77-99 % yields.

1-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(1,2,3,4-tetrahydroacridin-9-yl)urea (SC1)

Yellow solid, 80% yield, mp. 241-243^{0}C; IR: 3357, 3293, 1683, 1675, 1549, 1505, 1249, 1183, 1114, 788, 691 cm^{-1}; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.63 – 10.60 (m, 1H), 9.06 (s, 1H), 8.47 (s, 1H), 8.09 – 8.02 (m, 1H), 7.93 – 7.85 (m, 1H), 7.72 – 7.67 (m, 1H), 7.64 – 7.59 (m, 1H), 7.54 – 7.48 (m, 2H), 7.36 – 7.31 (m, 1H), 7.31 – 7.27 (m, 2H), 3.14 – 3.05 (m, 2H), 2.84 – 2.77 (m, 2H), 1.91 – 1.82 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.33, 25.07, 32.20, 114.87, 116.47, 116.75, 117.83, 119.80, 120.29, 122.25, 124.65, 125.29, 127.14, 129.29, 130.89, 132.62, 144.13, 144.41, 145.39, 147.07, 153.72, 155.19, 156.85, 161.01, 163.60; LC-MS (m/*z*): 469.13 [MH^+]. Anal. Calcd. for C26H20N4O3S: C, 66.65; H, 4.30; N, 11.96; O, 10.24; S, 6.84; found: C, 66.85; H, 4.56; N, 11.70; S, 6.80.

1-(6,8-dichloro-1,2,3,4-tetrahydroacridin-9-yl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (SC2)

Yellow solid, 85% yield, mp. 245-247^{0}C; IR: 3375, 3298, 1678, 1670, 1554, 1500, 1286, 1250, 1188, 1119, 760, 669 cm-1; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.61 (s, 1H), 8.72 (s, 1H), 8.47 (s, 1H), 7.68 (d, *J* = 2.2 Hz, 1H), 7.61 (dd, *J* = 7.8, 2.0 Hz, 1H), 7.51 (ddd, *J* = 8.9, 7.5, 2.0 Hz, 1H), 7.39 (d, *J* = 2.2 Hz, 1H), 7.36 – 7.31 (m, 1H), 7.31 – 7.27 (m, 2H), 3.14 – 3.05 (m, 2H), 2.84 – 2.77 (m, 2H), 1.91 – 1.82 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.32, 26.50, 32.49, 114.87, 115.53, 116.19, 116.47, 117.83, 119.80, 121.61, 125.29, 125.47, 129.29, 132.62, 132.92, 133.25, 142.35, 144.14, 145.39, 147.32, 153.72, 155.27, 158.24, 161.01, 163.60; LC-MS (m/*z*): 537.06 [MH^+]; Anal. Calcd. For C, 58.11; H, 3.38; Cl, 13.19; N, 10.43; O, 8.93; S, 5.97; found: C, 58.25; H, 3.40; Cl, 13.40; N, 10.12; S, 5.80.

1-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (SC3)

Yellow solid, 82% yield, mp. 243-245^{0}C; IR: 3370, 3293, 1673, 1665, 1549, 1495, 1281, 1245, 1183, 1114, 755, 664 cm^{-1}; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.63 – 10.60 (m, 1H), 9.08 (s, 1H), 8.47 (s, 1H), 8.03 (d, *J* = 9.0 Hz, 1H), 7.72 – 7.65 (m, 1H), 7.64 – 7.59 (m, 1H), 7.54 – 7.48 (m, 1H), 7.38 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.36 – 7.31 (m, 1H), 7.31 – 7.27 (m, 2H), 3.14 – 3.05 (m, 2H), 2.84 – 2.77 (m, 2H), 1.91 – 1.82 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.33, 25.07, 32.34, 114.87, 115.62, 116.47, 117.83, 119.44, 119.80, 123.54, 124.74, 124.94, 125.29, 129.29, 132.62, 137.59, 144.13, 145.11, 145.39, 146.66, 153.72, 155.23, 157.54, 161.01, 163.60; LC-MS (m/z): 503.09 [MH$^+$]; Anal. Calcd. For C, 62.09; H, 3.81; Cl, 7.05; N, 11.14; O, 9.54; S, 6.37; found: C, 62.20; H, 3.78; Cl, 7.17; N, 11.20; S, 6.51.

1-(7-methoxy-1,2,3,4-tetrahydroacridin-9-yl)-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (SC4)

Yellow solid, 78% yield, mp. 232-234 °C; IR: 3355, 3299, 1675, 1670, 1546, 1509, 1295, 1237, 1173, 1040, 1114, 753, 670 cm^{-1}; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.63 – 10.60 (m, 1H), 8.72 (s, 1H), 8.48 – 8.45 (m, 1H), 7.64 – 7.59 (m, 1H), 7.54 – 7.48 (m, 1H), 7.36 – 7.31 (m, 2H), 7.31 – 7.27 (m, 2H), 7.02 (dd, *J* = 8.3, 3.0 Hz, 1H), 3.80 (s, 5H), 3.14 – 3.05 (m, 2H), 2.84 – 2.77 (m, 2H), 1.91 – 1.82 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.36, 25.18, 32.21, 55.67, 104.55, 114.87, 116.47, 116.97, 117.83, 118.74, 119.64, 119.80, 125.29, 125.42, 129.29, 132.62, 142.36, 143.42, 144.14, 145.39, 153.72, 155.20, 156.24, 156.73, 161.01, 163.60; ; LC-MS (m/z): 499.14 [MH$^+$], Anal. Calcd. For C, 65.05; H, 4.45; N, 11.24; O, 12.84; S, 6.43; found: C, 65.17; H, 4.30; N, 11.37; S, 6.37.

1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(1,2,3,4-tetrahydroacridin-9-yl)urea (SC5)

Yellow solid, 61% yield, mp. 230-232 °C; IR: 3370, 3309, 1686, 1675, 1546, 1497, 1295, 1251, 1187, 1108, 763, 701 cm^{-1}; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.61 (s, 1H), 9.04 (s, 1H), 8.53 (s, 1H), 8.09 – 8.01 (m, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.76 – 7.64 (m, 1H), 7.57 – 7.46 (m, 1H), 7.34 – 7.27 (m, 1H), 7.24 (dd, *J* = 9.0, 7.9 Hz, 1H), 7.11 – 6.99 (m, 1H), 3.85 (s, 3H), 3.24 – 2.93 (m, 2H), 2.85 – 2.58 (m, 2H), 2.06 – 1.55 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.33, 25.07, 32.20, 56.10, 114.73, 115.32, 116.75, 117.76, 119.44, 120.29, 122.25, 123.14, 124.65, 125.24, 127.14, 130.89, 140.16, 142.79, 144.41, 145.47, 147.07, 147.46, 155.19, 156.85, 161.01, 162.82; ; LC-MS (m/z): 499.14 [MH$^+$]; Anal. Calcd. For C, 65.05; H, 4.45; N, 11.24; O, 12.84; S, 6.43 found: C, 65.11; H, 4.57; N, 11.32; S, 6.57.

1-(6,8-dichloro-1,2,3,4-tetrahydroacridin-9-yl)-3-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (SC6)

Yellow solid, 76% yield, mp. 235-237 °C; IR: 3389, 3296, 1680, 1675, 1556, 1520, 1284, 1252, 1186, 1111, 785, 646 cm^{-1}; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.63 – 10.60 (m, 1H), 8.72 (s, 1H), 8.53 (s, 1H), 7.69 – 7.66 (m, 1H), 7.56 – 7.48

(m, 1H), 7.38 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.35 – 7.27 (m, 1H), 7.27 – 7.20 (m, 1H), 7.06 (dd, *J* = 8.9, 1.7 Hz, 1H), 3.85 (s, 3H), 3.14 – 3.05 (m, 2H), 2.84 – 2.77 (m, 2H), 1.96 – 1.78 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.32, 26.50, 32.49, 56.10, 114.73, 115.32, 115.54, 116.20, 117.76, 119.44, 121.61, 123.14, 125.23, 125.47, 132.92, 133.25, 140.16, 142.35, 142.79, 145.47, 147.32, 147.46, 155.27, 158.24, 161.01, 162.82; ; LC-MS (m/z): 567.06 [MH$^+$]; Anal. Calcd. for C, 57.15; H, 3.55; Cl, 12.49; N, 9.87; O, 11.28; S, 5.65; found: C, 57.21; H, 3.58; Cl, 12.40; N, 9.80; S, 5.57.

