Bioorganic Chemistry 110 (2021) 104792

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Design and synthesis of novel coumarin derivatives as potential acetylcholinesterase inhibitors for Alzheimer's disease

Kamilia M. Amin, Doaa E. Abdel Rahman, Heba Abdelrasheed Allam, Haidy H. El-Zoheiry *

Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St., Cairo 11562, Egypt

ARTICLE INFO

Keywords:
Coumarin
Acetylcholinesterase
Neuroprotective
Alzheimer's disease

ABSTRACT

Twenty novel 7-benzyloxycoumarin based compounds were synthesized with a variety of bioactive chemical fragments. The synthesized compounds showed remarkable acetylcholinesterase (AChE) inhibitory activity. *In vitro* assay revealed that compounds 7-benzyloxy-4-{[(4-phenylthiazol-2(3*H*)-ylidene)hydrazono]methyl}-2*H*-chromen-2-one (**5b**, IC_{50}= 0.451μM), 7-benzyloxy-4-({[4-(4-methoxyphenyl)thiazol-2(3*H*)-ylidene]hydrazono}methyl)-2*H*-chromen-2-one (**5d,** IC_{50}= 0.625μM), 5-amino-1-[2-(7-benzyloxy-2-oxo-2*H*-chromen-4-yl)acetyl]-1*H*-pyrazole-4-carbonitrile (**13c**, IC_{50}= 0.466μM), 2-(7-benzyloxy-2-oxo-2*H*-chromen-4-yl)-*N*-(2-methylimino-4-phenylthiazol-3(2*H*)-yl)acetamide (**16a,** IC_{50}= 0.500μM) and 2-(7-benzyloxy-2-oxo-2*H*-chromen-4-yl)-*N*-[4-(4-methoxyphenyl)-2-methyliminothiazol-3(2*H*)-yl]acetamide (**16b,** IC_{50}= 0.590μM) exhibited promising AChE inhibitory activity even better than donepezil (IC_{50}= 0.711μM). Kinetic study for compound **5b** implied mixed type inhibitor which could bind peripheral anionic site (PAS) and catalytic active site (CAS) of AChE enzyme. In addition, *in vivo* evaluation of compounds **5b, 13c** and **16a** confirmed significant memory improvement in scopolamine-induced impairment model in tested mice. Furthermore, *in silico* studies were performed on the synthesized compounds which included molecular docking study at the active site of recombinant human acetylcholinesterase enzyme (rhAChE) as well as prediction of ADMET and other physicochemical parameters. A correlation between the docking results and IC_{50} of tested compounds was routinely observed and shared similar binding pattern to the co-crystallized ligand donepezil.

1. Introduction

Alzheimer's disease (AD) is a dramatically neurodegenerative brain disorder that is the driving inducer of dementia and is potentially fatal [1]. Decline in memory, language, thinking skills and other cognitive functions are considered as the distinctive symptoms of dementia [2]. This deterioration is due to damage to nerve cells in parts of the brain that are involved in cognitive function. Unfortunately, certain parts of the brain that allow a person to perform basic physical functions such as swallowing and walking are also impaired. As a result, people in the final stages of AD are bed-bound and require around-the-clock care [3]. Moreover, more than 18 million of the world population are projected to experience AD and numbers of patients will rise to 70 million by 2050 [4]. Officially, AD is ranked as the sixth trigger for death in the United States based on Alzheimer's association report in 2018 [3]. Consequently, AD is gaining overdue attention by researchers. Various parameters have been recognized to be involved in the etiology of AD. Deposits of abnormal proteins such as amyloid beta peptides (Aβ) and tau proteins besides cholinergic degeneration in the brain neurons are considered as the potential drivers of AD [5]. It is thought that the Aβ's accumulation interferes with the neuron-to-neuron communication at synapses which lead to cell death. In addition, tau tangles block nutrient and other essential molecules transport within neurons and also contribute to cell death [6]. On the other hand, around 30 years ago the cholinergic theory was conceived. It stated that, substantial loss of cholinergic activity in the central nervous system (CNS) contributed significantly to AD-associated cognitive impairment. This loss in cholinergic function is due to deficiency in choline acetyltransferase enzyme (ChAT) which is charged with the synthesis of acetylcholine (ACh) in addition to the reduction of choline uptake and ACh release [7,8]. Since a cholinergic hypothesis has been postulated as a crucial element in AD symptoms etiology, acetylcholinesterase inhibitors (AChEIs) have been the mainstay drugs used against AD until today [9]. So, AChEIs like donepezil, rivastigmine and galantamine are used presently in clinics for the symptomatic treatment of AD [10]. It seems however necessary today to complete this symptomatic approach with a

* Corresponding author.
E-mail address: haidi.abdelhamid@pharma.cu.edu.eg (H.H. El-Zoheiry).

https://doi.org/10.1016/j.bioorg.2021.104792
Received 16 November 2020; Received in revised form 23 February 2021; Accepted 2 March 2021
Available online 6 March 2021

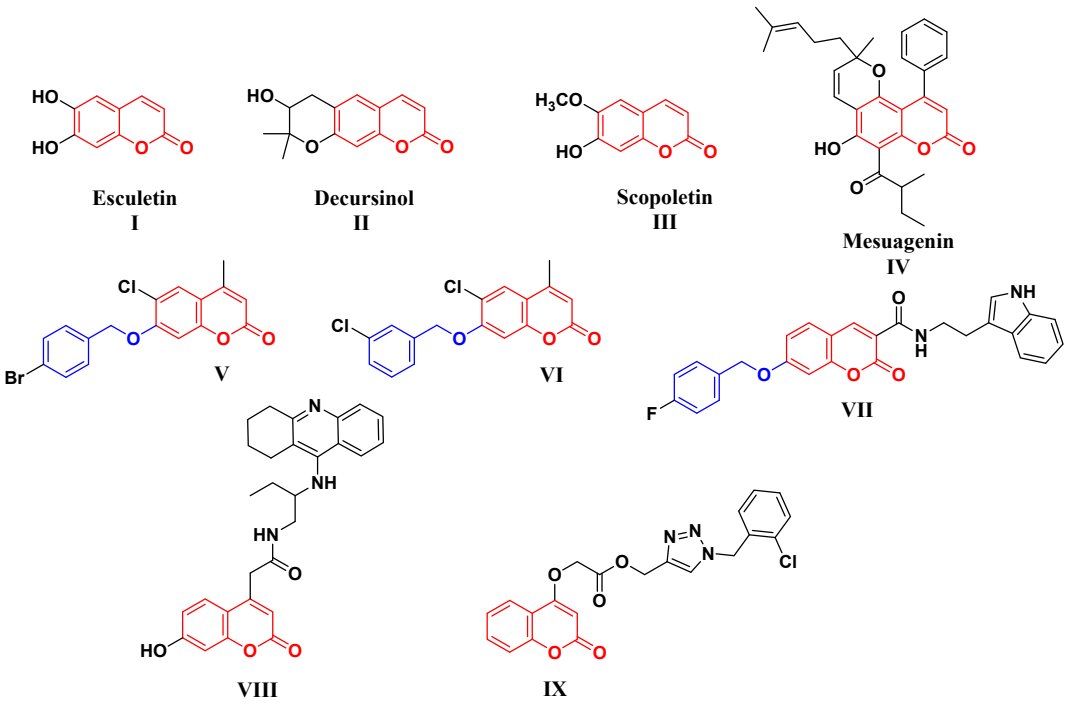


Fig. 1. The structure of different natural and synthetic coumarin compounds with potential AChE inhibitory activity.

more disease-modifying drugs [11]. Therefore, dual binding site AChEIs appear as promising anti-AD agents owing to their ability to restore the cholinergic deficit by inhibiting the catalytic active site (CAS) of AChE enzyme and at the same time to reduce the Aβ deposition and aggregation through their interaction with the peripheral anionic site (PAS) of the enzyme [12]. So, the design of dual binding site AChEIs could demonstrate an effective therapeutic approach for the symptomatic treatment of AD and delaying its development.

Interestingly, extensive researches have been directed towards the identification of AChEIs with the majority of them arising from the plant kingdom. There have also been many attempts to obtain semi-synthetic or synthetic derivatives from these naturally derived AChEIs aiming to improve the inhibitory potency and selectivity in addition to attaining dual modes of action relevant to AD therapy [13].

Coumarin, 2*H*-1-benzopyran-2-one, is a core structure in various natural products and synthetic compounds displaying a diverse array of biological activities such as anticancer [14,15], antioxidant, anti-aflatoxigenic [16], carbonic anhydrase inhibitory activity [17], antioxidant activity [18] in addition to AChE inhibitory activity. Moreover, the fact that chemical substitutions can occur at many positions of this core structure, has made coumarins interesting molecules for drug discovery in the field of AChEIs [19]. Among these natural products many coumarin derivatives such as esculetin **I**, decursinol **II**, scopoletin **III** and mesuagenin **IV** [20]. It has been already reported that the use of benzyloxy group at position 7 of coumarin scaffold has a great effect on AChE inhibitory activity as found in compounds **V**, **VI** [21] and **VII** [22]. A closer examination of binding mode of compound **VII** revealed that the 4-fluorophenyl ring formed a π-π stacking with Trp86 which may be contributed to the increase in AChE inhibitory activity [22]. Moreover, researches revealed that, substitution at position 4 of coumarin emphasize its AChE inhibitory activity as found in compounds **VIII** [13] and **IX** [23] Fig. 1.

Furthermore, literature review indicated that pharmacologically active heterocyclic rings such as thiazolines [24,25], thiazolidinones [24,25], cyclic imides [26,27], triazoles [28] and pyrazoles [29,30] were recognized in numerous AChEIs. In the same vein, from structural point of view versatile linkers especially nitrogenous linkers were incorporated in potent AChEIs. Among these linkers hydrazonomethyl [31], acetamide [32] or acetohydrazide [33].

Based on the above cited findings and with the aim to identify compounds with potent AChE inhibitory activity and safety profile for AD, it was considered valuable to present new compounds as promising potent AChEIs, having the 7-benzyloxycoumarin scaffold substituted with different distinct heterocycles at position 4 *via* appropriate linkers. These linkers are intended to stabilize the molecule inside the gorge of AChE enzyme and adjust the distance between PAS and CAS binding moieties, Fig. 2. The synthesized derivatives were tested for their AChE inhibitory activity and the promising compounds were subjected to *in*

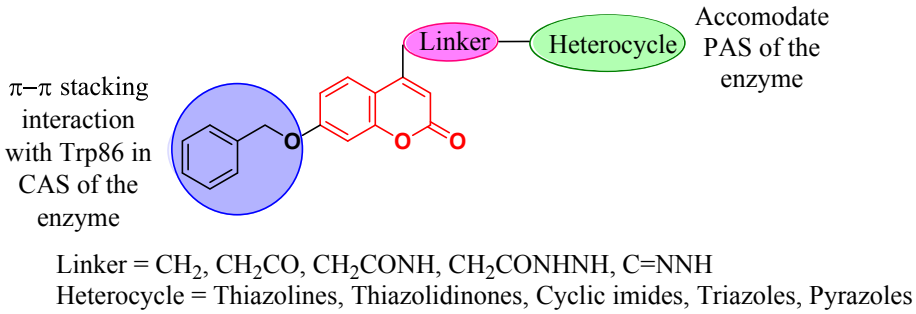
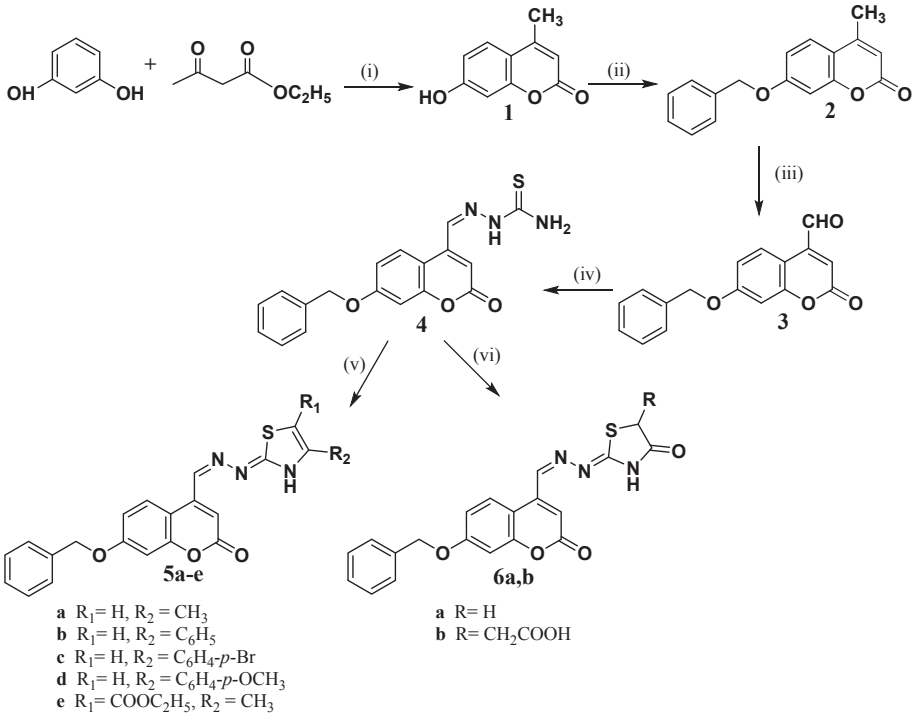


