Indian Journal of Chemical Technology
Vol. 31, May 2024, pp. 483-493
DOI: 10.56042/ijct.v31i3.3786

Design, synthesis and evaluation of novel coumarin-oxa/thiadiazole hybrids as AChE inhibitors for the treatment of alzheimer's disease

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Received 10 July 2023; accepted 11 March 2024

The most prevalent type of dementia is Alzheimer's disease. It is a central nervous system neurodegenerative disease marked mostly by progressive cognitive dysfunction. One of the well-defined targets for Alzheimer's disease management is the acetylcholinesterase enzyme. Coumarins are phytochemicals found naturally in many plant species that have a variety of biological actions, including acetylcholinesterase inhibition. To accomplish this goal, several 7-hydroxy coumarin derivatives as acetylcholinesterase (AChE) inhibitors have been synthesized using Pechmann condensation and conjugation with various thiadiazole and oxadiazole. Spectral analysis has been used to characterize these molecules. An *in silico* docking investigation against the AChE enzyme PDB 4EY7 demonstrates that the molecule interacts with the CAS and PAS sites of the acetylcholinesterase enzyme. The coumarin moiety interacts with the PAS site, whereas the thiadiazole/oxadiazole moiety interacts with the CAS site. Knowing the pharmacophoric requirements for inhibiting AChE, compounds have been developed and evaluated for anti-Alzheimer efficacy *in vitro* utilizing the Ellman assay. With an IC_{50} of 0.75 μM, compound **9b** have demonstrated great anti-Alzheimer efficacy. We conclude that the compounds described in this paper can be used to develop novel anti-Alzheimer agents.

Keywords: Coumarin derivatives, Molecular docking, Anti-Alzheimer Agents

Introduction

Alzheimer's disease is the most common type of dementia[1]. It is one of the major diseases that threaten the health of elderly people[2]. Alzheimer's disease (AD) is a neurodegenerative disease of the central nervous system characterized by progressive memory impairment[3]. British pathologists discovered that AD was not a remarkable illness of the young but rather what was regarded as "illness" in the 1960s. In the 1990s, specialists discovered that the β-amyloid protein played a role in AD. According to the World Health Organization (WHO) report, around 82 million people in 2030 and 152 million in 2050 will develop dementia (AD contributes 60% to 70% of cases). By 2050, there will be 106.2 million cases worldwide, with 1 in 85 people having AD[4]. The increase is a result of the aging of world's population. Using a Delphi consensus methodology, the international study on AD estimated that there were 24.3 million people worldwide who had dementia. According to medical standards for AD, laboratory and neurovascular research is primarily used for investigative functions, in particular to prevent structural brain damage and identify the "reversible" causes of dementia[5]. Only recently have pharmacological treatments for critical conditions like memory loss and cognitive impairment become available. Three of the five medications on the market Donepezil, Galantamine, and Rivastigmine come under the heading of "Cholinesterase Inhibitors." These medications prevent the brain's chemical messenger, which is important for memory and learning, from breaking down. The fourth medication, memantine, regulates a different chemical messenger in the brain that is also crucial for memory and learning. Although they function differently, both types of medications aid in symptom management. Cholinesterase Inhibitors are Tacrine (COGNEX) (1993), Donepezil (ARICEPT) (1996), Rivastigmine (EXELON) (2000), Galantamine (REMINYL)

(2001), NMDA antagonist: Memantine (NAMENDA) (2003)[6]. All autonomic ganglions, numerous autonomic innervation organs, neuromuscular junctions, and numerous synapses of the central nervous system contain the first neurotransmitter to be identified, acetylcholine (Ach). A family of enzymes known as cholinesterase is responsible for hydrolyzing the neurotransmitter ACh into choline and acetic acid. AChE is a serine hydrolase that is mainly present at cholinergic brain synapses and the neuromuscular junction[7]. Its primary biological function is the rapid hydrolysis of the neurotransmitter ACh to acetate and choline, which stops impulse transmission at cholinergic synapses. Particularly for cholinesterase, AChE has a very high degree of accuracy in catalytic activity, and each AChE molecule disintegrates about 25,000 ACh molecules per second, allowing the rate of diffusion-controlled reaction[8]. It is well known that AChE has 575 amino acids and two main binding sites: the peripheral anionic binding site (PAS) and the catalytic binding site (CBS), which are connected by a deep and hydrophobic gorge. Acetylcholinesterase inhibitors (AChEI) are the most trustworthy and possibly effective method for enhancing cholinergic transmission. The primary mechanism of action is to prevent the enzyme acetylcholinesterase from degrading acetylcholine[9].