1-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)-3-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (SC7)

Yellow solid, 80% yield, mp. 230-232 °C; IR: 3384, 3291, 1675, 1670, 1551, 1515, 1279, 1247, 1181, 1106, 780, 641 cm^{-1}; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.63 – 10.60 (m, 1H), 9.08 (s, 1H), 8.53 (s, 1H), 8.03 (d, *J* = 8.9 Hz, 1H), 7.71 – 7.65 (m, 1H), 7.56 – 7.49 (m, 1H), 7.40 – 7.34 (m, 1H), 7.34 – 7.29 (m, 1H), 7.27 – 7.20 (m, 1H), 7.11 – 7.01 (m, 1H), 3.85 (s, 3H), 3.19 – 3.00 (m, 2H), 2.84 – 2.71 (m, 2H), 2.02 – 1.71 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.33, 25.07, 32.34, 56.10, 114.73, 115.32, 115.62, 117.76, 119.44, 123.14, 123.54, 124.74, 124.94, 125.24, 137.59, 140.16, 142.79, 145.11, 145.47, 146.66, 147.46, 155.23, 157.54, 161.01, 162.82; ; LC-MS (m/z): 533.10 [MH$^+$]; Anal. Calcd. for C, 60.84; H, 3.97; Cl, 6.65; N, 10.51; O, 12.01; S, 6.02; found: C, 60.77; H, 3.84; Cl, 6.60; N, 10.59; S, 6.10.

1-(7-methoxy-1,2,3,4-tetrahydroacridin-9-yl)-3-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (SC8)

Yellow solid, 90% yield, mp. 226-228 °C; IR: 3353, 3295, 1683, 1665, 1546, 1509, 1283, 1227, 1183, 1114, 782, 645 cm^{-1}; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.65 – 10.59 (m, 1H), 8.72 (s, 1H), 8.53 (s, 1H), 7.74 – 7.66 (m, 1H), 7.55 – 7.49 (m, 1H), 7.37 – 7.33 (m, 1H), 7.33 – 7.29 (m, 1H), 7.27 – 7.20 (m, 1H), 7.10 – 6.97 (m, 2H), 3.89 – 3.77 (m, 6H), 3.14 – 3.01 (m, 2H), 2.84 – 2.71 (m, 2H), 1.97 – 1.75 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.36, 25.18, 32.21, 55.67, 56.10, 104.55, 114.73, 115.32, 116.97, 117.76, 118.74, 119.44, 119.64, 123.14, 125.23, 125.42, 140.16, 142.36, 142.79, 143.42, 145.47, 147.46, 155.20, 156.24, 156.73, 161.01, 162.82; ; LC-MS (m/z): 529.15 [MH$^+$]; Anal. Calcd. for C, 63.62; H, 4.58; N, 10.60; O, 15.13; S, 6.07 found: C, 63.52; H, 4.48; N, 10.63; S, 6.13.

1-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(1,2,3,4-tetrahydroacridin-9-yl)urea (SC9)

Yellow powder, 61% yield, mp. 256-258 °C; IR: 3365, 3300, 1732, 1699, 1609, 1547, 1487, 1344, 1295, 1000, 748 cm^{-1}; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.72 – 10.54 (m, 1H), 8.78 (s, 1H), 8.72 (s, 1H), 8.47 (s, 1H), 8.30 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.74 – 7.63 (m, 1H), 7.58 – 7.48 (m, 1H), 7.43 – 7.35 (m, 1H), 7.34 – 7.26 (m, 1H), 3.20 – 3.05 (m, 2H), 2.89 – 2.73 (m, 2H), 2.00 – 1.74 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.32, 26.50, 32.49, 114.73, 115.53, 116.19, 116.89, 117.46, 119.41, 121.61, 125.35, 125.47, 127.35, 132.92, 133.25, 142.35, 142.65, 143.09, 145.48, 147.32, 155.27, 157.33, 158.24, 161.01,

163.77; ; LC-MS (m/*z*): 514.12 [MH$^+$]; Anal. Calcd. for C, 60.81; H, 3.73; N, 13.64; O, 15.58; S, 6.24; found: C, 60.71; H, 3.78; N, 13.57; S, 6.34.

1-(6,8-dichloro-1,2,3,4-tetrahydroacridin-9-yl)-3-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (SC10)

Yellow solid, 61% yield, mp. 255-257 °C; IR: 3368, 3295, 1749, 1703, 1550, 1496, 1349, 1294, 1095, 844, 620 cm^{-1}, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.71 – 10.50 (m, 1H), 9.06 (s, 1H), 8.78 (s, 1H), 8.53 (d, *J* = 3.0 Hz, 1H), 8.30 (dd, *J* = 8.7, 2.4 Hz, 1H), 8.11 – 7.99 (m, 1H), 7.88 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.74 – 7.64 (m, 1H), 7.59 – 7.42 (m, 2H), 7.35 – 7.25 (m, 1H), 3.15 – 3.01 (m, 2H), 2.87 – 2.70 (m, 2H), 1.95 – 1.78 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.33, 25.07, 32.20, 114.73, 116.75, 116.89, 117.46, 119.41, 120.29, 122.25, 124.65, 125.36, 127.14, 127.35, 130.89, 142.65, 143.09, 144.41, 145.48, 147.07, 155.19, 156.84, 157.33, 161.01, 163.77; ; LC-MS (m/*z*): 582.04 [MH$^+$]; Anal. Calcd. for C, 53.62; H, 2.94; Cl, 12.17; N, 12.02; O, 13.74; S, 5.50; found: C, 53.54; H, 2.86; Cl, 12.05; N, 12.09; S, 5.53.

1-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)-3-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (SC11)

Yellow solid, 62% yield, mp. 257-259 °C; IR: 3363, 3290, 1744, 1698, 1545, 1491, 1344, 1289, 1090, 839, 615 cm^{-1}; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.75 – 10.48 (m, 1H), 9.08 (s, 1H), 8.78 (s, 1H), 8.53 (d, *J* = 3.0 Hz, 1H), 8.30 (dd, *J* = 8.7, 2.4 Hz, 1H), 8.03 (d, *J* = 9.0 Hz, 1H), 7.74 – 7.61 (m, 1H), 7.59 – 7.47 (m, 1H), 7.41 – 7.33 (m, 1H), 7.33 – 7.26 (m, 1H), 3.33 – 2.89 (m, 2H), 2.93 – 2.58 (m, 2H), 2.18 – 1.47 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.33, 25.07, 32.34, 114.73, 115.62, 116.89, 117.46, 119.41, 119.45, 123.54, 124.74, 124.94, 125.36, 127.35, 137.59, 142.65, 143.09, 145.11, 145.48, 146.66, 155.23, 157.32, 157.54, 161.01, 163.77; ; LC-MS (m/*z*): 548.08 [MH$^+$]; Anal. Calcd. for C, 56.99; H, 3.31; Cl, 6.47; N, 12.78; O, 14.60; S, 5.85; found: C, 56.89; H, 3.45; Cl, 6.31; N, 12.65; S, 5.70.