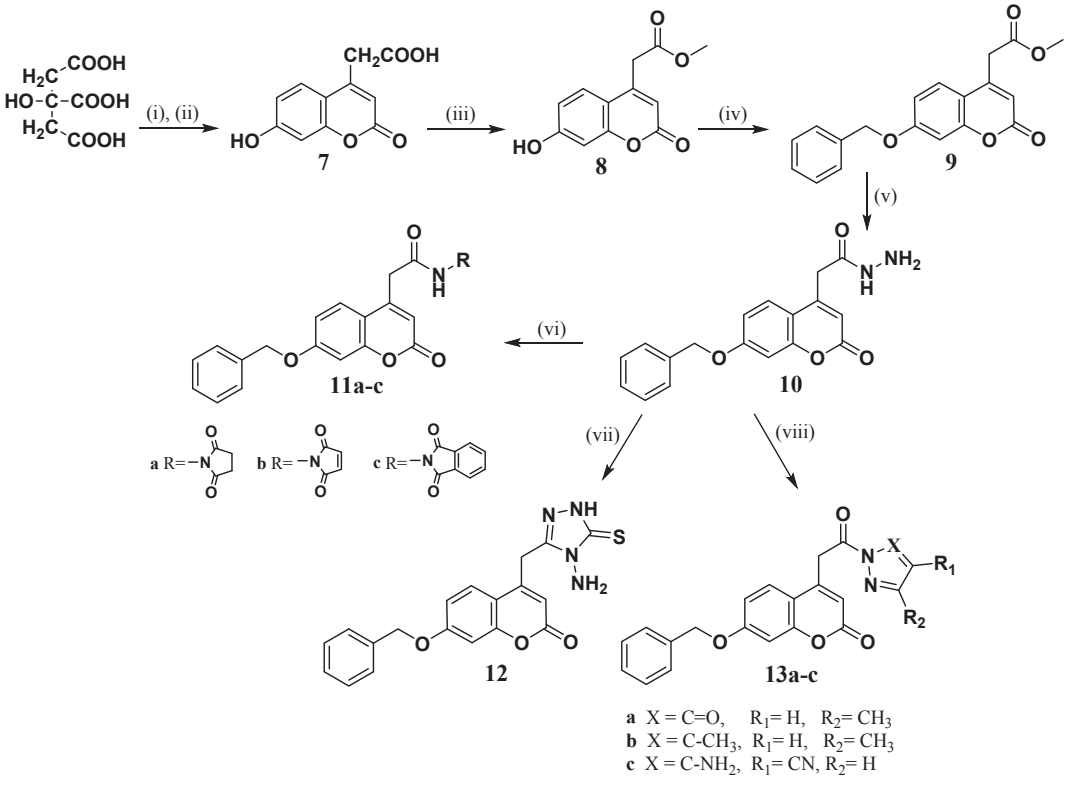
Fig. 2. Development strategy of the new compounds.



a R_1= H, R_2 = CH_3
b R_1= H, R_2 = C_6H_5
c R_1= H, R_2 = C_6H_4-*p*-Br
d R_1= H, R_2 = C_6H_4-*p*-OCH_3
e R_1= $COOC_2H_5$, R_2 = CH_3

a R= H
b R= CH_2COOH

Scheme 1. Reagents and conditions: (i) Sulfuric acid, stir, 30 min., store at 0 °C, 48 h; (ii) Benzyl chloride, potassium carbonate, acetone, reflux, 24 h; (iii) Selenium dioxide, xylene, reflux, 24 h; (iv) Thiosemicarbazide, glacial acetic acid, benzene/ethanol 1:1 mixture, reflux, 10 h; (v) α-halocarbonyl compounds (chloroacetone or appropriate phenacyl bromides or ethyl 2-chloroacetoacetate), anhydrous sodium acetate, ethanol, reflux, 48 h; (vi) Ethyl bromoacetate or maleic anhydride, anhydrous sodium acetate, ethanol, reflux, 48 h.



a R=
b R=
c R=

a X = C=O, R_1= H, R_2= CH_3
b X = C-CH_3, R_1= H, R_2= CH_3
c X = C-NH_2, R_1= CN, R_2= H

Scheme 2. Reagents and conditions: (i) Sulfuric acid, stir, 1 h, heat 70 °C, cool, (ii) Resorcinol, sulfuric acid, store at 0 °C, 16 h; (iii) Methanol, sulfuric acid, reflux, 10 h; (iv) Benzyl chloride, potassium carbonate, acetone, reflux, 24 h; (v) Hydrazine hydrate, methanol, r.t., 16 h; (vi) Appropriate cyclic acid anhydride, glacial acetic acid, reflux, 48 h; (vii) CS_2, KOH, ethanol, r.t., 12 h, hydrazine hydrate, ethanol, reflux, 24 h; (viii) Ethyl acetoacetate or acetylacetone or ethoxymethylene malononitrile, anhydrous sodium acetate, ethanol, reflux, 48 h.

vivo neurobehavioral assessment. Furthermore, the binding mode of the synthesized compounds was revealed through *in silico* molecular modeling study

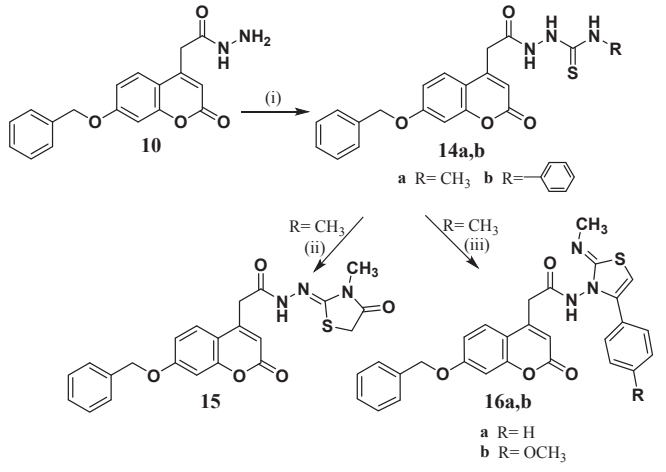
2. Results and discussion

2.1. Chemistry

In this work, synthesis of the target coumarin derivatives **5a-e**, **6a,b, 11a-c, 12, 13a-c, 15** and **16a,b** were achieved by different routes as illustrated in Schemes 1–3 using the key intermediates 1-[(7-benzyloxy-2-oxo-2*H*-chromen-4-yl)methylene]thiosemicarbazide **4** in Scheme 1 and 2-(7-benzyloxy-2-oxo-2*H*-chromen-4-yl)acetohydrazide **10** in Schemes 2 and 3.

In Scheme 1, the synthesis of the target thiazolines **5a-e** were adopted according to Hantzsch reaction in which an α-halocarbonyl compounds, namely chloroacetone, appropriate phenacyl bromides and ethyl 2-chloroacetoacetate, react with a compound bearing the N_C_S fragment such as thiosemicarbazone derivative **4**. ^{1}H NMR spectra of compounds **5a-d** revealed the characteristic CH proton of thiazoline ring at δ= 7.30–7.56 ppm. Cyclization of thiosemicarbazone derivative **4** with an α-haloester, ethyl bromoacetate, yielded the corresponding thiazolidinone derivative **6a** that was featured by presence of singlet signal at δ= 3.99 ppm corresponding to CH_2 protons of thiazolidinone ring in its ^{1}H NMR spectrum. Thia-Michael addition or conjugate (1,4) addition was adopted between maleic anhydride as Michael acceptor and thiosemicarbazone derivative **4** as Michael donor with the aid of sodium acetate as basic catalyst to obtain thiazolidinone derivative **6b** which showed a triplet signal corresponding to thiazolidinone CH proton at δ= 4.48 ppm in its ^{1}H NMR spectrum. The appearance of D_2O exchangeable NH of compounds **5a-e** and **6a,b** in the region δ 11.84–12.88 and 12.30–12.43, respectively, supported their assigned structure in Scheme 1 and excluded their possible tautomeric hydrazone structure.

In scheme 2, the reaction of appropriate cyclic anhydrides with the key acetohydrazide intermediate **10** yields derivatives **11a-c** which were structurally established by the disappearance of NH_2 singlet signals in ^{1}H NMR spectra. The 1,2,4-triazole derivative **12** was obtained from the acetohydrazide derivative **10,** carbon disulfide and ethanolic potassium hydroxide which lead to the formation of potassium dithiocarbazate salt then undergoes ring-closure by reaction with hydrazine hydrate in absolute ethanol. The target compound **12** was confirmed by the presence of C═S carbon signal at 166.45 ppm in the ^{13}C NMR spectrum. The target derivatives **13a,b** were obtained through condensation of the key acetohydrazide intermediate **10** with ethyl acetoacetate and acetylacetone, respectively. ^{1}H NMR spectra displayed singlet signal at 7.19 ppm attributed to pyrazolinone CH in compound **13a** spectrum and extra aromatic proton of pyrazole ring at 6.39 ppm in compound **13b** spectrum. Finally, synthesis of the pyrazole-4-carbonitrile derivative **13c** can be explained by the initial Michael addition in which the acetohydrazide derivative **10** which acts as Michael donor attacks β-carbon of ethoxymethylene malononitrile which acts as Michael acceptor. The structure of compound **13c** was ascertained by the existence of the CN characteristic band at 2206 cm^{-1} in its IR spectrum.



Scheme 3. Reagents and conditions: (i) Appropriate isothiocyanate derivatives, ethanol, reflux, 12 h; (ii) Ethyl bromoacetate, anhydrous sodium acetate, ethanol/chloroform 1:1 mixture, reflux, 48 h; (iii) Appropriate phenacyl bromide, ethanol/chloroform 1:1 mixture, reflux, 48 h.

In Scheme 3, the acetohydrazide key intermediate **10** reacted with appropriate isothiocyanates and yielded acylthiosemicarbazide derivatives **14a,b**. Afterwards, derivative **14a** underwent cyclization reaction with ethyl bromoacetate in 1:1 mixture of absolute ethanol/chloroform in presence of anhydrous sodium acetate to yield thiazolidinone derivative **15** which was confirmed by the appearance of thiazolidinone CH_2 protons at 3.97 ppm. On the other hand, condensation reaction of acylthiosemicarbazide derivative **14a** and appropriate phenacyl bromides in presence of anhydrous sodium acetate according to Hantzsch thiazoline synthesis gave derivatives **16a,b**. The IR spectra of the target derivatives **16a,b** revealed the disappearance of the 2NH and C═S bands of the acylthiosemicarbazide derivative **14a**.

2.2. Biological evaluation

2.2.1. In vitro biological studies

2.2.1.1. In vitro AChE inhibition assay. AChE inhibitory activities of the synthesized compounds were carried out according to an improved Ellman's method and calculated using Graphpad prism 8 software [34] and the results are proposed as IC_{50}±standard deviation (SD) in (Table 1). Results showed that the synthesized compounds showed good AChE inhibition in micromolar range (0.451–3.588μM) compared to donepezil as reference standard (IC_{50}= 0.711μM).

From the above mentioned IC_{50} results it was concluded that the thiosemicarbazone derivative **4** showed good AChE inhibitory activity and upon cyclization to 4-substituted thiazolines as in compounds **5a-d** better activity was achieved while cyclization to 4,5-disubstituted thiazoline or *N*-unsubstituted thiazolidin-4-one abolished activity as in compounds **5e** and **6a,b**, respectively. In addition, the 4-phenyl substituted thiazoline moiety as in compounds **5b-d** and **16a,b** exhibited better activity than the 4-methyl substituted thiazoline compound **5a** and even parent thiosemicarbazone compound **4**. Unsubstituted phenyl compound **5b** showed better activity than *p*-methoxy substituted one **5d** while *p*-bromophenyl compound **5c** showed comparable activity to 4-methylthiazoline compound **5a**. Cyclization of the acetohydrazide compound **10** to cyclic imides resulted in an active pyrrolidin-2,5-dione derivative **11a** and inactive derivatives 2,5-dihydropyrrole-2,5-dione

Table 1
IC_{50} values of the tested compounds and donepezil.

Compound No.	IC_{50} ± SD* (μM)	Compound No.	IC_{50} ± SD* (μM)
4	0.778 ± 0.022	**12**	0.722 ± 0.022
5a	1.911 ± 0.053	**13a**	3.278 ± 0.079
5b	0.451 ± 0.012	**13b**	1.419 ± 0.048
5c	1.812 ± 0.061	**13c**	0.466 ± 0.016
5d	0.625 ± 0.015	**14a**	1.720 ± 0.062
5e	2.272 ± 0.083	**14b**	3.213 ± 0.107
6a	2.310 ± 0.082	**15**	0.826 ± 0.021
6b	3.242 ± 0.067	**16a**	0.500 ± 0.018
11a	1.098 ± 0.066	**16b**	0.590 ± 0.013
11b	2.316 ± 0.057	**Donepezil**	0.711 ± 0.017
11c	3.588 ± 0.078		

* All results were found to be statistically significant at $P < 0.05$.

11b and isoindol-1,3-dione **11c**. Cyclization of the acetohydrazide compound **10** to 1,2,4-triazole compound **12** and pyrazole-4-carbonitrile compound **13c** provided good AChE inhibitory activity while activity of 3,5-dimethylpyrazole compound **13b** was moderate. On the contrary, cyclization to 3-methylpyrazolin-5-one **13a** yielded inactive compound. Cyclization of methyl substituted acylthiosemicarbazide derivative **14a** to 4-phenylthiazoline derivative produced active compounds **16a,b** that were more active than the *N*-methylthiazolidin-4-one cyclized product **15**. As observed before, unsubstituted phenyl compound **16a** showed better activity than *p*-methoxy substituted one **16b**. Briefly, it was observed that 4-methyl and phenyl thiazolines were active and unsubstituted phenyl was more active than 4-methoxyphenyl while 4,5-disubstituted thiazolines were inactive. In addition, pyrazole-4-carbonitrile, 1,2,4-triazole and pyrrolidine-2,5-dione were active. Moreover, thiosemicarbazone, methyl substituted acylthiosemicarbazide were active while phenyl substituted acylthiosemicarbazide was inactive. Furthermore, *N*-methylthiazolidin-4-one was active while the unsubstituted thiazolidin-4-ones were inactive. Interestingly, compounds **5b,d**, **13c** and **16a,b** showed better activity than donepezil.