To design new coumarin based derivatives (Fig. 1) first *in silico* molecular docking study carried out which revealed, coumarin derivatives in which the coumarin moiety binds with PAS site of AChE and tertiary nitrogen containing ring bind to CAS site of AChE. These two fragments are connected through an alkyl spacer. This knowledge allows us to assume that inhibition of AChE by acetylcholinesterase inhibitors occurs via competitive interaction with the CAS site of the enzyme. Our earlier report also supported us to design new derivatives[10, 11]. Coumarin belongs to a large class of phenolic substance founds in many plants isolated from a diverse range of plants sources including umbelliferae and rutaceae family. Coumarin scaffold represents a widely occurring and nature-friendly privileged structure, whose functionalization is straightforward. Coumarin derivatives have garnered attention due to their pharmacological properties, prompting exploration as potential AChE inhibitors[12, 13].

Our strategy is to design various derivatives with the basic skeleton of coumarin and it can give more potent anti-Alzheimer activity .So, based on this

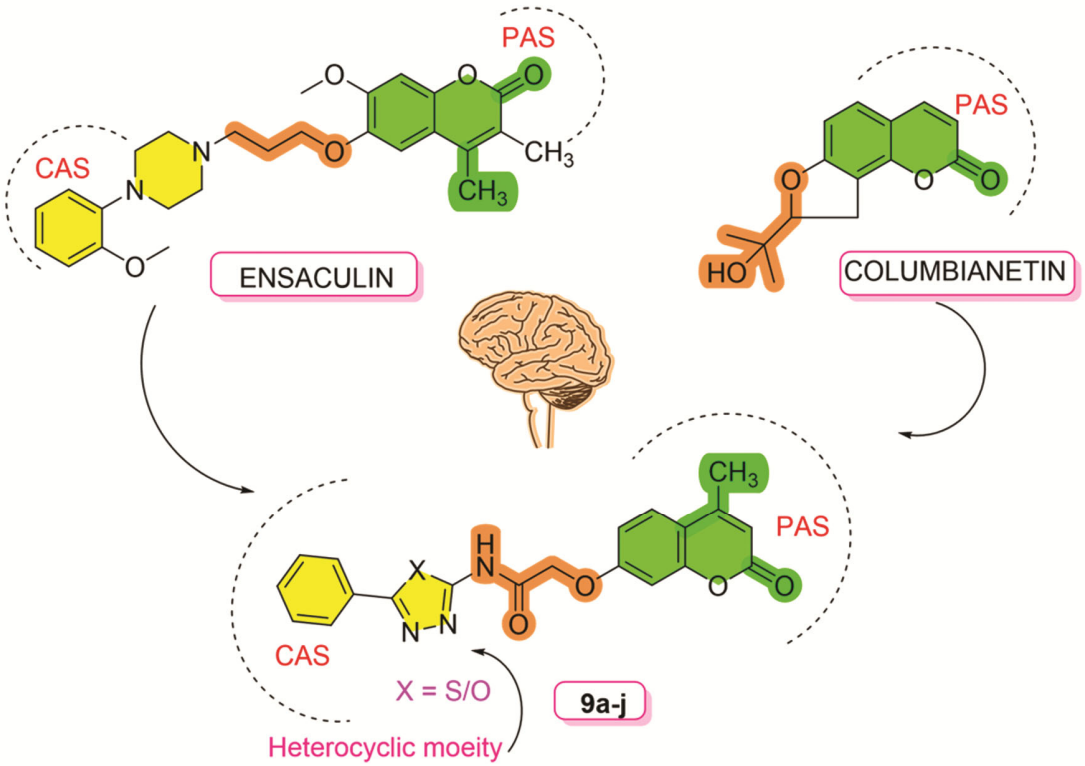


Fig. 1 — Design of coumarin based derivatives

knowledge we have proposed molecules which contain coumarin as a heterocyclic ring as one of the moieties capable of interacting with the PAS site of AChE in an effective manner .The other includes the thiadiazole or oxadiazole containing ring which goes interaction with the active site on CAS of AChE.