1-(7-methoxy-1,2,3,4-tetrahydroacridin-9-yl)-3-(4-(6-nitro-2-oxo-2H-chromen-3-yl)thiazol-2-yl)urea (SC12)

Yellow solid, 58% yield, mp. 243-245 °C; IR: 3369, 3145, 1738, 1703, 1617, 1558, 1348, 1247, 1094, 839, 749 cm^{-1}; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.66 – 10.57 (m, 1H), 8.78 (s, 1H), 8.72 (s, 1H), 8.53 (d, *J* = 2.9 Hz, 1H), 8.30 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.74 – 7.66 (m, 1H), 7.57 – 7.49 (m, 1H), 7.37 – 7.32 (m, 1H), 7.33 – 7.28 (m, 1H), 7.02 (dd, *J* = 8.3, 3.0 Hz, 1H), 3.80 (s, 3H), 3.13 – 3.02 (m, 2H), 2.83 – 2.70 (m, 2H), 1.93 – 1.80 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.97, 22.36, 25.18, 32.21, 55.67, 104.55, 114.73, 116.89, 116.97, 117.46, 118.74, 119.41, 119.64, 125.35, 125.42, 127.35, 142.36, 142.65, 143.09, 143.42, 145.48, 155.20, 156.24, 156.73, 157.33, 161.01, 163.77; ; LC-MS (m/*z*): 544.13 [MH$^+$]; Anal. Calcd. for C, 59.66; H, 3.89; N, 12.88; O, 17.66; S, 5.90; found: C, 59.55; H, 3.80; N, 12.73; S, 5.77.

Anticholinesterase activity assays

Acetyl- (AChE) and butyryl-cholinesterase (BuChE) inhibitory activities of the synthesized compounds were determined according to the literature method [111]. In this study, IC50 was determined by constructing an absorbance and/or inhibition (%) curve and examining the effect of different concentrations. IC50 values have been calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. The substrates of the reaction were acetylthiocholine iodide and butyrylthiocholine iodide. 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB) was used to measure the anticholinesterase activity. The solutions of the samples and galantamine in n-propanol were prepared at a concentration of 4000 µg/mL. Aliquots of 150 µL of 100 mM phosphate buffer (pH 8.0), 10 µL of sample solution and 20 µL AChE (2.476x10-4 U/µL) (or 3.1813x10-4 U/µL BChE) solution were mixed and incubated for 15 min at 25°C. 10 µL of DTNB solution was prepared by adding 2.0 mL of pH 7.0 and 4.0 mL of pH 8.0 phosphate buffers to a mixture of 1.0 mL of 16 mg/mL DTNB and 7.5 mg/mL NaHCO3 in pH 7.0 phosphate buffers. The reaction was initiated by adding 10 µL (7.1 mM) acetylthiocholine iodide (or 0.79 mM butyrylthiocholine iodide).

In this method, the activity was measured by following the yellow color produced as a result of the thio anion produced by reacting to the enzymatic hydrolysis of the substrate with DTNB. Propyl alcohol was used as a solvent to control. The hydrolysis of these substrates was monitored using a BioTek Power Wave XS at 412 nm. The results were calculated as IC50. Anticholinesterase activity assays Acetyl- (AChE) and butyryl-cholinesterase (BuChE) inhibitory activities of the synthesized compounds were determined according to the literature method [32]. IC50 of substance has been determined by constructing an absorbance and/or inhibition (%) curve and examining the effect of different concentrations. IC50 values have been calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. The substrates of the reaction were acetylthiocholine iodide and butyrylthiocholine iodide. 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB) was used to measure the anticholinesterase activity. The solutions of the samples and galantamine in n-propanol were prepared at a concentration of 4000 µg/mL. Aliquots of 150 µL of 100 mM phosphate buffer (pH 8.0), 10 µL of sample solution and 20 µL AChE (2.476x10-4 U/µL) (or 3.1813x10-4 U/µL BChE) solution were mixed and incubated for 15 min at 25°C. 10 µL of DTNB solution was prepared by adding 2.0 mL of pH 7.0 and 4.0 mL of pH 8.0 phosphate buffers to a mixture of 1.0 mL of 16 mg/mL DTNB and 7.5 mg/mL NaHCO3 in pH 7.0 phosphate buffers. The reaction was initiated by adding 10 µL (7.1 mM) acetylthiocholine iodide (or 0.79 mM butyrylthiocholine iodide). In this method, the activity was measured by following the yellow color produced as a result of the thio anion produced by reacting to the enzymatic hydrolysis of the substrate with DTNB. Propyl alcohol was used as a solvent to control. The hydrolysis of these substrates was monitored using a spectrophotometer at 412 nm. The results were calculated as IC50.

Results and Discussion

Chemistry

The synthetic procedures employed to obtain the target compounds SC(1-12) are depicted in Scheme 1. Coumarin-3-aldehyde derivatives I-2(a-c) were synthesized from substituted salicylaldehydes by Knoevenagel Condensation [85] & brominated with molecular bromine in chloroform to get 3-(2-bromoacetyl)coumarin derivatives I-3(a-c) by replacement of hydrogens alpha to the carbonyl group. 2-amino-4-(R1-coumarin-3-yl)thiazoles [I-4(a-c)] were obtained by the reactions of I-3(a-c) with thiourea. The condensation of I-3(a-c) with an active thiourea compound under Hantzsch reactions followed by an intramolecular heterocyclization, gave 2-amino-4-(coumarin-3-yl)thiazoles I-4(a-c). Simultaneously substituted Tacrines were synthesized from different benzonitriles by Friedländer condensation using zinc chloride in a microwave and further converted to isocyanates with the help of triphosgene. Finally, compounds I-4(a-c) were reacted with various substituted Tacrine-isocyanates II-3(a-d) in THF to get the product Tacrine-coumarin derivatives linked by thiazole and urea SC(1-12).

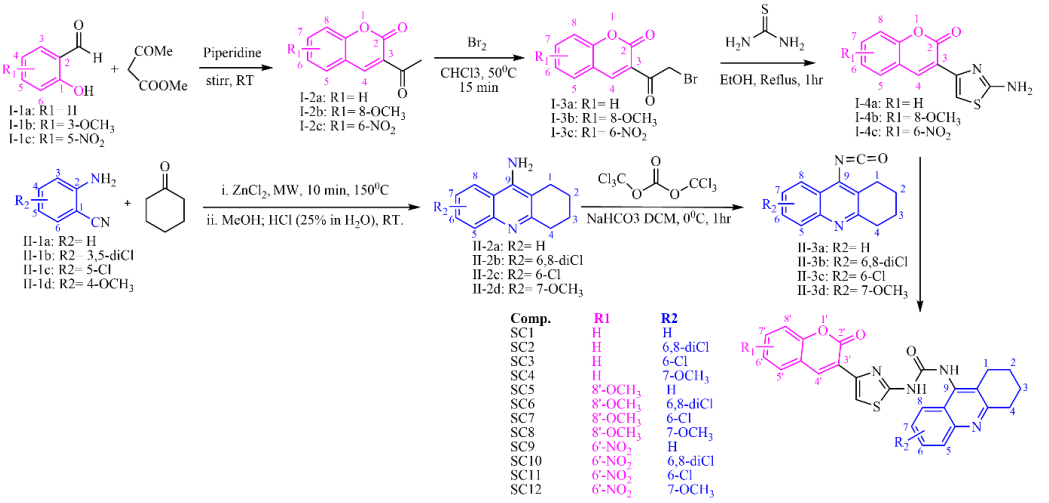


Figure 4. Synthetic scheme

Scheme 1. Synthesis of new Thiazolyl urea substituted Tacrine-coumarin analogs.

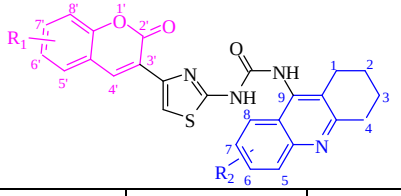
The new compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, MS, and elemental analysis. Infrared spectra of SC1 to SC12 show absorption between 3400 and 3350 cm-1 concerning N-H stretching, for urea derivatives, about 1610-1540 cm^{-1} relating to C=N stretch for thiazole, absorptions in about 1710 to 1740 cm^{-1} from coumarin carbonyl moiety stretch and absorptions between 1660 and 1705 cm^{-1} from urea carbonyl moiety stretching, Coumaryl C-O-C stretching shows absorption at about 1250 cm-1, while thiazole -S- shows stretching vibration at about 1100 cm-1. From the ^{1}H NMR spectra, a hydrogen attached to the urea nitrogen shows two singlets between 8.72 and 10.75 ppm, indicating N-H attached to thiazole shows a signal at more downfield than N-H nearby to Tacrine. The signals for aromatic hydrogens were observed between 8.78 to 7.011 ppm, and the signal for aromatic proton at the thiazole ring was detected at 7.30

ppm. The signals from the ^{13}C NMR spectra can be seen at 160-163 ppm, relating to coumarin carbonyl and thiazole ring is, followed by the sign of about 155 ppm for urea carbonyl and about 175 ppm.