2.2.1.2. Kinetic study of AChE inhibition. To investigate the AChE inhibitory mechanism for the synthesized compounds, the most potent compound **5b** was selected for kinetic study following the modified Ellman's method.[34] The overlaid reciprocal Lineweaver-Burk plots **(**Fig. 3**)** showed increasing slopes and increasing intercepts at increasing inhibitor concentration. This pattern suggested a mixed-type inhibition and hence concluded that compound **5b** might be eligible for binding to PAS as well as CAS of AChE enzyme.

2.2.2. In vivo behavioral studies on scopolamine-induced dementia model

Compounds **5b**, **13c** and **16a** that showed promising *in vitro* activity were further evaluated in AD animal models. The *in vivo* scopolamine model was used to evaluate the anticholinesterase activity in animals. Two behavioral tests were conducted Y-Maze [35] and Passive avoidance [36] using donepezil as positive control.

Y-Maze test results (Fig. 4**)**, mice in model group exhibited mean % alternations = 52.19 that is lower than mean % alternation showed by control group = 73.42 with P= 0.0077. Moreover, donepezil treated mice showed significant change in mean % alternations from 52.19 to 68.42 with P= 0.0487. On the other hand, treatment with compound **5b** exhibited a significant increase in mean % alternation from 52.19 to 70.51 with P= 0.0004. While treatment with compound **13c** showed significant increase in mean % alternations from 52.19 to 63.12 with P= 0.0266. Finally, compound **16a** increased mean % alternation significantly from 52.19 to 66.69 with P= 0.0464.

Passive avoidance test results (Fig. 5**)**, results showed that mice in model group showed shortness in mean transfer latency time (TLT = 217.5 s) compared to mice in control group (mean TLT = 360.5 s) with P < 0.0001. While donepezil group exhibited increase in mean TLT from 217.5 s to 336.0 s with P= 0.0001. In addition, treated group with compound **5b** reverse the lowered scopolamine mean TLT from 217.5 s to 301.3 s with P= 0.0104. Treated group with compound **13c** increased mean TLT from 217.5 s to 350.0 s with P = 0.0014. Furthermore, compound **16a** exhibited longer mean TLT from 217.5 s to 353.3 s with P= 0.0011.

In conclusion, a novel series of coumarin derivatives were assessed for their *in vitro* anticholinesterase activities and *in vivo* model using Y-maze and passive avoidance tests. *In vitro* assay revealed that compounds **5b** (IC_{50}= 0.451μM), **5d** (IC_{50}= 0.625μM), **13c** (IC_{50}= 0.466μM) and **16a** (IC_{50}= 0.500μM) and **16b** (IC_{50}= 0.590μM) exhibited promising AChE inhibitory activity even better than donepezil (IC_{50}= 0.711μM). Kinetic assay of compound **5b** showed mixed type of inhibition. *In vivo* evaluation of compounds **5b, 13c** and **16a** confirmed significant memory improvement in scopolamine-induced impairment model.

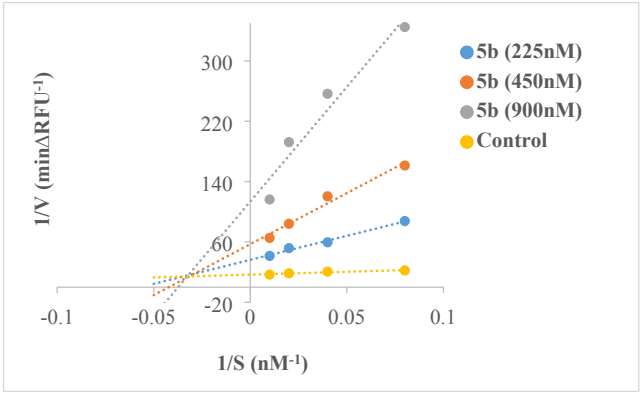


Fig. 3. Lineweaver-Burk plot for inhibition of AChE by compound **5b**.

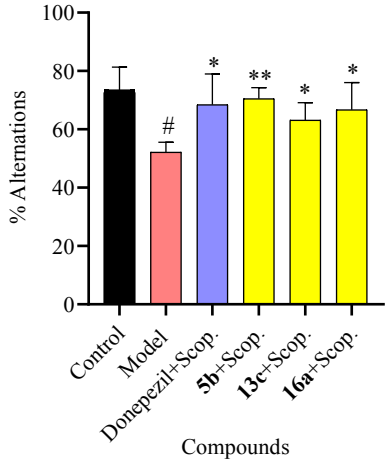


Fig. 4. Bar diagram shows the effect of compounds **5b**, **13c** and **16a** (1 mg/kg), donepezil (1 mg/Kg) and scopolamine (3 mg/Kg) on spontaneous alternation score (% alternation) in Y-maze test. Data are presented as Mean ± SD, (n = 4; #p < 0.01 *vs* control group and *p < 0.05, **p < 0.01 *vs* model group).

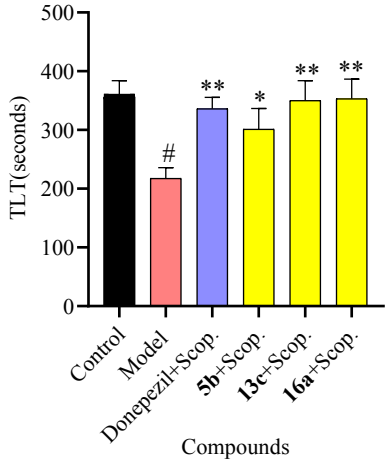


Fig. 5. Bar diagram shows the effect of compounds **5b, 13c** and **16a** (1 mg/kg), donepezil (1 mg/Kg) and scopolamine (3 mg/Kg) on transfer latency time (TLT) in seconds in passive avoidance test. Data are presented as Mean ± SD, (n = 4; #p < 0.01 *vs* control group and *p < 0.05, **p < 0.01 *vs* model group).

2.3. In silico studies

2.3.1. Molecular docking study

In silico docking study aims to rationalize the obtained biological data and help in understanding the possible interaction between small molecules commonly called ligands and the target macromolecules (receptor, enzymes and nucleic acids) fit together with the crystal structure of the target.

In this work, molecular docking study was conducted to realize the binding mode of the synthesized compounds in the active site of the AChE enzyme with the aim of explaining their inhibitory activity using Molecular Operating Environment (MOE, 2009.10) software.[37] Analysis of the binding mode elucidated that the docked compounds showed good fit in the active site of AChE enzyme like that of the native ligand donepezil. The most active compounds **5b,d, 13c** and **16a,b** showed a common predicted binding pattern in the AChE binding site with their benzyloxy moiety accommodated in the same region as the benzylpiperidine moiety of the co-crystalized inhibitor donepezil through π-π stacking interaction with Trp86. Also a water mediated hydrogen bond interaction exist between carbonyl group of coumarin nucleus, instead of protonated piperidine in donepezil and Asp74, Tyr337 and Tyr341. Besides two new hydrogen bonding interactions between oxygen of benzyloxy moiety in the docked compounds and Ser203 in AChE binding site and between carbonyl group of coumarin nucleus which acts as hydrogen bond acceptor and Tyr341. Finally, all of them were able to occupy both PAS and CAS sites like donepezil Figs. 6–8 Table 2.

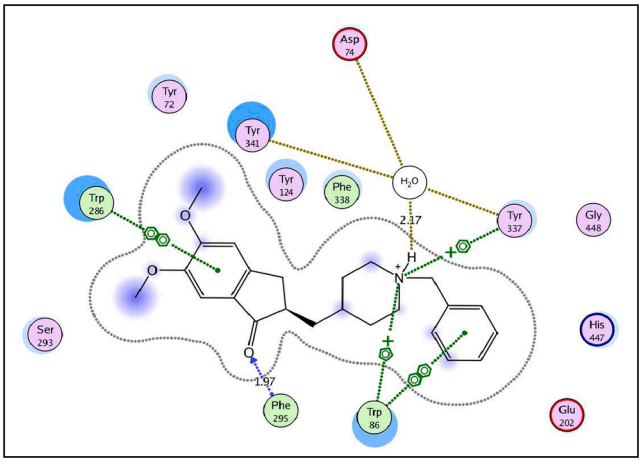


Fig. 6. 2D diagram of donepezil in the AChE binding site.

2.3.2. Physicochemical properties and pharmacokinetics prediction

Pharmacokinetic properties of the most active compounds **5b,d, 13c** and **16a,b** were calculated using the free accessible web server pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) compared with donepezil as the standard drug [38]. Results revealed that partition coefficient of the tested compounds showed good lipophilicity Clog P= 2.9052 to 5.3720 compared to 4.3611 for donepezil. Concerning the distribution, most of the compounds possess moderate penetration to the blood brain barrier (BBB) with values = −1.054 to −0.353 compared to 0.157 for donepezil. Besides, the tested compounds possess good CNS permeability values from −2.476 to −1.667 compared to −1.464 for donepezil. Finally, the tested compounds showed LD_{50} values from 2.193 to 3.137 compared to 2.759 for donepezil and LOEAL from 0.211 to 1.360 compared to 0.991 for donepezil Table 3.

3. Conclusion

Twenty novel coumarin derivatives were designed, synthesized and screened for their *in vitro* AChE inhibitory activity. The results elicited that compounds **5b,d 13c** and **16a,b** have promising anti-AD activity. Kinetic study for compound **5b** implied mixed type inhibitor which could bind PAS and CAS sites of AChE enzyme. In addition, *in vivo* scopolamine-induced dementia model for the three most active compounds showed that there is a significant improvement in cognitive functions in tested mice. A correlation between docking results and IC_{50} of tested compounds was routinely observed and shared similar binding pattern to the native co-crystallized ligand donepezil. Finally, predicted physicochemical and pharmacokinetic properties revealed that the synthesized compounds possessed good lipophilicity and moderate BBB penetration.

4. Experimental section

4.1. Chemistry

4.1.1. General

All chemicals and solvents were purchased from commercial suppliers and were used without further purification. All reactions were followed up by thin layer chromatography (TLC), using Macherey-Nagel Alugram Sil G/UV_{254} silica gel plates with fluorescent indicator UV_{254}

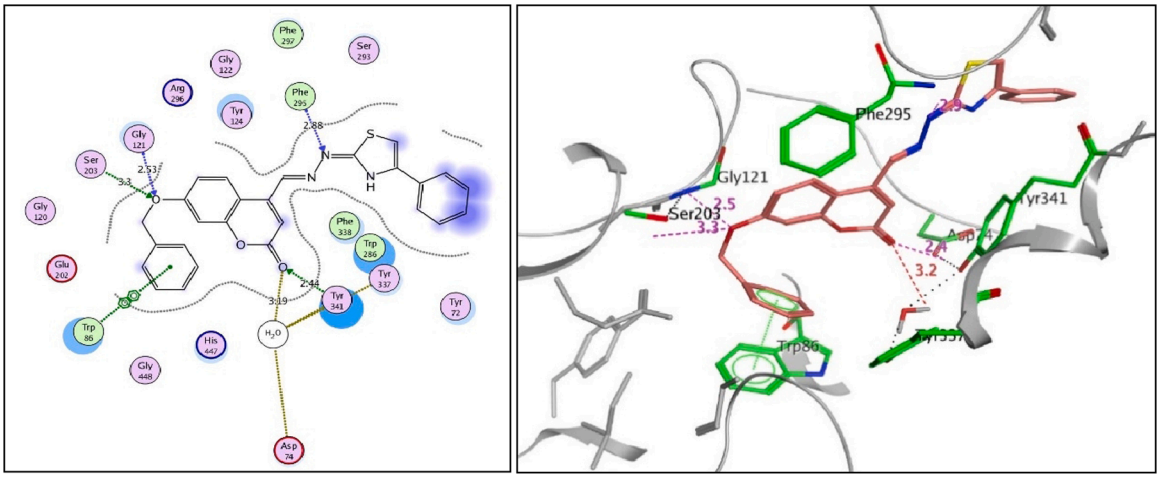


Fig. 7. 2D diagram **(a)** and 3D representation **(b)** of **5b** in the AChE binding site.