Experimental Section

Materials

All the raw ingredients and solvents needed for this work were procured from Merck, Sigma Aldrich. Thin-layer chromatography was used to monitor the reactions using pre-coated aluminium sheets with GF254 silica gel, 0.2 mm layer thickness (E. Merck). To determine melting points, a Veego melting point device was employed. The Agilent Technologies Infrared spectrometer was used to determine the IR spectra Cary630. The Bruker Advance-II 400 NMR Spectrometer at SICART, Vallabh Vidyanagar, Anand, Gujarat, running at 400 MHz was used to determine the ^{1}H-NMR spectra. The mass spectra of the synthetic chemicals were captured using an MS-Shimadzu mass spectrometer from Ribosome Research Center Pvt. Ltd. Kim.

Chemistry

Synthesis of new coumarin-oxa/thiadiazole hybrids were carried out as per scheme depicted in Fig 2. Each step involves specific reaction conditions and reagents tailored to the desired transformations. The resorcinol (**1**) reacted with ethyl acetoacetate in presence of sulphuric acid at 0-10°C to give 7-hydroxy-4methyl coumarin (**2**). Then ethyl bromoacetate reacted with compound 2 to form compound **5** in presence of K_2CO_3 and acetone. Seprately 1,3,4-thiadiazole and 1,3,4-oxadiazole (**8a-j**) were prepared by reacting various benzoic acids with thiosemicarbazide or semicarbazide in presence of $POCl_3$. Finally **9a-j** were prepared by reacting compound **5** with appropriate compund **8a-j** in presence of tertiary butoxide catalyst. All finally prepared derivatives chemical structure represented in Table 1

In-silico ADMET prediction

Discovery studio TOPKAT toxicity predictor used to calculate Mutagenicity, Carcinogenic potency of TD50 mouse, rat oral LD50, rat inhalational LC50, and Daphina EC50[14].

Molecular docking

Two-dimensional chemical structure for compounds **9a-j** was drawn in ChemDraw Ultra then conversion to 3D and optimization was carried out by using Maestro. The crystal structure of Recombinant Human Acetylcholinesterase in complex with Donepezil was retrieved from the protein data bank (PDB code: 4EY7)[15] and the structure was optimized by using Maestro of Schrödinger's Protein Preparation Wizard which correct the hydrogen atoms, missing