Biological activities

Inhibitory activities against AChE and BuChE, The inhibitory activities of the synthesized compounds (SC1 to SC12) against AChE and BuChE, were performed according to Ellman's method using galantamine as the reference compound [111]. The IC50 values for AChE and BuChE inhibitions are summarized in Table 1. The results showed that synthesized compounds exhibited inhibitory activity against ChEs. The IC50 values were 37.15 to 131.49 µM for AChE and between 5.89 and 89.60 µM for BuChE inhibitory activity. Among the synthesized compounds, SC10 (IC50 = 5.89 µM) showed the highest inhibitory activity against BuchE. Although the potency was less than those of tacrine (IC50 = 0.077 µM for AchE & 0.023 µM for BuchE) and galantamine (IC50 = 0.042 µM for AchE & 3.97 µM for BuchE).

Table 1
In vitro inhibition IC50 of compounds SC1 to SC12 for AChE and BuChE activities



COMPOUND	R1	R2	IC50 ± SD(µM)		SI
			AChE	BuChE	
SC1	H	H	74.20 ± 1.40	75.70 ± 0.47	1.02
SC2	H	6,8-diCl	102.14 ± 0.14	85.90 ± 2.52	0.84
SC3	H	6-Cl	39.15 ± 3.90	89.47 ± 5.13	2.29
SC4	H	7-OCH_3	37.03 ± 0.75	31.52 ± 1.57	0.85
SC5	8'-OCH_3	H	85.10 ± 0.7	78.62 ± 4.84	0.92
SC6	8'-OCH_3	6,8-diCl	116.02 ± 0.8	64.31 ± 1.04	0.55
SC7	8'-OCH_3	6-Cl	108.43 ± 1.10	71.63 ± 0.24	0.66
SC8	8'-OCH_3	7-OCH_3	84.78 ± 0.20	57.52 ± 0.28	0.68
SC9	6'-NO_2	H	103.78 ± 0.4	85.51 ± 0.28	0.82
SC10	6'-NO_2	6,8-diCl	131.49 ± 0.9	5.89 ± 0.49	0.04
SC11	6'-NO_2	6-Cl	130.24 ± 0.2	80.32 ± 0.67	0.62
SC12	6'-NO_2	7-OCH_3	39.70 ± 0.9	72.47 ± 0.87	1.83
TACRINE			0.077 ± 0.0034	0.023 ± 0.0019	
DONEPEZIL			0.042 ± 0.0012	3.97 ± 0.337	

* IC50 values are expressed as the mean ± standard deviation of the mean of three independent experiments.
* Selectivity index = IC50 (BuChE) / IC50 (AChE).

Structure-activity relationships (SAR)

The following conclusions should be noted regarding the ChEs inhibitory data in Table 1.

- The electron-donating group (methoxy) or electron-withdrawing group (nitro) at the coumarin ring did not cause a regular change in the inhibitory activity.
- All compounds show good activity against BuchE than AchE.
- Amongst 7-methoxy Tacrines, non-substituted coumarin & 6-Nitro coumarin derivative shows two times more potent activity than 8-Methoxy coumarin derivative against AchE.
- All 7-Methoxy Tacrine derivatives show moderately good activity against AchE and BuchE compared to other derivatives.

Conclusion

In conclusion, a series of 42 novel urea substituted coumarylthiazoles derivatives (SC1-SC12) were synthesized and evaluated their effects on AChE and BuChE. In addition, the structure-activity relationship was presented. All the synthesized compounds inhibited the AChE and BuChE activities. Among them, SC4 was the most active compound for AChE inhibitory activity, and SC10 exhibited the strongest inhibition against BuChE with an IC50 value of 5.89 μM. The SAR study revealed that the inhibitory activity of synthesized compounds could also be influenced by the type and position of substituent on the Tacrine and coumarin ring.

References

1. M. Goedert and M. G. Spillantini, "A century of Alzheimer's disease," *Science (80-.).*, vol. 314, no. 5800, pp. 777–781, 2006, doi: 10.1126/science.1132814.
2. P. Scheltens *et al.*, "Alzheimer's disease.," *Lancet (London, England)*, vol. 388, no. 10043, pp. 505–517, Jul. 2016, doi: 10.1016/S0140-6736(15)01124-1.
3. P. Muñoz-Ruiz *et al.*, "Design, synthesis, and biological evaluation of dual binding site acetylcholinesterase inhibitors: New disease-modifying agents for Alzheimer's disease," *J. Med. Chem.*, vol. 48, no. 23, pp. 7223–7233, Nov. 2005, doi: 10.1021/jm0503289.
4. L. Bertram and R. E. Tanzi, *Thirty years of Alzheimer's disease genetics: The implications of systematic meta-analyses*, vol. 9, no. 10. Nat Rev Neurosci, 2008, pp. 768–778.
5. U. Holzgrabe, P. Kapková, V. Alptüzün, J. Scheiber, and E. Kugelmann, "Targeting acetylcholinesterase to treat neurodegeneration," *Expert Opin. Ther. Targets*, vol. 11, no. 2, pp. 161–179, Feb. 2007, doi: 10.1517/14728222.11.2.161.
6. X. Zhu, A. K. Raina, G. Perry, and M. A. Smith, "Alzheimer's disease: The two-hit hypothesis," *Lancet Neurol.*, vol. 3, no. 4, pp. 219–226, 2004, doi: 10.1016/S1474-4422(04)00707-0.
7. G. Wang *et al.*, "Alzheimer's Disease: Scientific Breakthroughs and Translational Challenges Richard," *HHS Public Access*, vol. 311, no. 6, pp. 587–596, 2015, doi: 10.1016/j.mayocp.2017.02.011.Alzheimer.