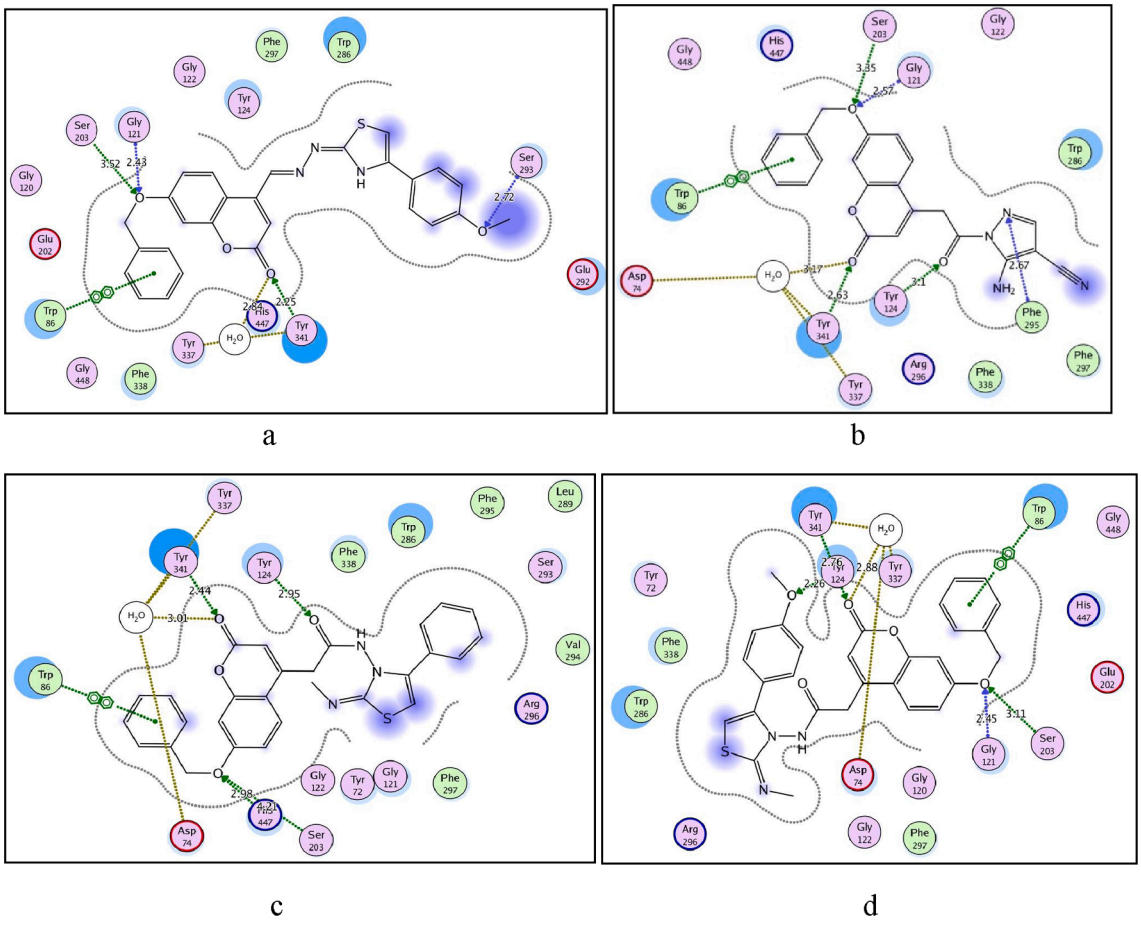


Fig. 8. 2D diagram of compounds (a) **5d**, (b) **13c**, (c) **16a**, and (d) **16b** in the AChE binding site.

and chloroform/methanol (90:10 v/v) or chloroform as the eluting system and the spots were visualized using Vilber Lourmet ultraviolet lamp at λ = 254 nm. Melting points were determined by open capillary tube method using Electrothermal Stuart SMP_3 digital melting point apparatus and were uncorrected. The IR spectra were recorded as KBr discs using Shimadzu Infrared spectrometer (IR-435) and FT-IR 1650 (Perkin Elmer) spectrophotometer and expressed in wave number (υ_{max}) cm^{-1} at Microanalytical unit, Faculty of Pharmacy, Cairo University. NMR spectra were performed on a Bruker spectrophotometer (Germany) in dimethyl sulfoxide (DMSO-d_6) at Microanalytical center, Faculty of Pharmacy, Cairo University. ^{1}H NMR Spectra were recorded in δ scale given in parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard at 400 MHz and ^{13}C NMR Spectra were recorded at 100 MHz. Mass spectra were performed on HP MODEL: MS_5988 mass spectrometer and on Shimadzu QP- 2010 Plus (EI, 70 ev), Micro Analytical Center, Faculty of Science, Cairo University. Elemental microanalysis were performed at the Regional Center for Mycology and Biotechnology, Al-Azhar University.

Compounds **1** [39], **2** [40], **3** [41], **7** [42], **8** [42,43] and **10** [44,45] were synthesized according to the previously reported methods.

4.1.2. 1-[(7-Benzyloxy-2-oxo-2H-chromen-4-yl)methylene] thiosemicarbazide (4)

To a solution of aldehyde compound **3** (2 g, 7 mmol) in 1:1 mixture of benzene/ absolute ethanol (50 mL), thiosemicarbazide (0.63 g, 7 mmol) and catalytic amount of glacial acetic acid were added. The mixture was refluxed for 10 h. The obtained solid was filtered, dried and crystallized from absolute ethanol.

Yellow powder, (yield 90%), m.p. 210–2 °C. IR (KBr, ν_{max}/cm^{-1}): 3471, 3371, 3275 (NH_2, NH), 3086 (CH aromatic), 2985, 2927 (CH aliphatic), 1720 (C═O), 1612, 1590, 1531 (C═N, NH, C═C), 1265 (C═S). ^{1}H NMR (DMSO-d_6) δ ppm: 5.24 (s, 2H, OCH_2), 6.99 (s, 1H, H-3 Ar), 7.09–7.11 (m, 2H, H-6,8 Ar), 7.35 (t, 1H, J = 7.2 Hz, H-4′ Ar), 7.42 (t, 2H, J = 7.5 Hz, H-3′,5′ Ar), 7.48 (d, 2H, J = 7.1 Hz, H-2′,6′ Ar), 7.89 (d, 1H, J = 8.8 Hz, H-5 Ar), 8.40 (s, 1H, CH = N), 8.56 (s, 2H, NH_2, D_2O exchang.), 11.77 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 70.37 (OCH_2), 102.65, 109.28, 111.15, 113.62, 126.31, 128.38, 128.60, 129.01, 136.12, 136.68, 145.13, 155.90 (aromatic carbons), 160.76 (C═O), 161.87 (CH═N), 179.12 (C═S). Anal. Calcd. for $C_{18}H_{15}N_3O_3S$ (353.39): C, 61.18; H, 4.28; N, 11.89; Found C, 60.89; H, 4.41; N, 12.15.

4.1.3. General procedure for synthesis of compounds (5a-e)

A mixture of thiosemicarbazone compound **4** (0.52 g, 1.5 mmol), chloroacetone (0.12 mL, 1.5 mmol), appropriate phenacyl bromide (1.65 mmol) or ethyl 2-chloroacetoacetate (0.2 mL, 1.5 mmol), respectively and anhydrous sodium acetate (0.24 g, 3 mmol) in absolute ethanol (50 mL) was refluxed for 48 h. The obtained solid was filtered while hot, washed with water to remove excess sodium acetate and dried. The product was crystallized from ethanol/chloroform mixture.

*4.1.3.1. 7-Benzyloxy-4-{[(4-methylthiazol-2(3H)-ylidene)hydrazono] methyl}-2H-chromen-2-one (**5a**).* Brown powder, (yield 90%), m.p. 285–6 °C. IR (KBr, ν_{max}/cm^{-1}): 3282 (NH), 3066 (CH aromatic), 2989, 2870 (CH aliphatic), 1701 (C═O), 1612, 1531 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 2.40 (s, 3H, CH_3), 5.25 (s, 2H, OCH_2), 6.22 (s, 1H, H-3 Ar), 7.03–7.08 (m, 2H, H-6,8 Ar), 7.34 (t, 1H, J = 7.0 Hz, H-4′ Ar), 7.37 (t, 2H, J = 7.3 Hz, H-3′,5′ Ar), 7.42 (d, 2H, J = 7.1 Hz, H-2′,6′ Ar), 7.47 (s, 1H, CH thiazoline), 7.69 (d, 1H, J = 8.7 Hz, H-5 Ar), 8.41 (s, 1H, CH = N), 11.84 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 18.58 (CH_3), 70.30 (OCH_2), 102.12, 111.70, 113.14, 113.74,

Table 2
The docking scores and interactions of compounds **5b,d, 13c, 16a,b** and native ligand donepezil.

Compound No.	Docking score (S) Kcal/mol	Binding amino acids	Interacting group	H-bond (Å)
5b	−14.9576	Asp74 (H_2O mediated)	C═O coumarin	3.19
		Trp86 (π–π)	Benzyl	-
		Gly121	OCH_2	2.53
		Ser203	OCH_2	3.30
		Phe295	C═N	2.88
		Tyr337 (H_2O mediated)	C═O coumarin	3.19
		Tyr341 (H_2O mediated)	C═O coumarin	3.19
		Tyr341	C═O coumarin	2.44
5d	−16.8714	Asp74 (H_2O mediated)	C═O coumarin	2.84
		Trp86 (π–π)	Benzyl	-
		Gly121	OCH_2	2.43
		Gly122	OCH_2	3.08
		Ser203	OCH_2	3.52
		Ser293	OCH_3	2.72
		Tyr337 (H_2O mediated)	C═O coumarin	2.84
		Tyr341 (H_2O mediated)	C═O coumarin	2.84
		Tyr341	C═O coumarin	2.25
13c	−16.3673	Asp74 (H_2O mediated)	C═O coumarin	3.17
		Trp86 (π–π)	Benzyl	-
		Gly121	OCH_2	2.57
		Tyr124	C═O amide	3.10
		Ser203	OCH_2	3.35
		Phe295	C═N	2.67
		Tyr337 (H_2O mediated)	C═O coumarin	3.17
		Tyr341 (H_2O mediated)	C═O coumarin	3.17
		Tyr341	C═O coumarin	2.63
16a	−17.3909	Asp74 (H_2O mediated)	C═O coumarin	3.01
		Trp86 (π–π)	Benzyl	-
		Tyr124	C═O amide	2.95
		Ser203	OCH_2	4.21
		Tyr337 (H_2O mediated)	C═O coumarin	3.01
		Tyr341 (H_2O mediated)	C═O coumarin	3.01
		Tyr341	C═O coumarin	2.44
		His447	OCH_2	2.98
16b	−19.6007	Asp74 (H_2O mediated)	C═O coumarin	2.88
		Trp86 (π–π)	Benzyl	-
		Gly121	OCH_2	2.45
		Tyr124	OCH_3	2.26
		Ser203	OCH_2	3.11
		Tyr337 (H_2O mediated)	C═O coumarin	2.88
		Tyr341 (H_2O mediated)	C═O coumarin	2.88
		Tyr341	C═O coumarin	2.76
Donepezil	−13.0561	Asp74 (H_2O mediated)	N of Piperidine	2.17
		Trp86 (π–π)	Benzyl	-
		Trp86 (π -cation)	N of Piperidine	-
		Trp286 (π–π)	Indanone	-
		Phe295	C═O indanone	1.97
		Tyr337 (π -cation)	N of Piperidine	-
		Tyr337 (H_2O mediated)	N of Piperidine	2.17
		Tyr341 (H_2O mediated)	N of Piperidine	2.17

126.95, 128.32, 128.36, 128.54, 128.98, 136.76, 153.84 (aromatic carbons, C-4,5 thiazoline), 155.12 (CH = N), 160.58 (C═O), 161.80 (C═N). Anal. Calcd. for $C_{21}H_{17}N_3O_3S$ (391.43): C, 64.43; H, 4.38; N, 10.73; Found C, 64.71; H, 4.57; N, 11.02.

*4.1.3.2. 7-Benzyloxy-4-{[(4-phenylthiazol-2(3H)-ylidene)hydrazono] methyl}-2H-chromen-2-one (**5b**).* Yellowish brown powder, (yield 90%), m.p. 239 °C (decomp.). IR (KBr, ν_{max}/cm^{-1}): 3197 (NH), 3062 (CH aromatic), 2931, 2873 (CH aliphatic), 1710 (C═O), 1670, 1612, 1558, 1543 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 5.26 (s, 2H, OCH_2), 6.51 (s, 1H, H-3 Ar), 7.12–7.14 (m, 2H, H-6,8 Ar), 7.31–7.45 (m, 9H, CH thiazoline, Ar-H), 7.49 (d, 2H, J = 7.2 Hz, H-2′',6′' Ar), 7.88 (d, 1H, J = 7.2 Hz, H-5 Ar), 8.16 (s, 1H, CH = N), 12.87 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 70.34 (OCH_2), 102.61, 105.53, 110.38, 111.66, 113.32, 126.05, 127.95, 128.24, 128.38, 128.58, 128.75, 129.00, 129.15, 136.72, 145.06 (aromatic carbons, C-4,5 thiazoline), 156.00 (CH = N), 160.68 (C═O), 161.77 (C═N). Anal. Calcd. for $C_{26}H_{19}N_3O_3S$ (453.51): C, 68.86; H, 4.22; N, 9.27; Found C, 68.52; H, 4.34; N, 9.50.

*4.1.3.3. 7-Benzyloxy-4-({[4-(4-bromophenyl)thiazol-2(3H)-ylidene] hydrazono} methyl)-2H-chromen-2-one (**5c**).* Yellowish brown powder, (yield 90%), m.p. 260 °C (decomp.). IR (KBr, ν_{max}/cm^{-1}): 3190 (NH), 3066 (CH aromatic), 2935, 2870 (CH aliphatic), 1718 (C═O), 1670, 1612, 1558, 1535 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 5.26 (s, 2H, OCH_2), 6.52 (s, 1H, H-3 Ar), 7.10–7.16 (m, 2H, H-6,8 Ar), 7.36 (t, 1H, J = 7.2 Hz, H-4′ Ar), 7.42 (t, 2H, J = 7.4 Hz, H-3′,5′ Ar), 7.49 (d, 2H, J = 7.2 Hz, H-2′,6′ Ar), 7.56 (s, 1H, CH thiazoline), 7.62 (d, 2H, J = 8.4 Hz, H-2′',6′' Ar), 7.82 (d, 2H, J = 8.3 Hz, H-3′',5′' Ar), 8.17 (s, 1H, CH = N), 8.48 (d, 1H, J = 8.0 Hz, H-5 Ar), 12.88 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 70.33 (OCH_2), 102.57, 106.39, 110.34, 111.61, 113.31, 121.25, 127.90, 128.04, 128.35, 128.58, 129.00, 132.05, 133.95, 136.68, 137.64, 145.00 (aromatic carbons, C-4,5 thiazoline), 155.96 (CH = N), 160.69 (C═O), 161.75 (C═N). Anal. Calcd. for $C_{26}H_{18}BrN_3O_3S$ (532.41): C, 58.65; H, 3.41; N, 7.89; Found C, 58.96; H, 3.64; N, 8.12.

Table 3
BBB and CNS permeability, LD_{50}, LOEAL and Clog P for the most active compounds.