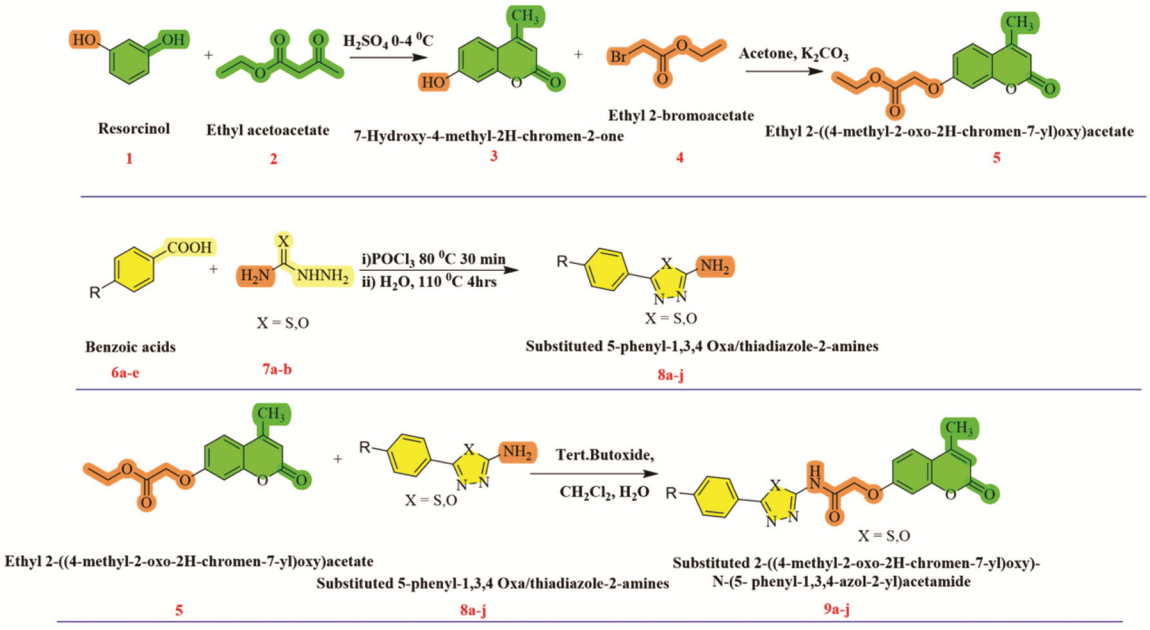
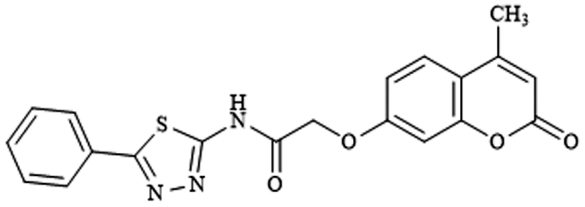
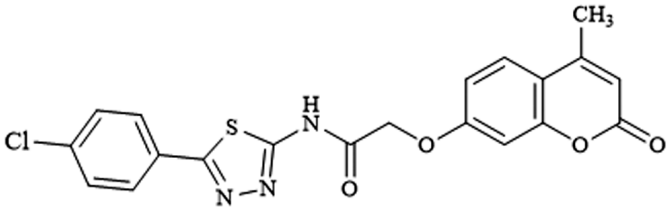
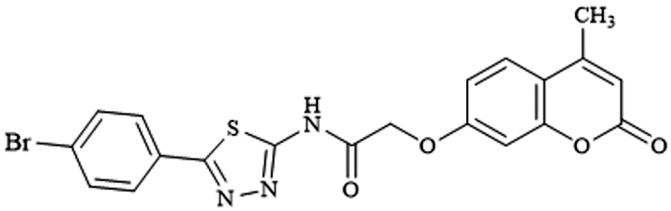
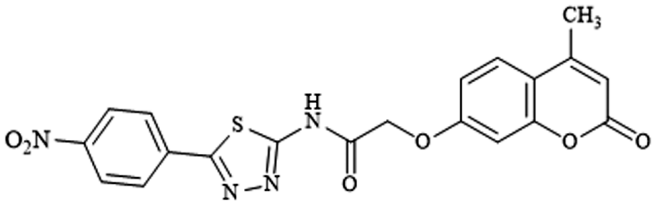
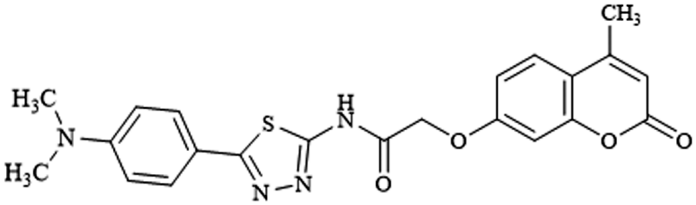
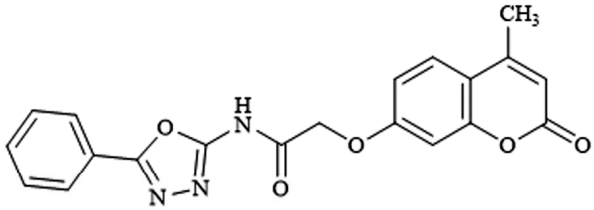
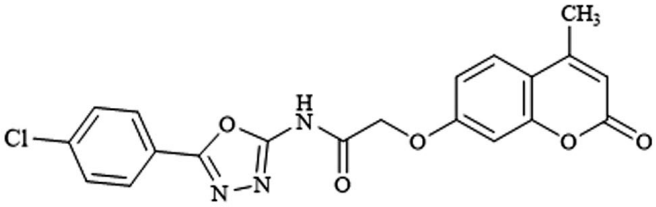