8. X. Zhang, K. P. Rakesh, S. N. A. Bukhari, M. Balakrishna, H. M. Manukumar, and H. Qin, "Bioorganic Chemistry Multi-targetable chalcone analogs to treat deadly Alzheimer ' s disease : Current view and upcoming advice," *Bioorg. Chem.*, vol. 80, no. April, pp. 86–93, 2018, doi: 10.1016/j.bioorg.2018.06.009.
9. G. S. Bloom, "Amyloid-β and tau: The trigger and bullet in Alzheimer disease pathogenesis," *JAMA Neurol.*, vol. 71, no. 4, pp. 505–508, 2014, doi: 10.1001/jamaneurol.2013.5847.
10. M. Jouanne, S. Rault, and A. S. Voisin-Chiret, "Tau protein aggregation in Alzheimer's disease: An attractive target for the development of novel therapeutic agents," *Eur. J. Med. Chem.*, vol. 139, no. July, pp. 153–167, 2017, doi: 10.1016/j.ejmech.2017.07.070.
11. W. J. Huang, X. Zhang, and W. W. Chen, "Role of oxidative stress in Alzheimer's disease (review)," *Biomed. Reports*, vol. 4, no. 5, pp. 519–522, 2016, doi: 10.3892/br.2016.630.
12. P. Zatta, D. Drago, S. Bolognin, and S. L. Sensi, "Alzheimer's disease, metal ions and metal homeostatic therapy," *Trends Pharmacol. Sci.*, vol. 30, no. 7, pp. 346–355, 2009, doi: 10.1016/j.tips.2009.05.002.
13. M. T. Heneka, M. K. O'Banion, D. Terwel, and M. P. Kummer, "Neuroinflammatory processes in Alzheimer's disease," *J. Neural Transm.*, vol. 117, no. 8, pp. 919–947, 2010, doi: 10.1007/s00702-010-0438-z.
14. A. Contestabile, "The history of the cholinergic hypothesis," *Behav. Brain Res.*, vol. 221, no. 2, pp. 334–340, 2011, doi: 10.1016/j.bbr.2009.12.044.
15. N. H. Greig *et al.*, "A new therapeutic target in Alzheimer's disease treatment: Attention to butyryloholinesterase," *Curr. Med. Res. Opin.*, 2001, doi: 10.1185/03007990152673800.
16. R. M. Lane, S. G. Potkin, A. Enz, R. M. Lane, S. G. Potkin, and A. Enz, "Neuropsychopharmacology : Targeting acetylcholinesterase and butyrylcholinesterase in dementia How to cite this article : Targeting acetylcholinesterase and butyrylcholinesterase in dementia," no. August 2005, 2012, doi: 10.1017/S1461145705005833.
17. P. De La Torre *et al.*, "A novel class of selective acetylcholinesterase inhibitors: Synthesis and evaluation of (E)-2-(benzo[d]thiazol-2-yl)-3-heteroarylacrylonitriles," *Molecules*, vol. 17, no. 10, pp. 12072–12085, Oct. 2012, doi: 10.3390/molecules171012072.
18. J. L. Sussman *et al.*, "Atomic structure of acetylcholinesterase from Torpedo californica: A prototypic acetylcholine-binding protein," *Science (80-.).*, vol. 253, no. 5022, pp. 872–879, 1991, doi: 10.1126/science.1678899.
19. Y. Nicolet, O. Lockridge, P. Masson, J. C. Fontecilla-Camps, and F. Nachon, "Crystal Structure of Human Butyrylcholinesterase and of Its Complexes with Substrate and Products," *J. Biol. Chem.*, vol. 278, no. 42, pp. 41141–41147, 2003, doi: 10.1074/jbc.M210241200.
20. H. Dvir, I. Silman, M. Harel, T. L. Rosenberry, and J. L. Sussman, "Acetylcholinesterase: From 3D structure to function," *Chem. Biol. Interact.*, vol. 187, no. 1–3, pp. 10–22, 2010, doi: 10.1016/j.cbi.2010.01.042.
21. A. Mary, D. Z. Renko, C. Guillou, and C. Thal, "Potent acetylcholinesterase inhibitors: design, synthesis, and structure-activity relationships of bis-interacting ligands in the galanthamine series.," *Bioorg. Med. Chem.*, vol. 6, no. 10, pp. 1835–50, Oct. 1998, doi: 10.1016/s0968-0896(98)00133-3.
22. A. Asadipour *et al.*, "Novel coumarin-3-carboxamides bearing N-

benzylpiperidine moiety as potent acetylcholinesterase inhibitors," *Eur. J. Med. Chem.*, vol. 70, pp. 623–630, 2013, doi: 10.1016/j.ejmech.2013.10.024.
23. I. W. Wyman and D. H. Macartney, "Host-guest complexes and pseudorotaxanes of cucurbit[7]uril with acetylcholinesterase inhibitors," *J. Org. Chem.*, vol. 74, no. 21, pp. 8031–8038, Nov. 2009, doi: 10.1021/jo901861e.
24. N. C. Inestrosa *et al.*, "Acetylcholinesterase accelerates assembly of amyloid-β-peptides into Alzheimer's fibrils: Possible role of the peripheral site of the enzyme," *Neuron*, vol. 16, no. 4, pp. 881–891, 1996, doi: 10.1016/S0896-6273(00)80108-7.
25. M. Bartolini, C. Bertucci, V. Cavrini, and V. Andrisano, "beta-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies.," *Biochem. Pharmacol.*, vol. 65, no. 3, pp. 407–416, Feb. 2003, doi: 10.1016/s0006-2952(02)01514-9.
26. G. A. Reid and S. Darvesh, "Butyrylcholinesterase-knockout reduces brain deposition of fibrillar β-amyloid in an Alzheimer mouse model," *Neuroscience*, vol. 298, pp. 424–435, 2015, doi: 10.1016/j.neuroscience.2015.04.039.
27. A. Toxicol *et al.*, "SAR study to find optimal cholinesterase reactivator against organophosphorous nerve agents and pesticides," *Arch. Toxicol.*, 2016, doi: 10.1007/s00204-016-1827-3.
28. W. J. Krall, J. J. Sramek, and N. R. Cutler, "Cholinesterase Inhibitors: A Therapeutic Strategy for Alzheimer Disease," vol. 33, pp. 441–450, 1999.
29. A. V. T. Jr *et al.*, "The Cholinergic Hypothesis of Age and Alzheimer ' s Disease- Related Cognitive Deficits : Recent Challenges and Their Implications for Novel Drug Development," vol. 306, no. 3, pp. 821–827, 2003, doi: 10.1124/jpet.102.041616.For.
30. [30] I. Churcher, "Tau Therapeutic Strategies for the Treatment of Alzheimer ' s Disease," pp. 579–595, 2006.
31. D. F. Swaab, E. J. G. Dubelaar, E. J. W. Van Someren, and R. W. H. Verwer, "Therapeutic Strategies for Alzheimer Disease," vol. 17, no. September, pp. 114–122, 2003.
32. M. Bolognesi, A. Minarini, M. Rosini, V. Tumiatti, and C. Melchiorre, "From Dual Binding Site Acetylcholinesterase Inhibitors to Multi-Target-Directed Ligands (MTDLs): A Step Forward in the Treatment of Alzheimers Disease," *Mini-Reviews Med. Chem.*, vol. 8, no. 10, pp. 960–967, 2008, doi: 10.2174/138955708785740652.
33. R. A. Hansen, G. Gartlehner, A. P. Webb, L. C. Morgan, C. G. Moore, and D. E. Jonas, "Efficacy and safety of donepezil , galantamine , and rivastigmine for the treatment of Alzheimer ' s disease : A systematic review and meta-analysis," vol. 3, no. 2, pp. 211–225, 2008.
34. J. L. Cummings, T. Morstorf, and K. Zhong, "Alzheimer ' s disease drug-development pipeline : few candidates , frequent failures," pp. 1–7, 2014.
35. A. Więckowska *et al.*, "Novel Multitarget-Directed Ligands Aiming at Symptoms and Causes of Alzheimer's Disease," *ACS Chem. Neurosci.*, vol. 9, no. 5, pp. 1195–1214, 2018, doi: 10.1021/acschemneuro.8b00024.
36. K. Diseases, "LiverTox: A Website on Drug-Induced Liver Injury," pp. 873–874, doi: 10.1002/hep.26175.
37. A. Burns, "Treatment of cognitive impairment in Alzheimer's disease Alistair," pp. 35–43.
38. F. Monacelli, P. Odetti, M. Sartini, A. Parodi, C. Brunelli, and G. Rosa,