Compound No.	Distribution		Toxicity		CLogP
	BBB permeability	CNS permeability	Oral rat acute toxicity (LD_{50}) mol/Kg	Oral rat chronic toxicity (LOAEL) log mg/Kg_bw/day	
5b	−0.668	−1.667	2.193	1.360	5.3634
5d	−0.824	−1.949	2.808	0.382	5.3720
13c	−1.054	−2.476	2.574	0.820	2.9052
16a	−0.353	−1.988	3.137	0.255	4.7453
16b	−0.556	−2.158	3.034	0.211	4.7539
Donepezil	0.157	−1.464	2.753	0.991	4.3611

*4.1.3.4. 7-Benzyloxy-4-({[4-(4-methoxyphenyl)thiazol-2(3H)-ylidene] hydrazono} methyl)-2H-chromen-2-one (**5d**).* Yellowish brown powder, (yield 90%), m.p. 159 °C (decomp.). IR (KBr, ν_{max}/cm^{-1}): 3282 (NH), 3062 (CH aromatic), 2935, 2835 (CH aliphatic), 1720 (C═O), 1670, 1604, 1558, 1553 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 3.79 (s, 3H, OCH_3), 5.26 (s, 2H, OCH_2), 6.51 (s, 1H, H-3 Ar), 6.99 (d, 3H, $J = 8.1$ Hz, H-6,3'',5'' Ar), 7.12 (s, 1H, H-8 Ar), 7.30 (s, 1H, CH thiazoline), 7.37–7.44 (m, 3H, H-3′,4′,5′ Ar), 7.50 (d, 2H, $J = 6.7$ Hz, H-2′,6′ Ar) 7.80 (d, 2H, $J = 7.7$ Hz, H-2'',6'' Ar), 8.16 (s, 1H, CH = N), 8.49 (d, 1H, $J = 8.9$ Hz, H-5 Ar), 12.83 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 55.61 (OCH_3), 70.34 (OCH_2), 102.60, 110.39, 111.59, 113.30, 114.50, 127.40, 127.97, 128.37, 128.58, 129.00, 136.72, 145.08, 156.00 (aromatic carbons, C-4,5 thiazoline), 159.43 (CH = N), 160.69 (C═O), 161.75 (C═N). Anal. Calcd. for $C_{27}H_{21}N_3O_4S$ (483.54): C, 67.07; H, 4.38; N, 8.69; Found C, 67.30; H, 4.56; N, 8.76.

*4.1.3.5. Ethyl-2-{2-[(7-benzyloxy-2-oxo-2H-chromen-4-yl)methylene] hydrazono}-4-methyl-2,3-dihydrothiazole-5-carboxylate (**5e**).* Yellow powder, (yield 90%), m.p. 290 °C (decomp.). IR (KBr, ν_{max}/cm^{-1}): 3159 (NH), 3035 (CH aromatic), 2924, 2870 (CH aliphatic), 1720, 1693 (2C = O), 1670, 1612, 1558, 1535 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 1.28 (t, 3H, $J = 7.0$ Hz, $CH_2\underline{CH_3}$), 2.49 (s, 3H, CH_3), 4.23 (q, 2H, $J = 7.0$ Hz, $\underline{CH_2}CH_3$), 5.25 (s, 2H, OCH_2), 6.55 (s, 1H, H-3 Ar), 7.09–7.12 (m, 2H, H-6,8 Ar), 7.36 (t, 1H, $J = 7.1$ Hz, H-4′), 7.42 (t, 2H, $J = 7.5$ Hz, H-3′,5′ Ar), 7.49 (d, 2H, $J = 7.1$ Hz, H-2′,6′ Ar), 8.24 (s, 1H, CH = N), 8.41 (d, 1H, $J = 8.4$ Hz, H-5 Ar), 12.84 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 14.70 ($\underline{CH_3}CH_2$, CH_3), 60.91 ($\underline{CH_2}CH_3$), 70.35 (OCH_2), 102.57, 110.34, 112.36, 113.25, 127.79, 128.32, 128.56, 128.98, 136.70, 144.79 (aromatic carbons, C-4,5 thiazoline), 155.95 (CH = N), 160.56 (C═O), 161.79 (C═N), 162.05 (C═O). Anal. Calcd. for $C_{24}H_{21}N_3O_5S$ (463.51): C, 62.19; H, 4.57; N, 9.07; Found C, 62.43; H, 4.73; N, 9.21.

*4.1.4. General procedure for synthesis of compounds (**6a,b**)*

A mixture of thiosemicarbazone compound **4** (0.52 g, 1.5 mmol), ethyl bromoacetate (0.18 mL, 1.65 mmol) or maleic anhydride (0.14 g, 1.5 mmol), respectively and anhydrous sodium acetate (0.24 g, 3 mmol) in absolute ethanol (50 mL) was refluxed for 48 h. The obtained solid was filtered while hot, washed with water to remove excess sodium acetate and dried. The product was crystallized from ethanol/chloroform mixture.

*4.1.4.1. 2-{2-[(7-Benzyloxy-2-oxo-2H-chromen-4-yl)methylene]hydrazono} thiazolidin-4-one (**6a**).* Yellow powder, (yield 90%), m.p. 305 °C (decomp.). IR (KBr, ν_{max}/cm^{-1}): 3421 (NH), 3040 (CH aromatic), 2927, 2870 (CH aliphatic), 1720, 1708 (2C = O), 1640, 1612, 1558 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 3.99 (s, 2H, CH_2 thiazolidine), 5.26 (s, 2H, OCH_2), 6.71 (s, 1H, H-3 Ar), 7.07 (d, 1H, $J = 9.0$ Hz, H-6), 7.14 (s, 1H, H-8 Ar), 7.36 (t, 1H, $J = 7.2$ Hz, H-4′), 7.42 (t, 2H, $J = 7.4$ Hz, H-3′,5′ Ar), 7.49 (d, 2H, $J = 7.3$ Hz, H-2′,6′ Ar), 8.59 (s, 1H, CH = N), 8.65 (d, 1H, $J = 9.0$ Hz, H-5 Ar), 12.30 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 33.89 (CH_2 thiazolidine), 70.34 (OCH_2), 102.54, 110.37, 113.31, 115.71, 128.36, 128.59, 128.76, 129.01, 136.69, 145.08, 154.59 (aromatic carbons), 156.03 (CH═N), 160.62 (C═O), 161.82 (C═N), 174.79 (C═O). Anal. Calcd. for $C_{20}H_{15}N_3O_4S$ (393.42) C, 61.06; H, 3.84; N, 10.68; Found C, 61.32; H, 4.07; N, 10.93.

*4.1.4.2. 2-(2-{[(7-Benzyloxy-2-oxo-2H-chromen-4-yl)methylene]hydrazono}-4-oxothiazolidin-5-yl) acetic acid (**6b**).* Yellow powder, (yield 90%), m.p. 310 °C (decomp.). IR (KBr, ν_{max}/cm^{-1}): 3433 (NH), 3059 (CH aromatic), 2974, 2920 (CH aliphatic), 2758–2627 (carboxylic OH), 1720, 1708, 1693 (3C = O), 1612, 1604, 1558 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 2.95–3.08 (m, 2H, $C\underline{H_2}COOH$), 4.48 (t, 1H, $J = 4.1$ Hz, CH thiazolidine), 5.25 (s, 2H, OCH_2), 6.69 (s, 1H, H-3 Ar), 7.05 (d, 1H, $J = 9.0$ Hz, H-6), 7.11 (s, 1H, H-8 Ar), 7.35 (t, 1H, $J = 7.1$ Hz, H-4′), 7.41 (t, 2H, $J = 7.5$ Hz, H-3′,5′ Ar), 7.48 (d, 2H, $J = 7.2$ Hz, H-2′,6′ Ar), 8.60 (d, 2H, $J = 8.7$ Hz, CH = N, H-5 Ar), 12.43 (s, 2H, NH, COOH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 36.70 ($\underline{CH_2}COOH$), 44.45 (CH thiazolidine), 70.34 (OCH_2), 102.65, 110.41, 113.22, 115.57, 128.34, 128.57, 128.63, 129.00, 136.71, 145.07, 154.72, (aromatic carbons), 156.01 (CH═N), 160.58 (C═O), 161.85 (C═N), 172.07, 176.01 (2C═O). Anal. Calcd. for $C_{22}H_{17}N_3O_6S$ (451.45) C, 58.53; H, 3.80; N, 9.31; Found C, 58.78; H, 3.69; N, 9.47.

*4.1.5. Methyl 2-(7-benzyloxy-2-oxo-2H-chromen-4-yl) acetate (**9**)*

A mixture of compound **8** (2.34 g, 10 mmol), benzyl chloride (4.6 mL, 40 mmol), anhydrous potassium carbonate (2.76 g, 20 mmol) in dry acetone (50 mL) was refluxed for 24 h. The reaction mixture was poured onto ice (50 mL). The precipitated solid was filtered, washed with ethanol and dried. The crude product was crystallized from absolute ethanol.

White powder, (yield 60%), m.p. 98–102 °C. IR (KBr, ν_{max}/cm^{-1}): 3062 (CH aromatic), 2920, 2850 (CH aliphatic), 1724, 1693 (2C═O), 1608, 1558 (C═C).

^{1}H NMR (DMSO-d_6) δ ppm: 3.58 (s, 3H, CH_3), 3.96 (s, 2H, CH_2CO), 4.09 (s, 2H, CH_2CO), 5.22 (s, 2H, OCH_2), 6.31 (s, 1H, H-3 Ar), 7.04 (d, 1H, $J = 8.9$ Hz, H-6 Ar), 7.08 (s, 1H, H-8 Ar), 7.35 (t, 1H, $J = 7.0$ Hz, H-4′ Ar), 7.41 (t, 2H, $J = 7.4$ Hz, H-3′,5′ Ar), 7.47 (d, 2H, $J = 7.0$ Hz, H-2′,6′ Ar), 7.80 (d, 1H, $J = 9.0$ Hz, H-5 Ar). ^{13}C NMR (DMSO-d_6) δ ppm: 36.86 (CH_3), 52.93 ($\underline{CH_2}CO$), 70.39 (OCH_2), 102.53, 111.81, 112.28, 113.50, 128.39, 128.60, 128.73, 129.00, 129.34, 136.66, 138.31, 153.02, 155.39 (aromatic carbons), 160.34, 171.85 (2C═O). Anal. Calcd. for $C_{19}H_{16}O_5$ (324.33) C, 70.36; H, 4.97; Found C, 70.53; H, 5.13.

*4.1.6. General procedure for synthesis of compounds (**11a-c**)*

A solution of carboxazide compound **10** (0.32 g, 1 mmol) in glacial acetic acid (20 mL) and appropriate cyclic acid anhydride (succinic, maleic or phthalic anhydrides) (2 mmol) was refluxed for 48 h. The solution was poured onto ice (20 mL) then filtered, dried and crystallized from absolute ethanol.

*4.1.6.1. 2-(7-Benzyloxy-2-oxo-2H-chromen-4-yl)-N-(2,5-dioxopyrrolidin-1-yl) acetamide (**11a**).* Buff powder, (yield 89%), m.p. 225–8 °C. IR (KBr, ν_{max}/cm^{-1}): 3255 (NH), 3062 (CH aromatic), 2927, 2858 (CH aliphatic), 1710, 1693 (4C═O), 1604, 1558 (NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 2.77 (s, 4H, $C\underline{H_2}C\underline{H_2}$), 3.96 (s, 2H, CH_2CO), 4.06 (s, 2H, CH_2CO), 5.25 (s, 2H, OCH_2), 7.09 (s, 1H, H-3 Ar), 7.19–7.20 (m, 2H, H-6,8 Ar), 7.35 (t, 1H, $J = 6.8$ Hz, H-4′ Ar), 7.41 (t, 2H, $J = 6.8$ Hz, H-

3′,5′ Ar), 7.48 (d, 2H, $J = 6.7$ Hz, H-2′,6′ Ar), 7.78 (d, 1H, $J = 8.8$ Hz, H-5 Ar), 10.90 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 26.75 ($\underline{C}H_2\underline{C}H_2$), 32.88, 33.72 ($\underline{C}H_2CO$), 70.30 ($OCH_2$), 101.91, 113.23, 113.66, 123.97, 126.65, 127.52, 128.29, 128.65, 128.85, 129.00, 136.76, 139.07, 145.12, 153.86 (aromatic carbons), 161.18, 167.26, 174.52 (4C═O). Anal. Calcd. for $C_{22}H_{18}N_2O_6$ (406.39) C, 65.02; H, 4.46; N, 6.89; Found C, 64.87; H, 4.78; N, 7.13.

*4.1.6.2. 2-(7-Benzyloxy-2-oxo-2H-chromen-4-yl)-N-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (**11b**).* Buff powder, (yield 89%), m.p. 115–7 °C. IR (KBr, ν_{max}/cm^{-1}): 3421 (NH), 3032 (CH aromatic), 2931, 2877 (CH aliphatic), 1740, 1716, 1700, 1693 (4C = O), 1604, 1558 (NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 3.91 (s, 2H, CH_2CO), 3.95 (s, 2H, CH_2CO), 5.24 (s, 2H, OCH_2), 7.08 (s, 1H, H-3 Ar), 7.18–7.19 (m, 2H, H-6,8 Ar), 7.26 (d, 2H, $J = 3.2$ Hz, C$\underline{H}$ = C$\underline{H}$), 7.35 (t, 1H, $J = 6.8$ Hz, H-4′ Ar), 7.41 (t, 2H, $J = 7.0$ Hz, H-3′,5′ Ar), 7.48 (d, 2H, $J = 6.8$ Hz, H-2′,6′ Ar), 7.77 (d, 1H, $J = 8.7$ Hz, H-5 Ar), 10.21 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 32.87, 33.97 ($\underline{C}H_2CO$), 70.27 (OCH_2), 101.94, 113.16, 113.85, 123.72, 126.58, 127.55, 128.29, 128.66, 128.99, 134.22, 136.82, 139.29, 145.91, 153.87 (aromatic carbons), 161.11, 167.18, 168.34, 168.49 (4C = O). Anal. Calcd. for $C_{22}H_{16}N_2O_6$ (404.37) C, 65.34; H, 3.99; N, 6.93; Found C, 65.71; H, 4.17; N, 7.20.