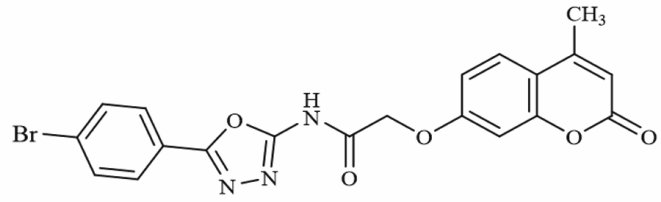
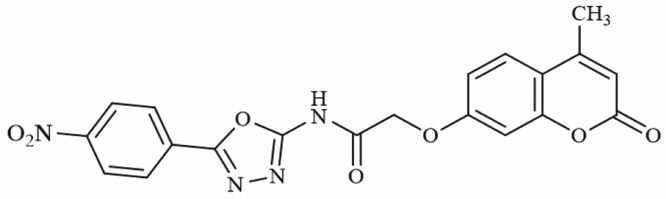
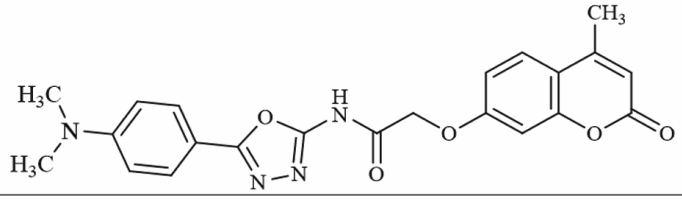
Fig. 2 — Synthetic route for new coumarin-oxa/thiadiazole hybrids

Table 1 — Chemical structure for all the molecules under investigation

Compounds	Chemical Structure	Mol. wt (g/mol)
9a		393.42
9b		427.86
9c		472.31
9d		438.41
9e		436.48
9f		377.35
9g		411.80

(*Contd.*)

Table 1 — Chemical structure for all the molecules under investigation (*Contd*.)

Compounds	Chemical Structure	Mol. wt (g/mol)
9h		456.25
9i		422.35
9j		420.42

atoms, bonds and computed charges and OPLS2005 force field was applied[16,17]. To see the various compounds binding mode in acetylcholine-sterase active site molecular docking study was performed using Glide docking simulation integrated into Schrodinger molecular modeling package[18,19]. The SP mode (Standard precision) docking protocols with default parameters defining the grid box generated using ligand present on active site and all other settings as default to Glide receptor grid generation.

Procedure for the synthesis of compound 3a[20]

150 mL of concentrated H_2SO_4 was taken in a three necked flask with a thermometer, mechanical stirrer and a dropping funnel. The flask was immersed in an ice bath. When the temperature decreased below 10 ℃, a solution of 15.3 g (0.91 mol) of resorcinol in 20 g (19.5 mL, 1.03 mol) β-keto esters was added drop wise with stirring. The temperature was maintained below 10 ℃ by means of an ice bath during the addition. The reaction mixture was kept at room temperature for about 18 h and then poured it with vigorous stirring into a mixture of crushed ice and water. The crude precipitates were collected by filtration at pump, washed it with cold water and dry at 100 ℃.

7-Hydroxy 4-methyl coumarin (3a)

Yield 92%; mp 183-185 °C; IR [(KBr) υ_{max}/ cm^{-1}]: 3429.2 (broad –OH group), 3157.1 (aromatic C–H), 1573 (C=N), 1662.4(C=O), 1274.7(C-O-C) cm^{-1}

Procedure for synthesis of compound 5a[21]

Ethyl bromoacetate (0.7 mL, 6.8 mmol) and anhydrous K_2CO_3 (1.9 g, 11.36 mmol) were added to a solution of 7-hydroxy-4 methyl coumarin (1.4 g) in distilled acetone (15 mL) & mixture was stirred under reflux at 6 h. Removal of solvent by evaporation left a pale-yellow solid residue that was extracted with ethyl acetate (3 x 20 mL) and dried over Na_2SO_4. The combined organic layer was concentrated in vacuoto give a white colour solid purified by recrystallization with ethanol.

Ethyl 2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetate (5a)

Yield 85%; mp 167-169 °C; IR [(KBr) υ_{max}/ cm^{-1}]: 3075.1 (aromatic C–H), 1379.1 (CH_3), 1703.4(C=O), 1058.0(C-O) cm^{-1}

General procedure for synthesis of compounds 8a-8j[22]

Thiosemicarbazide or semicarbazide (0.1 mol) and benzoic acid (0.1 mol) were added into a round bottom flask & then $POCl_3$ was added dropwise with stirring. The mixture was refluxed at 90 ºC for 25-30 min. After that the reaction mixture was cooled at room temperature then H_2O was added & the reflux was continued for 3-4 h. The reaction mixture was cooled & neutralized using NaOH solution. The precipitate was filtered, washed with water & recrystallized from ethanol.