"Cardioprotection and Anticholinesterases in Patients with Alzheimer ' s Disease : Time for Reappraisal," no. DiMI, pp. 45–50, 2014, doi: 10.1159/000357124.
39. A. Ferreira, L. Vilela, C. Lúcia, and C. Mateo, "Sensors and Actuators B : Chemical An immobilized acetylcholinesterase as test system to screen new inhibitor drugs to treat Alzheimer ' s disease," *Sensors Actuators B. Chem.*, vol. 278, no. May 2018, pp. 196–201, 2019, doi: 10.1016/j.snb.2018.09.071.
40. D. A. Casey, D. Antimisiaris, and J. O. Brien, "Drugs for Alzheimer ' s Disease : Are They Effective ?," vol. 35, no. 4, pp. 208–211, 2010.
41. S. S. Chaudhaery *et al.*, "Novel carbamates as orally active acetylcholinesterase inhibitors found to improve scopolamine-induced cognition impairment: Pharmacophore-based virtual screening, synthesis, and pharmacology," *J. Med. Chem.*, vol. 53, no. 17, pp. 6490–6505, 2010, doi: 10.1021/jm100573q.
42. M. Rosini *et al.*, "Inhibition of acetylcholinesterase, β-amyloid aggregation, and NMDA receptors in Alzheimer's disease: A promising direction for the multi-target-directed ligands gold rush," *J. Med. Chem.*, vol. 51, no. 15, pp. 4381–4384, 2008, doi: 10.1021/jm800577j.
43. L. Piazzi *et al.*, "Multi-target-directed coumarin derivatives: hAChE and BACE1 inhibitors as potential anti-Alzheimer compounds," *Bioorganic Med. Chem. Lett.*, vol. 18, no. 1, pp. 423–426, Jan. 2008, doi: 10.1016/j.bmcl.2007.09.100.
44. T. Rees, P. I. Hammond, H. Soreq, S. Younkin, and S. Brimijoin, "Acetylcholinesterase promotes beta-amyloid plaques in cerebral cortex," vol. 24, pp. 777–787, 2003, doi: 10.1016/S0197-4580(02)00230-0.
45. A. Romero, R. Cacabelos, M. J. Oset-Gasque, A. Samadi, and J. Marco-Contelles, "Novel tacrine-related drugs as potential candidates for the treatment of Alzheimer's disease," *Bioorganic Med. Chem. Lett.*, vol. 23, no. 7, pp. 1916–1922, 2013, doi: 10.1016/j.bmcl.2013.02.017.
46. M. Sadafi Kohnehshahri *et al.*, "Novel tacrine-based acetylcholinesterase inhibitors as potential agents for the treatment of Alzheimer's disease: Quinolotacrine hybrids," *Mol. Divers.*, vol. 26, no. 1, pp. 489–503, 2022, doi: 10.1007/s11030-021-10307-2.
47. A. Rastegari *et al.*, "Synthesis and evaluation of novel arylisoxazoles linked to tacrine moiety: in vitro and in vivo biological activities against Alzheimer's disease," *Mol. Divers.*, vol. 26, no. 1, pp. 409–428, 2022, doi: 10.1007/s11030-021-10248-w.
48. H. Abbasi and M. Fereidoonnezhad, "Vilazodone-Tacrine Hybrids as Potential Anti-Alzheimer Agents: QSAR, Molecular Docking, and Molecular Dynamic (MD) Simulation Studies," *Biointerface Res. Appl. Chem.*, vol. 12, no. 1, pp. 588–607, 2021, doi: 10.33263/briac121.588607.
49. P. Zhang *et al.*, "Bioorganic Chemistry Design and synthesis of novel tacrine-dipicolylamine dimers that are multiple-target-directed ligands with potential to treat Alzheimer ' s disease," *Bioorg. Chem.*, vol. 116, no. April, p. 105387, 2021, doi: 10.1016/j.bioorg.2021.105387.
50. O. Ozten, B. Zengin, F. Sonmez, B. Dogan, and S. Durdagi, "Bioorganic Chemistry Synthesis , molecular docking and molecular dynamics studies of novel tacrine-carbamate derivatives as potent cholinesterase inhibitors," *Bioorg. Chem.*, vol. 115, no. July, p. 105225, 2021, doi: 10.1016/j.bioorg.2021.105225.

51. A. Rani *et al.*, “1H-1,2,3-triazole grafted tacrine-chalcone conjugates as potential cholinesterase inhibitors with the evaluation of their behavioral tests and oxidative stress in mice brain cells,” *Bioorg. Chem.*, vol. 114, no. September 2020, p. 105053, 2021, doi: 10.1016/j.bioorg.2021.105053.
52. Z. Xuan *et al.*, “Dimeric Tacrine(10)-hupyridone as a Multitarget-Directed Ligand To Treat Alzheimer ’ s Disease,” no. 10, 2021, doi: 10.1021/acschemneuro.1c00182.
53. H. Yao *et al.*, “Discovery of Novel Tacrine − Pyrimidone Hybrids as Potent Dual AChE/GSK - 3 Inhibitors for the Treatment of Alzheimer ’ s Disease,” 2021, doi: 10.1021/acs.jmedchem.1c00160.
54. L. Gorecki *et al.*, “Phenothiazine-Tacrine Heterodimers: Pursuing Multitarget Directed Approach in Alzheimer ’ s Disease,” 2021, doi: 10.1021/acschemneuro.1c00184.
55. S. Hamulakova *et al.*, “Synthesis and biological evaluation of novel tacrine derivatives and tacrine-coumarin hybrids as cholinesterase inhibitors,” *J. Med. Chem.*, vol. 57, no. 16, pp. 7073–7084, 2014, doi: 10.1021/jm5008648.
56. M. I. Fernández-Bachiller, C. Pérez, L. Monjas, J. Rademann, and M. I. Rodríguez-Franco, “New tacrine-4-oxo-4H-chromene hybrids as multifunctional agents for the treatment of Alzheimer’s disease, with cholinergic, antioxidant, and β-amyloid-reducing properties,” *J. Med. Chem.*, 2012, doi: 10.1021/jm201460y.
57. S. Thiratmatrakul *et al.*, “Synthesis, biological evaluation and molecular modeling study of novel tacrine-carbazole hybrids as potential multifunctional agents for the treatment of Alzheimer’s disease,” *Eur. J. Med. Chem.*, vol. 75, pp. 21–30, Mar. 2014, doi: 10.1016/j.ejmech.2014.01.020.
58. S. KhanYusufzai *et al.*, “Design, characterization, in vitro antibacterial, antitubercular evaluation and structure–activity relationships of new hydrazinyl thiazolyl coumarin derivatives,” *Med. Chem. Res.*, vol. 26, no. 6, pp. 1139–1148, 2017, doi: 10.1007/s00044-017-1820-2.
59. K. V. Sashidhara *et al.*, “Discovery and synthesis of novel 3-phenylcoumarin derivatives as antidepressant agents,” *Bioorganic Med. Chem. Lett.*, vol. 21, no. 7, pp. 1937–1941, 2011, doi: 10.1016/j.bmcl.2011.02.040.
60. P. Manojkumar, T. Ravi, and G. Subbuchettiar, “Synthesis of coumarin heterocyclic derivatives with antioxidant activity and in vitro cytotoxic activity against tumour cells,” *Acta Pharm.*, 2009, doi: 10.2478/v10007-009-0018-7.
61. M. Khoobi, S. Emami, G. Dehghan, A. Foroumadi, A. Ramazani, and A. Shafiee, “Synthesis and free radical scavenging activity of coumarin derivatives containing a 2-methylbenzothiazoline motif,” *Arch. Pharm. (Weinheim).*, 2011, doi: 10.1002/ardp.201000271.
62. M. Cacic, M. Trkovnik, F. Cacic, and E. Has-Schon, “Synthesis and Antimicrobial Activity of Some Derivatives of (7- Hydroxy-2-oxo-2,” *Molecules*, vol. 11, pp. 134–147, 2006.
63. L. El-Ansary, E. Aly, M. H.- EGYPTIAN, and undefined 1992, “New Coumarin derivatives as antibacterial agents Sohair,” *NIDOC-NATIONAL Inf. & ….*
64. B. Sandhya, D. Giles, V. Mathew, G. Basavarajaswamy, and R. Abraham, “Synthesis, pharmacological evaluation and docking studies of coumarin derivatives,” *Eur. J. Med. Chem.*, 2011, doi: 10.1016/j.ejmech.2011.07.013.
65. K. Fylaktakidou, D. Hadjipavlou-Litina, K. Litinas, and D. Nicolaides, “Natural and Synthetic Coumarin Derivatives with Anti-Inflammatory / Antioxidant Activities,” *Curr. Pharm. Des.*, 2005, doi:

10.2174/1381612043382710.
66. M. Ghate, D. Manohar, V. Kulkarni, R. Shobha, and S. Y. Kattimani, “Synthesis of vanillin ethers from 4-(bromomethyl) coumarins as anti-inflammatory agents,” *Eur. J. Med. Chem.*, 2003, doi: 10.1016/S0223-5234(03)00016-3.
67. M. Ghate, R. A. Kusanur, and M. V. Kulkarni, “Synthesis and in vivo analgesic and anti-inflammatory activity of some bi heterocyclic coumarin derivatives,” *Eur. J. Med. Chem.*, 2005, doi: 10.1016/j.ejmech.2005.03.025.
68. Z. M. Nofal, M. I. El-Zahar, and S. S. Abd El-Karim, “Novel coumarin derivatives with expected biological activity,” *Molecules*, 2000, doi: 10.3390/50200099.
69. T. A. De Almeida Barros *et al.*, “Antinociceptive and anti-inflammatory properties of 7-hydroxycoumarin in experimental animal models: potential therapeutic for the control of inflammatory chronic pain,” *J. Pharm. Pharmacol.*, vol. 62, no. 2, pp. 205–213, 2010, doi: 10.1211/jpp.62.02.0008.
70. C. Riviere, L. Goossens, N. Pommery, C. Fourneau, A. Delelis, and J. P. Henichart, “Antiproliferative effects of isopentenylated coumarins isolated from Phellolophium madagascariense Baker,” *Nat. Prod. Res.*, vol. 20, no. 10, pp. 909–916, 2006, doi: 10.1080/14786410500277787.
71. A. Manvar *et al.*, “Synthesis, anti-tubercular activity and 3D-QSAR study of coumarin-4-acetic acid benzylidene hydrazides,” *Eur. J. Med. Chem.*, vol. 43, no. 11, pp. 2395–2403, 2008, doi: https://doi.org/10.1016/j.ejmech.2008.01.016.
72. J. F. Vasconcelos *et al.*, “Effects of umbelliferone in a murine model of allergic airway inflammation,” *Eur. J. Pharmacol.*, vol. 609, no. 1–3, pp. 126–131, 2009, doi: 10.1016/j.ejphar.2009.03.027.
73. S. Hamulakova, M. Kozurkova, and K. Kuca, “Coumarin Derivatives in Pharmacotherapy of Alzheimer´s Disease,” *Curr. Org. Chem.*, vol. 21, no. 7, pp. 602–612, 2017, doi: 10.2174/1385272820666160601155411.
74. A. Husain, A. Balushi K, M. J. Akhtar, and S. A. Khan, “Coumarin linked heterocyclic hybrids: A promising approach to develop multi target drugs for Alzheimer’s disease,” *J. Mol. Struct.*, vol. 1241, p. 130618, 2021, doi: 10.1016/j.molstruc.2021.130618.
75. P. Anand, B. Singh, and N. Singh, “A review on coumarins as acetylcholinesterase inhibitors for Alzheimer’s disease,” *Bioorganic Med. Chem.*, vol. 20, no. 3, pp. 1175–1180, Feb. 2012, doi: 10.1016/j.bmc.2011.12.042.
76. A. Kumar, M. K. Gupta, and M. Kumar, “An efficient non-ionic surfactant catalyzed multicomponent synthesis of novel benzylamino coumarin derivative via Mannich type reaction in aqueous media,” *Tetrahedron Lett.*, vol. 52, no. 35, pp. 4521–4525, Aug. 2011, doi: 10.1016/j.tetlet.2011.06.040.
77. A. Parvez *et al.*, “Pharmacophores modeling in terms of prediction of theoretical physico-chemical properties and verification by experimental correlations of novel coumarin derivatives produced via Betti’s protocol,” *Eur. J. Med. Chem.*, vol. 45, no. 9, pp. 4370–4378, Sep. 2010, doi: 10.1016/j.ejmech.2010.06.004.
78. L. Piazzi *et al.*, “3-(4-{[benzyl(methyl)amino]methyl}-phenyl)-6,7-dimethoxy-2H-2-chromenone (AP2238) inhibits both acetylcholinesterase and acetylcholinesterase-induced β-amyloid aggregation: A dual function lead for Alzheimer’s disease therapy,” *J. Med. Chem.*, vol. 46, no. 12, pp. 2279–2282,

2003, doi: 10.1021/jm0340602.
79. T. Mohamed, W. Osman, G. Tin, and P. P. N. Rao, “Selective inhibition of human acetylcholinesterase by xanthine derivatives: In vitro inhibition and molecular modeling investigations,” *Bioorganic Med. Chem. Lett.*, vol. 23, no. 15, pp. 4336–4341, Aug. 2013, doi: 10.1016/j.bmcl.2013.05.092.
80. B. Kaboudin, M. Arefi, S. Emadi, and V. Sheikh-Hasani, “Synthesis and inhibitory activity of ureidophosphonates, against acetylcholinesterase: Pharmacological assay and molecular modeling,” *Bioorg. Chem.*, vol. 41–42, pp. 22–27, Apr. 2012, doi: 10.1016/j.bioorg.2012.01.003.
81. P. Anand and B. Singh, “Synthesis and evaluation of novel carbamate-substituted flavanone derivatives as potent acetylcholinesterase inhibitors and anti-amnestic agents,” *Med. Chem. Res.*, vol. 22, no. 4, pp. 1648–1659, Apr. 2013, doi: 10.1007/s00044-012-0162-3.
82. S. Yılmaz *et al.*, “Synthesis and inhibitory properties of some carbamates on carbonic anhydrase and acetylcholine esterase,” *J. Enzyme Inhib. Med. Chem.*, vol. 31, no. 6, pp. 1484–1491, Nov. 2016, doi: 10.3109/14756366.2016.1149477.
83. X.-Y. Y. Jiang *et al.*, “Dual GSK-3β/AChE Inhibitors as a New Strategy for Multitargeting Anti-Alzheimer’s Disease Drug Discovery,” *ACS Med. Chem. Lett.*, vol. 9, no. 3, pp. 171–176, Mar. 2018, doi: 10.1021/acsmedchemlett.7b00463.
84. B. Z. Kurt *et al.*, *Potential of aryl-urea-benzofuranylthiazoles hybrids as multitasking agents in Alzheimer’s disease*, vol. 102. Elsevier Ltd, 2015.
85. B. Z. Kurt, I. Gazioglu, F. Sonmez, and M. Kucukislamoglu, “Synthesis, antioxidant and anticholinesterase activities of novel coumarylthiazole derivatives,” *Bioorg. Chem.*, vol. 59, pp. 80–90, 2015, doi: 10.1016/j.bioorg.2015.02.002.
86. M. Shidore *et al.*, “Benzylpiperidine-Linked Diarylthiazoles as Potential Anti-Alzheimer’s Agents: Synthesis and Biological Evaluation,” *J. Med. Chem.*, vol. 59, no. 12, pp. 5823–5846, 2016, doi: 10.1021/acs.jmedchem.6b00426.
87. S. Liu, R. Shang, L. Shi, D. C. C. Wan, and H. Lin, “Synthesis and biological evaluation of 7H-thiazolo[3,2-b]-1,2,4-triazin-7- one derivatives as dual binding site acetylcholinesterase inhibitors,” *Eur. J. Med. Chem.*, vol. 81, pp. 237–244, 2014, doi: 10.1016/j.ejmech.2014.05.020.
88. W. Luo *et al.*, “Design, synthesis and evaluation of novel 4-dimethylamine flavonoid derivatives as potential multi-functional anti-Alzheimer agents,” *Bioorganic Med. Chem.*, vol. 21, no. 23, pp. 7275–7282, 2013, doi: 10.1016/j.bmc.2013.09.061.
89. B. Tasso *et al.*, “Quinolizidinyl derivatives of bi- and tricyclic systems as potent inhibitors of acetyl- and butyrylcholinesterase with potential in Alzheimer’s disease,” *Eur. J. Med. Chem.*, vol. 46, no. 6, pp. 2170–2184, Jun. 2011, doi: 10.1016/j.ejmech.2011.02.071.
90. S. S. Xie, X. B. Wang, J. Y. Li, L. Yang, and L. Y. Kong, “Design, synthesis and evaluation of novel tacrine-coumarin hybrids as multifunctional cholinesterase inhibitors against Alzheimer’s disease,” *Eur. J. Med. Chem.*, vol. 64, pp. 540–553, 2013, doi: 10.1016/j.ejmech.2013.03.051.
91. S. Liu, R. Shang, L. Shi, D. C. C. Wan, and H. Lin, “Synthesis and biological evaluation of 7H-thiazolo[3,2-b]-1,2,4-triazin-7- one derivatives as dual binding site acetylcholinesterase inhibitors,” *Eur. J. Med. Chem.*, vol. 81, pp. 237–244, Jun. 2014, doi: 10.1016/j.ejmech.2014.05.020.