*4.1.6.3. 2-(7-Benzyloxy-2-oxo-2H-chromen-4-yl)-N-(1,3-dioxoisoindolin-2-yl) acetamide (**11c**).* White powder, (yield 89%), m.p. 245–7 °C. IR (KBr, ν_{max}/cm^{-1}): 3414 (NH), 3016 (CH aromatic), 2927, 2858 (CH aliphatic), 1747, 1720, 1670 (4C = O), 1604, 1558 (NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 3.99 (s, 2H, C$\underline{H}_2$CO), 4.15 (s, 2H, C$\underline{H}_2$CO), 5.25 (s, 2H, OCH_2), 7.10 (s, 1H, H-3 Ar), 7.20–7.29 (m, 2H, H-6,8 Ar), 7.35 (t, 1H, $J = 6.9$ Hz, H-4′ Ar), 7.41 (t, 2H, $J = 7.4$ Hz, H-3′,5′ Ar), 7.49 (d, 2H, $J = 7.0$ Hz, H-2′,6′ Ar), 7.83 (d, 1H, $J = 8.7$ Hz, H-5 Ar), 7.95–7.96 (m, 4H, H-3′′,4′′,5′′,6′′ Ar), 11.15 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 32.94, 33.71 ($\underline{C}H_2CO$), 70.32 (OCH_2), 101.94, 113.25, 113.67, 124.05, 124.25, 126.64, 127.52, 128.33, 128.55, 128.69, 128.85, 128.99, 129.91, 135.79, 136.80, 139.14, 145.05, 153.91 (aromatic carbons), 161.22, 161.37, 165.45, 168.13 (4C = O). Anal. Calcd. for $C_{26}H_{18}N_2O_6$ (454.43) C, 68.72; H, 3.99; N, 6.16; Found C, 69.01; H, 4.16; N, 6.38.

*4.1.7. 4-[(4-Amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]-7-benzyloxy-2H-chromen-2-one (**12**)*

Carbon disulfide (0.08 mL, 1.5 mmol) was added to ice cold mixture of potassium hydroxide (0.08 g, 1.5 mmol) and carboxazide compound **10** (0.32 g, 1 mmol) in absolute ethanol (30 mL). The mixture was stirred at room temperature for 12 h. Ether (20 mL) was added to this mixture, the separated solid was filtered and washed with ether. The obtained precipitate was refluxed with hydrazine hydrate 99% (0.1 mL, 2 mmol) in absolute ethanol (20 mL) for 24 h, then cooled and poured onto ice. The obtained solid was filtered, dried and crystalized from absolute ethanol.

Yellowish brown powder, (yield 70%), m.p. 230–2 °C. IR (KBr, ν_{max}/cm^{-1}): 3417, 3336 (NH_2, NH), 3062 (CH aromatic), 2927, 2860 (CH aliphatic), 1708 (C═O), 1640, 1606, 1580, 1535 (C═N, NH, C═C), 1284 (C═S). ^{1}H NMR (DMSO-d_6) δ ppm: 3.98 (s, 2H, NH_2, D_2O exchang.), 4.29 (s, 2H, CH_2), 5.23 (s, 2H, OCH_2), 7.10 (s, 1H, H-3 Ar), 7.16–7.18 (m, 2H, H-6,8 Ar), 7.34 (t, 1H, $J = 7.1$ Hz, H-4′ Ar), 7.40 (t, 2H, $J = 7.3$ Hz, H-3′,5′ Ar), 7.47 (d, 2H, $J = 7.2$ Hz, H-2′,6′ Ar), 7.64 (d, 1H, $J = 9.0$ Hz, H-5 Ar), 13.47 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 32.88 (CH_2), 70.31 (OCH_2), 101.99, 113.33, 123.81, 126.60, 128.30, 128.50, 128.54, 128.79, 128.99, 136.79, 139.26, 145.50, 149.46, 153.99 (aromatic carbons), 161.20 (C═N), 161.58 (C═O), 166.45 (C═S). Anal. Calcd. for $C_{19}H_{16}N_4O_3S$ (380.42) C, 59.99; H, 4.24; N, 14.73; Found C, 60.21; H, 4.53; N, 14.97.

*4.1.8. General procedure for synthesis of compounds (**13a-c**)*

A mixture of carboxazide compound **10** (0.32 g, 1 mmol), ethyl acetoacetate (0.12 mL, 1 mmol), acetylacetone (0.1 mL, 1 mmol) or ethoxymethylene malononitrile (0.12 g, 1 mmol), respectively and anhydrous sodium acetate (0.16 g, 2 mmol) in absolute ethanol (30 mL) was refluxed for 48 h. The precipitated solid was filtered, washed with water to remove excess sodium acetate, dried and crystalized from absolute ethanol.

*4.1.8.1. 1-[2-(7-Benzyloxy-2-oxo-2H-chromen-4-yl)acetyl]-3-methyl-1,2-dihydropyrazol-5-one (**13a**).* Buff powder, (yield 60%), m.p. 113–5 °C. IR (KBr, ν_{max}/cm^{-1}): 3414 (NH), 3040 (CH aromatic), 2974, 2839 (CH aliphatic), 1720, 1700, 1685 (3C = O), 1608, 1562 (NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 3.96 (s, 5H, CH_3, CH_2CO), 4.07 (s, 2H, CH_2CO), 5.23 (s, 2H, OCH_2), 7.10 (s, 1H, H-3 Ar), 7.19 (s, 1H, CH pyrazolinone), 7.21 (s, 1H, NH, D_2O exchang.), 7.24 (d, 2H, $J = 7.0$ Hz, H-6,8 Ar), 7.35 (t, 1H, $J = 7.1$ Hz, H-4′ Ar), 7.41 (t, 2H, $J = 7.3$ Hz, H-3′,5′ Ar), 7.47 (d, 2H, $J = 7.4$ Hz, H-2′,6′ Ar), 7.64 (d, 1H, $J = 8.8$ Hz, H-5 Ar). ^{13}C NMR (DMSO-d_6) δ ppm: 19.14 (CH_3), 32.68, 34.71 ($\underline{C}H_2CO$), 70.31 (OCH_2), 101.99, 107.25, 113.39, 113.64, 123.53, 126.58, 127.31, 128.32, 128.51, 128.78, 128.99, 136.78, 139.23, 145.22, 153.91 (aromatic carbons), 161.22, 161.65, 169.31 (3C = O). Anal. Calcd. for $C_{22}H_{18}N_2O_5$ (390.39) C, 67.69; H, 4.65; N, 7.18; Found C, 67.96; H, 4.83; N, 7.39.

*4.1.8.2. 7-Benzyloxy-4-[2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethyl]-2H-chromen-2-one (**13b**).* Buff powder, (yield 60%), m.p. 118–120 °C. IR (KBr, ν_{max}/cm^{-1}): 3062 (CH aromatic), 2927, 2873 (CH aliphatic), 1701 (2C = O), 1635, 1620, 1562 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 1.69 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 3.96 (s, 2H, CH_2CO), 4.08 (s, 2H, CH_2CO), 5.24 (s, 2H, OCH_2), 6.39 (s, 1H, CH pyrazole), 7.10 (s, 1H, H-3 Ar), 7.17–7.21 (m, 2H, H-6,8 Ar), 7.35 (t, 1H, $J = 7.2$ Hz, H-4′ Ar), 7.41 (t, 2H, $J = 7.3$ Hz, H-3′,5′ Ar), 7.47 (d, 2H, $J = 7.1$ Hz, H-2′,6′ Ar), 7.58 (d, 1H, $J = 8.9$ Hz, H-5 Ar). ^{13}C NMR (DMSO-d_6) δ ppm: 16.38 (CH_3), 26.37 (CH_3), 32.91, 35.30 ($\underline{C}H_2CO$), 70.31 (OCH_2), 101.86, 113.28, 114.25, 123.21, 126.56, 127.57, 128.26, 128.53, 128.79, 128.99, 136.82, 139.31, 145.23, 147.43, 153.90, 155.87 (aromatic carbons), 161.22 (C═N), 161.68, 169.31 (2C = O). Anal. Calcd. for $C_{23}H_{20}N_2O_4$ (388.42) C, 71.12; H, 5.19; N, 7.21; Found C, 70.91; H, 5.47; N, 7.49.

*4.1.8.3. 5-Amino-1-[2-(7-benzyloxy-2-oxo-2H-chromen-4-yl)acetyl]-1H-pyrazole-4-carbonitrile (**13c**).* Brown powder, (yield 65%), m.p. 79–81 °C. IR (KBr, ν_{max}/cm^{-1}): 3340, 3217 (NH_2), 3028 (CH aromatic), 2916, 2873 (CH aliphatic), 2206 (CN), 1716, 1708 (2C = O), 1620, 1597, 1562 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 3.91 (s, 2H, CH_2CO), 3.93 (s, 2H, CH_2CO), 5.23 (s, 2H, OCH_2), 7.09 (s, 1H, H-3 Ar), 7.19–7.25 (m, 3H, CH pyrazole, H-6,8 Ar), 7.34 (t, 1H, $J = 7.1$ Hz, H-4′ Ar), 7.40 (t, 2H, $J = 7.3$ Hz, H-3′,5′ Ar), 7.46 (d, 2H, $J = 7.1$ Hz, H-2′,6′ Ar), 7.74 (d, 1H, $J = 7.9$ Hz, H-5 Ar), 10.39 (s, 2H, NH_2, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 32.89, 33.93 ($\underline{C}H_2CO$), 70.29 (OCH_2), 101.96, 110.67, 113.21, 113.83, 123.77, 126.59, 127.47, 128.30, 128.54, 128.66, 128.99, 136.81, 139.24, 145.73, 153.91 (CN, aromatic carbons), 161.15 (C═N), 161.45, 167.10 (2C═O). Anal. Calcd. for $C_{22}H_{16}N_4O_4$ (400.39) C, 66.00; H, 4.03; N, 13.99; Found C, 66.24; H, 4.24; N, 14.23.

*4.1.9. General procedure for synthesis of compounds (**14a,b**)*

A mixture of carboxazide compound **10** (0.32 g, 1 mmol) and appropriate isothiocyanate derivatives (methyl and phenyl isothiocyanate) (1 mmol) in absolute ethanol (30 mL) was refluxed for 12 h. The precipitated solid was filtered, dried and crystallized from absolute ethanol.

*4.1.9.1. 1-[2-(7-Benzyloxy-2-oxo-2H-chromen-4-yl)acetyl]-4-methyl-thiosemicarbazide (**14a**).* White powder, (yield 95%), m.p. 228–230 °C.

IR (KBr, ν_{max}/cm^{-1}): 3350, 3263, 3163 (3 NH), 3066 (CH aromatic), 2960, 2935 (CH aliphatic), 1724, 1678 (2C = O), 1608, 1558 (NH, C═C), 1288 (C═S). ^{1}H NMR (DMSO-d_6) δ ppm: 2.89 (s, 3H, CH_3), 3.91 (s, 2H, CH_2CO), 3.94 (s, 2H, CH_2CO), 5.25 (s, 2H, OCH_2), 7.04 (s, 1H, H-3 Ar), 7.20–7.24 (m, 2H, H-6,8 Ar), 7.35 (t, 1H, J = 7.1 Hz, H-4′ Ar), 7.41 (t, 2H, J = 7.3 Hz, H-3′,5′ Ar), 7.47 (d, 2H, J = 7.2 Hz, H-2′,6′ Ar), 7.68 (d, 1H, J = 8.9 Hz, H-5 Ar), 7.99 (s, 1H, NH, D_2O exchang.), 9.28 (s, 1H, NH, D_2O exchang.), 10.10 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 31.16 (CH_3), 32.92, 34.21 ($\underline{C}H_2CO$), 70.28 (OCH_2), 102.56, 105.22, 113.89, 126.47, 126.64, 128.55, 128.99, 129.17, 136.60, 137.56, 145.88, 153.87, 156.30, 158.31 (aromatic carbons), 161.12, 161.44 (2C = O), 171.75 (C═S). Anal. Calcd. for $C_{20}H_{19}N_3O_4S$ (397.45) C, 60.44; H, 4.82; N, 10.57; Found C, 60.73; H, 4.96; N, 10.80.

*4.1.9.2. 1-[2-(7-Benzyloxy-2-oxo-2H-chromen-4-yl)acetyl]-4-phenylthiosemicarbazide (**14b**).* Buff powder, (yield 95%), m.p. 195–7 °C. IR (KBr, ν_{max}/cm^{-1}): 3421, 3350, 3147 (NH), 3062 (CH aromatic), 2938, 2877 (CH aliphatic), 1724, 1685 (2C = O), 1608, 1558 (NH, C═C), 1284 (C═S). ^{1}H NMR (DMSO-d_6) δ ppm: 3.96 (s, 2H, CH_2CO), 3.98 (s, 2H, CH_2CO), 5.24 (s, 2H, OCH_2), 7.10 (s, 1H, H-3 Ar), 7.18–7.20 (m, 2H, H-6,8 Ar), 7.35 (t, 2H, J = 7.3 Hz, H-4′,4'' Ar), 7.41 (t, 4H, J = 7.5 Hz, H-3′,5′,3'',5'' Ar), 7.48 (d, 4H, J = 7.2 Hz, H-2′,6′,2'',6'' Ar), 7.74 (d, 1H, J = 7.2 Hz, H-5 Ar), 9.65 (s, 2H, 2NH, D_2O exchang.), 10.28 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 32.96, 34.26 ($\underline{C}H_2CO$), 70.29 (OCH_2), 101.94, 113.24, 113.90, 122.06, 123.54, 125.04, 128.29, 128.54, 128.60, 128.85, 128.99, 136.82, 138.27, 139.22, 139.48, 153.89, 154.32, 158.25, 159.76 (aromatic carbons), 161.13, 161.99 (2C=O), 170.05 (C═S). Anal. Calcd. for $C_{25}H_{21}N_3O_4S$ (459.52) C, 65.34; H, 4.61; N, 9.14; Found C, 65.02; H, 4.88; N, 9.45.