General procedure for synthesis of compounds 9a-9j

KOt-Bu (6.6 mmol) was dissolved in THF with stirring in air at room temperature for 1 min.

Then substituted appropriate 5-phenyl-1,3,4 thia/oxadiazole-2-amine (3.3 mmol) and ethyl 2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetate (3.3 mmol) were added immediately and the mixture was stirred at room temperature until the amine was consumed. After evaporating THF under reduced pressure, H_2O (50 mL) and CH_2Cl_2 (50 mL) were added and the organic layer was separated and dried over anhydrous $MgSO_4$.

2-((4-Methyl-2-oxo-2H-chromen-7-yl) oxy)-N-(5-phenyl-1,3,4 thiadiazole-2-yl) acetamide (9a)

Yield 85%; mp 162-164 °C; IR [(KBr) υ_{max}/ cm^{-1}]: 3164.5 (N-H), 3041.5 (aromatic C–H), 1662.4, (C=O), 1561.8 (C=N), 685.8 (C-S) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (s, 1H, NH), 8.02-7.94 (m, 3H, Phenyl C_2, C_6 & Coumarin C_5-H), 7.51-7.44 (m, 3H, Phenyl C_3, C_4& C_5-H), 7.03 (d, 1H, Coumarin-C_8-H), 6.83 (dd, 1H, Coumarin-C_6-H), 6.10 (q, 1H, Coumarin-C_3-H), 4.81 (s, 2H, CH_2O), 2.41 (s, 3H, CH_3). MS *m/z* $[M]^+$calcd for $C_{20}H_{15}N_3O_4S$: 394

N-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetamide(9b)

Yield 60 %; mp174-176 °C IR [(KBr) υ_{max}/ cm^{-1}]: 3220.4 (N-H); 3052.7 (aromatic C–H), 1750, 1650 (C=O); 1580.4 (C=N), 738.0 (C-S); 825.1 (C-Cl) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (s, 1H, NH), 7.94-7.85 (m, 2H, Phenyl C_2,C_6-H), 7.49 (d, 1H, Coumarin C_5-H), 7.51-7.42 (m, 2H, Phenyl C_3& C_5-H), 7.02 (d, 1H, Coumarin-C_8-H), 6.87 (dd, 1H, Coumarin-C_6-H), 6.09 (q, 1H, Coumarin-C_3-H), 4.82 (s, 2H, CH_2O), 2.40 (s, 3H, CH_3).

N-(5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl)-2-((4-methyl-2-oxo-2H-chromen-7yl)oxy) acetamide (9c)

Yield 62 %; mp 168-170 °C; IR [(KBr) υ_{max}/ cm^{-1}]: 3179.4 (N-H); 3071.3 (aromatic C–H), 1729.5, 1648 (C=O); 738.0 (C-S); 693.3 (C-Br) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.89-7.83 (m, 3H, NH, Phenyl C_2,C_6-H), 7.69-7.63 (m, 2H, Phenyl C_3& C_5-H), 7.51 (d, 1H, Coumarin C_5-H), 7.01 (d, 1H, Coumarin-C_8-H), 6.85 (dd, 1H, Coumarin-C_6-H), 6.11 (q, 1H, Coumarin-C_3-H), 4.85 (s, 2H, CH_2O), 2.43 (s, 3H, CH_3).

N-(5-(4-Nitrophenyl)-1,3,4-thiadiazol-2-yl)-2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetamide (9d)

Yield 65 %; mp 190-192 °C; IR [(KBr) υ_{max}/ cm^{-1}]: 3201.8 (N-H); 3034.1 (aromatic C–H), 1715, 1649 (C=O); 1528.2 (C=N) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.67 (s, 1H, NH), 8.35-8.29 (m, 2H, Phenyl C_3,C_5-H), 8.20-8.14 (m, 2H, Phenyl C_2, & C_6-H), 7.51 (d, 1H, Coumarin C_5-H), 7.02 (d, 1H, Coumarin-C_8-H), 6.86 (dd, 1H, Coumarin-C_6-H), 6.09 (q, 1H, Coumarin-C_3-H), 4.86 (s, 2H, CH_2O), 2.39 (s, 3H, CH_3).