92. F. Mao, J. Li, H. Wei, L. Huang, and X. Li, “Tacrine-propargylamine derivatives with improved acetylcholinesterase inhibitory activity and lower hepatotoxicity as a potential lead compound for the treatment of Alzheimers disease,” *J. Enzyme Inhib. Med. Chem.*, vol. 30, no. 6, pp. 995–1001, Nov. 2015, doi: 10.3109/14756366.2014.1003212.
93. M. L. Bolognesi *et al.*, “Multi-target-directed drug design strategy: From a dual binding site acetylcholinesterase inhibitor to a trifunctional compound against Alzheimer’s disease,” *J. Med. Chem.*, vol. 50, no. 26, pp. 6446–6449, Dec. 2007, doi: 10.1021/jm701225u.
94. M. Gümüş, M. Yakan, and I. Koca, “Recent advances of thiazole hybrids in biological applications,” *Future Med. Chem.*, vol. 11, no. 16, pp. 1979–1998, 2019, doi: 10.4155/fmc-2018-0196.
95. L. G. de Souza, M. N. Rennã, and J. D. Figueroa-Villar, “Coumarins as cholinesterase inhibitors: A review,” *Chem. Biol. Interact.*, vol. 254, pp. 11–23, 2016, doi: 10.1016/j.cbi.2016.05.001.
96. M. Ignasik, M. Bajda, N. Guzior, M. Prinz, U. Holzgrabe, and B. Malawska, “Design, synthesis and evaluation of novel 2-(Aminoalkyl)-isoindoline-1,3-dione derivatives as dual-binding site acetylcholinesterase inhibitors,” *Arch. Pharm. (Weinheim).*, vol. 345, no. 7, pp. 509–516, 2012, doi: 10.1002/ardp.201100423.
97. T. Mohamed, W. Osman, G. Tin, and P. P. N. Rao, “Bioorganic & Medicinal Chemistry Letters Selective inhibition of human acetylcholinesterase by xanthine derivatives : In vitro inhibition and molecular modeling investigations,” *Bioorg. Med. Chem. Lett.*, vol. 23, no. 15, pp. 4336–4341, 2013, doi: 10.1016/j.bmcl.2013.05.092.
98. S. Hamulakova *et al.*, “Targeting copper(II)-induced oxidative stress and the acetylcholinesterase system in Alzheimer’s disease using multifunctional tacrine-coumarin hybrid molecules,” *J. Inorg. Biochem.*, no. Ii, 2016, doi: 10.1016/j.jinorgbio.2016.05.001.
99. S. Hamulakova, L. Janovec, O. Soukup, D. Jun, and K. Kuca, “Synthesis, in vitro acetylcholinesterase inhibitory activity and molecular docking of new acridine-coumarin hybrids,” *Int. J. Biol. Macromol.*, vol. 104, pp. 333–338, 2017, doi: 10.1016/j.ijbiomac.2017.06.006.
100. S. Hamulakova *et al.*, “Tacrine-coumarin and Tacrine-7-chloroquinoline Hybrids with Thiourea Linkers: Cholinesterase Inhibition Properties, Kinetic Study, Molecular Docking and Permeability Assay for Blood-brain Barrier,” *Curr. Alzheimer Res.*, vol. 15, no. 12, pp. 1096–1105, 2018, doi: 10.2174/1567205015666180711110750.
101. M. Fereidoonnezhad, A. Mostoufi, M. Eskandari, S. Zali, and F. Aliyan, “Multitarget drug design, molecular docking and PLIF studies of novel Tacrine−Coumarin hybrids for the treatment of Alzheimer’s disease,” *Iran. J. Pharm. Res.*, vol. 17, no. 4, pp. 1217–1228, Jun. 2018, doi: 10.22037/ijpr.2018.2308.
102. Z. Najafi *et al.*, “Novel tacrine-coumarin hybrids linked to 1,2,3-triazole as anti-Alzheimer’s compounds: In vitro and in vivo biological evaluation and docking study,” *Bioorg. Chem.*, vol. 83, no. August 2018, pp. 303–316, 2019, doi: 10.1016/j.bioorg.2018.10.056.
103. Q. Sun, D. Y. Peng, S. G. Yang, X. L. Zhu, W. C. Yang, and G. F. Yang, “Syntheses of coumarin-tacrine hybrids as dual-site acetylcholinesterase inhibitors and their activity against butylcholinesterase, Aβ aggregation, and

β-secretase," *Bioorganic Med. Chem.*, vol. 22, no. 17, pp. 4784–4791, Sep. 2014, doi: 10.1016/j.bmc.2014.06.057.
104. P. P. Babitha, M. M. Sahila, S. Bandaru, and S. Sureshkumar, "Molecular Docking and Pharmacological Investigations of Rivastigmine-Fluoxetine and Coumarin – Tacrine hybrids against Acetyl Choline Esterase," vol. 11, no. 8, pp. 378–386, 2015.
105. S. S. Xie *et al.*, "Multi-target tacrine-coumarin hybrids: Cholinesterase and monoamine oxidase B inhibition properties against Alzheimer's disease," *Eur. J. Med. Chem.*, vol. 95, pp. 153–165, 2015, doi: 10.1016/j.ejmech.2015.03.040.
106. T. Sugino and K. Tanaka, "Solvent-free coumarin synthesis," *Chem. Lett.*, no. 2, pp. 110–111, 2001, doi: 10.1246/cl.2001.110.
107. C. F. Koelsch, "Bromination of 3-Acetocoumarin," *J. Am. Chem. Soc.*, vol. 72, no. 7, pp. 2993–2995, Jul. 1950, doi: 10.1021/ja01163a053.
108. [108] I. O. Zhuravel, S. M. Kovalenko, S. V Vlasov, and V. P. Chernykh, "Solution-phase Synthesis of a Combinatorial Library of 3-[4- (Coumarin-3-yl)-1,3-thiazol-2-ylcarbamoyl]propanoic acid Amides," *molecules*, vol. 10, pp. 444–456, 2005.
109. L. Gorecki *et al.*, "Structure-activity relationships of dually-acting acetylcholinesterase inhibitors derived from tacrine on N-methyl-D-Aspartate receptors," *Eur. J. Med. Chem.*, vol. 219, p. 113434, 2021, doi: 10.1016/j.ejmech.2021.113434.
110. P. Trivedi *et al.*, "Design, synthesis, biological evaluation and molecular docking study of arylcarboxamido piperidine and piperazine-based hydroxamates as potential HDAC8 inhibitors with promising anticancer activity," *Eur. J. Pharm. Sci.*, vol. 138, no. May, p. 105046, 2019, doi: 10.1016/j.ejps.2019.105046.
111. G. L. Ellman, K. D. Courtney, V. Andres, and R. M. Featherstone, "A new and rapid colorimetric determination of acetylcholinesterase activity," *Biochem. Pharmacol.*, vol. 7, no. 2, pp. 88–95, 1961, doi: 10.1016/0006-2952(61)90145-9.
112. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
113. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. *International Journal of Health Sciences*, *5*(2), vi-ix. https://doi.org/10.53730/ijhs.v5n2.2937