*4.1.10. 2-(7-Benzyloxy-2-oxo-2H-chromen-4-yl)-N'-(3-methyl-4-oxothiazolidin-2-ylidene) acetohydrazide (**15**)*

A mixture of acylthiosemicarbazide derivative **14a** (0.65 g, 1.5 mmol), ethyl bromoacetate (0.18 mL, 1.65 mmol) and anhydrous sodium acetate (0.24 g, 3 mmol) in 1:1 mixture of absolute ethanol/chloroform (30 mL) was refluxed for 48 h. The obtained solid was filtered while hot, washed with water to remove excess sodium acetate and dried. The product was crystallized from absolute ethanol.

Buff powder, (yield 90%), m.p. 270–2 °C. IR (KBr, ν_{max}/cm^{-1}): 3209 (NH), 3062 (CH aromatic), 2943, 2912 (CH aliphatic), 1716 (3C = O), 1651, 1604, 1550, 1510 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 3.11 (s, 3H, CH_3), 3.97 (s, 4H, CH_2 thiazolidine, CH_2CO), 4.05 (s, 2H, CH_2CO), 5.24 (s, 2H, OCH_2), 7.09 (s, 1H, H-3 Ar), 7.24–7.27 (m, 2H, H-6,8 Ar), 7.35 (t, 1H, J = 7.1 Hz, H-4′ Ar), 7.41 (t, 2H, J = 7.0 Hz, H-3′,5′ Ar), 7.47 (d, 2H, J = 7.1 Hz, H-2′,6′ Ar), 7.77 (d, 1H, J = 8.7 Hz, H-5 Ar), 10.63 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 29.61 (CH_3), 32.71 (CH_2 thiazolidine), 33.35, 34.48 ($\underline{C}H_2CO$), 70.28 (OCH_2), 101.93, 113.28, 123.63, 126.59, 127.42, 128.30, 128.54, 128.63, 128.81, 128.99, 136.78, 139.35, 146.29, 154.03 (aromatic carbons), 159.53 (C═N), 161.99, 164.62, 172.24 (3C = O). Anal. Calcd. for $C_{22}H_{19}N_3O_5S$ (437.47) C, 60.40; H, 4.38; N, 9.61; Found C, 60.27; H, 4.51; N, 9.89.

*4.1.11. General procedure for synthesis of compounds (**16a,b**)*

A mixture of acylthiosemicarbazide derivative **14a** (0.39 g, 1 mmol), appropriate phenacyl bromides (1 mmol) and anhydrous sodium acetate (0.16 g, 2 mmol) in 1:1 mixture of absolute ethanol/chloroform (30 mL) was refluxed for 48 h. The precipitated solid was filtered, washed with water to remove excess sodium acetate, dried and crystallized from ethanol/chloroform mixture.

*4.1.11.1. 2-(7-Benzyloxy-2-oxo-2H-chromen-4-yl)-N-(2-methylimino-4-phenylthiazol-3(2H)-yl)acetamide (**16a**).* Buff powder, (yield 70%), m.p. 226–8 °C. IR (KBr, ν_{max}/cm^{-1}): 3228 (NH), 3059 (CH aromatic), 2939, 2877 (CH aliphatic), 1724, 1705 (2C = O), 1651, 1612, 1589, 1573 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 3.19 (s, 3H, CH_3), 3.92 (s, 2H, CH_2CO), 4.01 (s, 2H, CH_2CO), 5.25 (s, 2H, OCH_2), 6.26 (s, 1H, CH thiazoline), 7.10 (s, 1H, H-3 Ar), 7.19–7.21 (m, 2H, H-6,8 Ar), 7.35 (t, 1H, J = 7.0 Hz, H-4′ Ar), 7.41 (t, 2H, J = 7.1 Hz, H-3′,5′ Ar), 7.48 (br s, 7H, H-2′,6′,2'',3'',4'',5'',6'' Ar), 7.84 (d, 1H, J = 9.0 Hz, H-5 Ar), 10.63 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 32.90 (CH_3), 33.59, 34.75 ($\underline{C}H_2CO$), 70.30 (OCH_2), 101.91, 113.22, 114.01, 123.55, 126.57, 127.51, 128.32, 128.53, 128.69, 128.81, 128.98, 129.05, 129.68, 130.96, 136.83, 139.46, 140.91, 146.53, 153.92 (aromatic carbons), 161.12 (C═N), 161.58, 168.00 (2C = O). Anal. Calcd. for $C_{28}H_{23}N_3O_4S$ (497.56) C, 67.59; H, 4.66; N, 8.45; Found C, 67.81; H, 4.85; N, 8.72.

*4.1.11.2. 2-(7-Benzyloxy-2-oxo-2H-chromen-4-yl)-N-[4-(4-methoxyphenyl)-2-methyliminothiazol-3(2H)-yl]acetamide (**16b**).* Orange powder, (yield 70%), m.p. 189–191 °C. IR (KBr, ν_{max}/cm^{-1}): 3201 (NH), 3062 (CH aromatic), 2966, 2840 (CH aliphatic), 1712 (C═O), 1620, 1558, 1508 (C═N, NH, C═C). ^{1}H NMR (DMSO-d_6) δ ppm: 3.17 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2CO), 4.00 (s, 2H, CH_2CO), 5.25 (s, 2H, OCH_2), 6.16 (s, 1H, CH thiazoline), 7.04 (d, 2H, J = 8.4 Hz, H-3'',5'' Ar), 7.09 (s, 1H, H-3 Ar), 7.22–7.27 (m, 2H, H-6,8 Ar), 7.35 (t, 1H, J = 7.2 Hz, H-4′ Ar), 7.41 (t, 4H, J = 7.9 Hz, H-3′,5′,2'',6'' Ar), 7.48 (d, 2H, J = 7.2 Hz, H-2′,6′ Ar), 7.83 (d, 1H, J = 8.8 Hz, H-5 Ar), 10.34 (s, 1H, NH, D_2O exchang.). ^{13}C NMR (DMSO-d_6) δ ppm: 32.88 (CH_3), 33.45, 34.69 ($\underline{C}H_2CO$), 55.74 (OCH_3), 70.28 (OCH_2), 101.90, 113.26, 113.97, 114.69, 123.14, 123.55, 126.61, 128.30, 128.55, 128.64, 128.83, 129.00, 130.55, 136.78, 139.38, 140.78, 146.51, 153.89, 160.33 (aromatic carbons), 161.10 (C═N), 161.62, 168.17 (2C = O). Anal. Calcd. for $C_{29}H_{25}N_3O_5S$ (527.59) C, 66.02; H, 4.78; N, 7.96; Found C, 65.88; H, 4.97; N, 8.15.

4.2. In vitro biological studies

4.2.1. In vitro AChE inhibition assay

The method of Ellman was used to determine the *in vitro* cholinesterase inhibitory potencies of the target compounds. This method is based on the calculation of production rate of thiocholine as acetylthiocholine is hydrolyzed. This is achieved by the ongoing reaction of the thiol with 5,5′-dithiobis-2-nitrobenzoate ion (**I**) (DTNB) to produce the yellow anion of 5-thio-2-nitro-benzoic acid (**II**). The color production rate is determined at 412 nm using photometer [34]. Records of the full assay can be retrieved by detecting the output of the photometer on a continuous basis. Donepezil was used as reference drug. The IC_{50} was calculated by plotting an absorbance and % inhibition curve and assessing the influence of four different concentrations. The IC_{50} values were computed by measuring the required concentration to suppress half of the full biological response of the substrate.

4.2.2. Kinetic study

Kinetic characterization of AChE was carried out experimentally using Ellman's method [34] with three different concentrations of the inhibitor (225, 450, 900 nM) in addition to a parallel experiment that is carried out in absence of inhibitor. Lineweaver-Burk reciprocal plot was built by implementing 1/velocity against 1/[Substrate]. Then the results were analyzed using Microsoft Office Excel 2013.

4.3. In vivo behavioral studies on scopolamine-induced dementia model

4.3.1. Experimental design

4.3.1.1. Chemicals and reagents. Donepezil hydrochloride (CAS Number: 120011-70-3), scopolamine hydrobromide (CAS Number: 6533-68-2), acetylthiocholine iodide (ATCI) (CAS Number: 1866-15-5), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) (CAS Number: 69-78-3) and dimethyl sulfoxide (DMSO) (CAS Number: 67-68-5) were all purchased

from Sigma-Aldrich (Egypt).

4.3.1.2. Animals. Healthy male Balb c mice, weighing 25–30 g were provided by the Laboratory Animal House of VACSERA (Egypt). All animals were held in standard environmental regulated conditions: temperature (25 ± 2 °C), relative humidity (60 ± 10%), room air change (12–18 times/h) and light/dark cycle (12/12). Food and water were available *ad libitum*. This study was performed according to approved protocols by the research ethics committee for experimental and clinical studies at Faculty of Pharmacy, Cairo University (Approval number: PC1334).

4.3.1.3. Experimental protocol. Animals were divided into 6 groups containing 4 animals each:

(i) Control
(ii) Model (Scopolamine) (3 mg/kg)
(iii) Scopolamine plus donepezil (1 mg/kg)
(iv) Scopolamine plus compound **5b** (1 mg/kg)
(v) Scopolamine plus compound **13c** (1 mg/kg)
(vi) Scopolamine plus compound **16a** (1 mg/kg)

The route of drug administration was intraperitoneal injection (i.p.) for all the groups. Donepezil and test compounds were administered in equimolar concentration (1 mg/kg) once daily in different groups for seven days. Scopolamine (3 mg/kg) was dissolved in distilled water and administered i.p. on the seventh day, 30 min. after test or donepezil administration [11,35].

All the group animals except control were administered with scopolamine on the seventh day to induce amnesia. The different animal sets were adopted for Y-maze and passive avoidance experiments. The experimental findings are presented as Mean ± SD. The significance of difference between groups was determined using unpaired student *t*-test using GraphPad Prism 8 software.[46]

4.3.2. Behavioral studies

4.3.2.1. Y-Maze test. The Y-maze study aims to detect rodents' spontaneous alternations. Spontaneous alternations is a behavioral test for measuring exploratory behavior. Normal rodents will prefer to experience a different arm of the maze than the one they visited on their previous entry. Y-maze is a horizontal three-armed labyrinth (45 cm long, 14 cm wide, 16 cm high) wherein the arms are positioned in a symmetric manner at 120° angles one from another. Training session of 15 min. was carried out in which animals were placed in Y-maze with novel arm closed. Main study was performed after 5 min. of scopolamine injection. In this session the animal was positioned in the middle of the arm to explore all three of those arms. The experiment was performed for 15 min. and entry of the mice in each of the arms was recorded with in camera and repeated arm entry was considered as the sign of memory impairment. Further, consecutive arm choices (ABC, BCA, CAB not BAB) and novel arm entry were considered as the memory improvement. The memory improvement score was calculated using the equation [35]:

$$\% \text{ alternation} = (\text{No. of alternations/Total arm entries} - 2) \times 100$$

4.3.2.2. Passive avoidance test. Passive avoidance is experienced as a rodent tries to avoid a species-typical behavior (i.e., crossing to the darkened area of the chamber) so as to deter an associated disinterested trigger (i.e., mild foot-shock). This paradigm consists of a two chamber apparatus, an illuminated chamber connected by a hole to a large dark one with a 2 mm stainless steel rods of 1 cm spacing. A guillotine door divides the two chambers. The mice alternate between two separate experimental tests incorporate a training or acquisition trial besides a test or retention trial 24 h later. For the acquisition trial, mice were positioned in the lighted chamber firstly. 10 s later, the door separating the two chambers opened. As mice reached the darkened area, the door was immediately shut down and a 3 s electric foot shock (0.5 mA) was conducted *via* the stainless steel rods. 24 h following the acquisition phase, mice were returned to the illuminated area for retention testing. Transfer latency time (TLT) was defined as the period of time needed by mice to go through the darkened area which expressed in seconds and indicating memory improved performance [36].

4.4. In silico studies

4.4.1. Molecular docking study

The molecular docking study were implemented using Molecular Operating Environment (MOE, 2009.10) software [37]. In the current work, we used (PDB ID: 4EY7) [47] which has recombinant human AChE (rhAChE) co-crystallized with donepezil downloaded from PDB (https://www.rcsb.org/). All minimizations were carried out with MOE until RMSD gradient of 0.05 kcal·mol^{-1}Å^{-1} with MMFF94x force field and the partial charges were automatically calculated. The score function or dock function (s, kcal/mol), developed by MOE program was used for testing the binding affinity of the ligand. The enzyme was prepared for docking study by acting on only one chain of amino acids containing one molecule of E20 and deleting all water of crystallization away from the active site. Prior to docking of coumarin derivatives, validation of the molecular docking protocol was performed by re-docking of the co-crystallized ligand donepezil in the AChE active site. The most stable conformer obtained for each compound was virtually docked into the predefined active site. Triangle Matcher placement method and London dG scoring function were adopted by the docking protocol. The developed dock models were energetically minimized and then used to predict the interaction of the ligand with the amino acids in the active site of the enzyme.

4.4.2. Physicochemical properties and pharmacokinetics prediction

Pharmacokinetic properties such as absorption, distribution, metabolism, excretion and toxicity (ADMET) play an integral role in the development of active therapeutic reagents. Therefore, pharmacokinetic properties of the finally targeted compounds were calculated using the free accessible web server pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) compared with donepezil as standard drug [38].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2021.104792.

References

[1] M. Goedert, M.G. Spillantini, A century of Alzheimer's disease, Science 314 (5800) (2006) 777–781.
[2] L. Pisani, M. Catto, I. Giangreco, F. Leonetti, O. Nicolotti, A. Stefanachi, S. Cellamare, A. Carotti, Design, synthesis, and biological evaluation of coumarin derivatives tethered to an edrophonium-like fragment as highly potent and selective dual binding site acetylcholinesterase inhibitors, ChemMedChem 5 (9) (2010) 1616–1630.
[3] J. Gaugler, B. James, T. Johnson, A. Marin, J. Weuve, Alzheimer's disease facts and figures, Alzheimer's Dement. 14 (2018) (2018) 367–429.
[4] A. Wimo, L. Jönsson, J. Bond, M. Prince, B. Winblad, The worldwide economic impact of dementia 2010, Alzheimer's Dement. 9 (1) (2013) 1–11.e3.
[5] L.G. de Souza, M.N. Rennó, J.D. Figueroa-Villar, Coumarins as cholinesterase inhibitors: A review, Chem. Biol. Interact. 254 (2016) 11–23.
[6] J. Gaugler, B. James, T. Johnson, K. Scholz, J. Weuve, Alzheimer's disease facts and figures, Alzheimer's Dement. 11 (2015) (2015) 332–384.
[7] D. Viña, M.J. Matos, M. Yáñez, L. Santana, E. Uriarte, 3-Substituted coumarins as dual inhibitors of AChE and MAO for the treatment of Alzheimer's disease, MedChemComm. 3 (2) (2012) 213–218.

[8] R. Bartus, R. Dean, B. Beer, A. Lippa, The cholinergic hypothesis of geriatric memory dysfunction, Science 217 (4558) (1982) 408–414.

[9] L. Waite, Treatment for Alzheimer's disease: Has anything changed ? Aust. Prescr. 38 (2) (2015) 60–63.

[10] A. Aliabadi, A. Mohammadi-Farani, M.J. Ahmadvand, M. Rahmani-Khajouei, Synthesis, docking and acetylcholinesterase inhibitory evaluation of (E)-3-(4-(diethylamino) phenyl)-1-phenylprop-2-en-1-one derivatives with probable anti-Alzheimer effects, J. Reports Pharm. Sci. 6 (2017) 134–141.

[11] D. Wang, M. Hu, X. Li, D. Zhang, C. Chen, J. Fu, S. Shao, G. Shi, Y.u. Zhou, S. Wu, T. Zhang, Design, synthesis, and evaluation of isoflavone analogs as multifunctional agents for the treatment of Alzheimer's disease, Eur. J. Med. Chem. 168 (2019) 207–220.

[12] D. Genest, C. Rochais, C. Lecoutey, J.-d. Oliveira Santos, C. Ballandonne, S. Butt-Gueulle, R. Legay, M. Since, P. Dallemagne, Design, synthesis and biological evaluation of novel indano- and thiaindano-pyrazoles with potential interest for Alzheimer's disease, Medchemcomm. 4 (6) (2013) 925, https://doi.org/10.1039/c3md00041a.

[13] S. Hamulakova, L. Janovec, M. Hrabinova, K. Spilovska, J. Korabecny, P. Kristian, K. Kuca, J. Imrich, Synthesis and biological evaluation of novel tacrine derivatives and tacrine-coumarin hybrids as cholinesterase inhibitors, J. Med. Chem. 57 (16) (2014) 7073–7084.

[14] B.Z. Kurt, N. Ozten Kandas, A. Dag, F. Sonmez, M. Kucukislamoglu, Synthesis and biological evaluation of novel coumarin-chalcone derivatives containing urea moiety as potential anticancer agents, Arab. J. Chem. 13 (1) (2020) 1120–1129.

[15] V. Luque-Agudo, J. Albarrán-Velo, M.E. Light, J.M. Padrón, E. Román, J. A. Serrano, M.V. Gil, Synthesis and antiproliferative activity of new 2-glyco-3-nitro-2*H*-chromenes, Bioorg. Chem. 87 (2019) 112–116.

[16] B.Z. Kurt, I. Gazioglu, N.O. Kandas, F. Sonmez, Synthesis, anticholinesterase, antioxidant, and anti-aflatoxigenic activity of novel coumarin carbamate derivatives, ChemistrySelect. 3 (14) (2018) 3978–3983.

[17] A. Nocentini, D. Vullo, S. Del Prete, S.M. Osman, F.A.S. Alasmary, Z. AlOthman, C. Capasso, F. Carta, P. Gratteri, C.T. Supuran, Inhibition of the β-carbonic anhydrase from the dandruff-producing fungus Malassezia globosa with monothiocarbamates, J. Enzyme Inhib. Med. Chem. 32 (1) (2017) 1064–1070.

[18] B.Z. Kurt, I. Gazioglu, F. Sonmez, M. Kucukislamoglu, Synthesis, antioxidant and anticholinesterase activities of novel coumarylthiazole derivatives, Bioorg. Chem. 59 (2015) 80–90.

[19] R. Raza, A. Saeed, M. Arif, S. Mahmood, M. Muddassar, A. Raza, J. Iqbal, Synthesis and biological evaluation of 3-thiazolocoumarinyl schiff-base derivatives as cholinesterase inhibitors, Chem. Biol. Drug Des. 80 (4) (2012) 605–615.

[20] E. Jameel, T. Umar, J. Kumar, N. Hoda, Coumarin: A privileged scaffold for the design and development of antineurodegenerative agents, Chem. Biol. Drug Des. 87 (2016) 21–38.

[21] AU-Rehman, S. Magsi, M.A. Abbasi, S. Rasool, A. Malik, G. Hussain, M. Ashraf, N. Khalid, Synthesis, characterization and enzyme inhibition study of O-substituted derivatives of chlorinated coumarin, Pak. J. Pharm. Sci. 27 (2014) 271–278.

[22] Samaneh Ghanei-Nasab, Mehdi Khoobi, Farzin Hadizadeh, Azam Marjani, Alireza Moradi, Hamid Nadri, Saeed Emami, Alireza Foroumadi, Abbas Shafiee, Synthesis and anticholinesterase activity of coumarin-3-carboxamides bearing tryptamine moiety, Eur. J. Med. Chem. 121 (2016) 40–46.

[23] Sahar Mohammad Bagheri, Mehdi Khoobi, Hamid Nadri, Alireza Moradi, Saeed Emami, Leili Jalili-Baleh, Farnaz Jafarpour, Farshad Homayouni Moghadam, Alireza Foroumadi, Abbas Shafiee, Synthesis and anticholinergic activity of 4-hydroxycoumarin derivatives containing substituted benzyl-1,2,3-triazole moiety, Chem. Biol. Drug Des. 86 (5) (2015) 1215–1220.

[24] G.L. Turan-Zitouni, L. Yurttaş, A. Tabbi, G.L. Akalin Çiftçi, H.E. Temel, Z. A. Kaplancikli, New thiazoline-tetralin derivatives and biological activity evaluation, Molecules 23 (2018) 1–15.

[25] K.S. Sharath Kumar, C.D. Mohan, S. Jagadish, K.S. Rakesh, A. Hanumappa, K.S. Rangappa Basappa, Synthesis and acetylcholinesterase/butyrylcholinesterase inhibition activity of arecoline-, 4-thiazolidinone- and piperidine- based conjugates, Asian J. Pharm. Clin. Res. 8 (2015) 142–148.

[26] Zeynep Soyer, Sirin Uysal, Sulunay Parlar, Ayse Hande Tarikogullari Dogan, Vildan Alptuzun, Synthesis and molecular docking studies of some 4-phthalimidobenzenesulfonamide derivatives as acetylcholinesterase and butyrylcholinesterase inhibitors, J. Enzyme Inhib. Med. Chem. 32 (1) (2017) 13–19.

[27] A. Sadiq, F. Mahmood, F. Ullah, M. Ayaz, S. Ahmad, F.U. Haq, G. Khan, M.S. Jan, Synthesis, anticholinesterase and antioxidant potentials of ketoesters derivatives of succinimides: A possible role in the management of Alzheimer's, Chem. Cent. J. 9 (2015) 9–31.

[28] Ayşe Hande Tarikoğulları, Murat Çizmecioğlu, Merve Saylam, Sülünay Parlar, Vildan Alptüzün, Zeynep Soyer, Synthesis and cholinesterase inhibitory activity of some phenylacetamide derivatives bearing 1*H*-pyrazole and 1*H*–1,2,4-triazole, Marmara Pharm. J. 20 (1) (2016) 21, https://doi.org/10.12991/mpj.2016202105828.

[29] Dilan Ozmen Ozgun, Halise Inci Gul, Cem Yamali, Hiroshi Sakagami, Ilhami Gulcin, Murat Sukuroglu, Claudiu T. Supuran, Synthesis and bioactivities of pyrazoline benzensulfonamides as carbonic anhydrase and acetylcholinesterase inhibitors with low cytotoxicity, Bioorg. Chem. 84 (2019) 511–517.

[30] M.S. Shah, S.U. Khan, S.A. Ejaz, S. Afridi, S. Umar, F. Rizvi, M. Najam-ul-haq, J. Iqbal, Cholinesterases inhibition and molecular modeling studies of piperidyl-thienyl and 2-pyrazoline derivatives of chalcones, Biochem. Biophys. Res. Commun. 482 (2017) 615–624.

[31] T. Fo, O. Em, O. Ij, I. Da, I. To, Design, synthesis and biological evaluation of some novel 3-methlyquinoxaline-2-hydrazone derivatives, Org. Chem. Curr. Res. 6 (2017) 6–11.

[32] F. Sonmez, B.Z. Kurt, I. Gazioglu, L. Basile, A. Dag, V. Cappello, T. Ginex, M. Kucukislamoglu, F. Sonmez, B.Z. Kurt, I. Gazioglu, L. Basile, V. Cappello, T. Ginex, M. Kucukislamoglu, S. Guccione, Design, synthesis and docking study of novel coumarin ligands as potential selective acetylcholinesterase inhibitors, J. Enzyme Inhib. Med. Chem. 32 (2017) 285–297.

[33] Betül Kaya, Yusuf Özkay, Halide Edip Temel, Zafer Asım Kaplancıklı, Synthesis and biological evaluation of novel piperazine containing hydrazone derivatives, J. Chem. 2016 (2016) 1–7.

[34] George L. Ellman, K.Diane Courtney, Valentino Andres, Robert M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (2) (1961) 88–95.

[35] Devendra Kumar, Sukesh K. Gupta, Ankit Ganeshpurkar, Gopichand Gutti, Sairam Krishnamurthy, Gyan Modi, Sushil K. Singh, Development of piperazinediones as dual inhibitor for treatment of Alzheimer's disease, Eur. J. Med. Chem. 150 (2018) 87–101.

[36] Seung Ok Nam, Dong Hyun Park, Young Hun Lee, Jong Hoon Ryu, Yong Sup Lee, Synthesis of aminoalkyl-substituted coumarin derivatives as acetylcholinesterase inhibitors, Bioorg. Med. Chem. 22 (4) (2014) 1262–1267.

[37] M.O.E. (MOE), Molecular Operating Environment (MOE), Chemical computing Group (2009) http://www.chemcomp.com.

[38] D.E.V. Pires, T.L. Blundell, D.B. Ascher, pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures, J. Med. Chem. 58 (2015) 4066–4072.

[39] B.S. Furniss, A.J. Hannaford, P.W.G. Smith, A.R. Tatchell, Vogel's textbook of practical organic chemistry, Fifth Edn (1989) 1193.

[40] Corinne Brühlmann, Frédéric Ooms, Pierre-Alain Carrupt, Bernard Testa, Marco Catto, Francesco Leonetti, Cosimo Altomare, Angelo Carotti, Coumarins derivatives as dual inhibitors of acetylcholinesterase and monoamine oxidase, J. Med. Chem. 44 (19) (2001) 3195–3198.

[41] Ye Zhang, Biqun Zou, Zhenfeng Chen, Yingming Pan, Hengshan Wang, Hong Liang, Xianghui Yi, Synthesis and antioxidant activities of novel 4-Schiff base-7-benzyloxy-coumarin derivatives, Bioorg. Med. Chem. Lett. 21 (22) (2011) 6811–6815.

[42] Atul Manvar, Alpeshkumar Malde, Jitender Verma, Vijay Virsodia, Arun Mishra, Kuldip Upadhyay, Hrishikesh Acharya, Evans Coutinho, Anamik Shah, Synthesis, anti-tubercular activity and 3D-QSAR study of coumarin-4-acetic acid benzylidene hydrazides, Eur. J. Med. Chem. 43 (11) (2008) 2395–2403.

[43] I.P. Dubovik, M.M. Garazd, V.P. Khilya, Modified coumarins. 14. Synthesis of 7-hydroxy-[4,3′]dichromenyl-2,2′-dione derivatives, Chem. Nat. Compd. 40 (2004) 434–443.

[44] M. Cacic, M. Trkovnik, F. Cacic, E. Has-Schon, Synthesis and antimicrobial activity of some derivatives of (7-hydroxy-2-oxo-2*H*-chromen-4-yl)-acetic acid hydrazide, Molecules 11 (2006) 134–147.

[45] L. Pisani, G. Muncipinto, T.F. Miscioscia, O. Nicolotti, F. Leonetti, M. Catto, C. Caccia, P. Salvati, R. Soto-Otero, E. Mendez-Alvarez, C. Passeleu, A. Carotti, Discovery of a novel class of potent coumarin monoamine oxidase B inhibitors: Development and biopharmacological profiling of 7-[(3-chlorobenzyl)oxy]-4-[(methylamino)methyl]-2*H*-chromen-2-one methanesulfonate (NW-1772) as a highly potent, selective, revers, J. Med. Chem. 52 (2009) 6685–6706.

[46] GraphPad Prism version 8.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

[47] Jonah Cheung, Michael J. Rudolph, Fiana Burshteyn, Michael S. Cassidy, Ebony N. Gary, James Love, Matthew C. Franklin, Jude J. Height, Structures of human acetylcholinesterase in complex with pharmacologically important ligands, J. Med. Chem. 55 (22) (2012) 10282–10286.