N-(5-(4-(Dimethylamino)phenyl)-1,3,4-thiadiazol-2-yl)-2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetamide (9e)

Yield 68 %; mp 165-168 °C; IR [(KBr) υ_{max}/ cm^{-1}]: 3296.3 (N-H); 3042.6 (aromatic C–H), 1760, 1653 (C=O); 1523.2 (C=N) cm^{-1} ; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.66 (s, 1H, NH), 7.90-7.82 (m, 2H, Phenyl C_2,C_6-H), 7.49 (d, 1H, Coumarin C_5-H), 7.07 (d, 1H, Coumarin-C_8-H), 6.84 (dd, 1H, Coumarin-C_6-H), 6.84-6.78 (m, 2H, Phenyl C_3& C_5-H), 6.13 (q, 1H, Coumarin-C_3-H), 4.79 (s, 2H, CH_2O), 2.43 (s, 9H, CH_3).

2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy)-N-(5-phenyl-1,3,4-oxadiazol-2-yl) acetamide (9f)

Yield 68 %; mp155-158 °C; IR [(KBr) υ_{max}/ cm^{-1}]: 3290.6 (N-H); 3030.3 (aromatic C–H), 1737, 1644 (C=O); 1591.6 (C=N); 1248.2 (C-O-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.89 (s, 1H, NH), 7.96-7.90 (m, 2H, Phenyl C_2,C_6-H), 7.64-7.56 (m, 2H, Phenyl C_3,C_5-H), 7.58-7.40 (m, 2H, Phenyl-C_4-H, & Coumarin C_5-H), 7.09 (d, *J* = 2.3 Hz, 1H, Coumarin-C_8-H), 6.76 (dd, 1H, Coumarin-C_6-H), 6.05 (q, 1H, Coumarin-C_3-H), 4.73 (s, 2H, CH_2O), 2.42 (s, 3H, CH_3).

N-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetamide (9g)

Yield 64 %; mp 174-176 °C; IR [(KBr) υmax/ cm^{-1}]: 3260 (N-H); 3049 (aromatic C–H), 1744, 1648 (C=O); 1510 (C=N); 1346.2 (C-O-C); 850 (C-Cl) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.89 (s, 1H, NH), 8.06-8.00 (m, 2H, Phenyl C_2,C_6-H), 7.51 – 7.42 (m, 3H, Phenyl C_3,C_5-H & Coumarin C_5-H), 7.08 (d, 1H, Coumarin-C_8-H), 6.84 (dd, 1H, Coumarin-C_6-H), 6.10 (q, 1H, Coumarin-C_3-H), 4.77 (s, 2H, CH_2O), 2.41 (s, 3H, CH_3).

N-(5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetamide (9h)

Yield 70%; mp 164-166°C; IR [(KBr) υ_{max}/ cm^{-1}]: 3275 (N-H); 3030 (aromatic C–H), 1765, 1647 (C=O); 1558.1 (C=N); 1248 (C-O-C); 689 (C-Br) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.84 (s, 1H, NH), 7.85 – 7.82 (m, 2H, Phenyl C_2,C_6-H), 7.65 – 7.58 (m, 2H, Phenyl C_3,C_5-H), 7.47 (d, 1H, Coumarin C_5-H), 7.05 (d, 1H, Coumarin-C_8-H), 6.81 (dd, 1H, Coumarin-C_6-H), 6.07 (q, Coumarin-C_3-H) 4.76 (s, 2H, CH_2O), 2.40 (s, 3H, CH_3).

N-(5-(4-Nitrophenyl)-1,3,4-thiadiazol-2-yl)-2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetamide (9i)

Yield 67%; mp 201-203 °C; IR [(KBr) υ_{max}/ cm^{-1}]: 3290 (N-H); 3051 (aromatic C-H), 1590 (C=N); 1755, 1660 (C=O); 1350 (NO_2); 1210 (C-O-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.85 (s, 1H, NH), 8.40-8.34 (m, 2H, Phenyl C_3,C_5-H), 8.24-8.18 (m, 2H, Phenyl C_2,C_6-H), 7.50 (d, 1H, Coumarin C_5-H), 7.04 (d, 1H, Coumarin-C_8-H), 6.88 (dd, 1H, Coumarin-C_6-H), 6.10 (q, 1H, Coumarin-C_3-H) 4.73 (s, 2H, CH_2O), 2.41 (s, 3H, CH_3).

N-(5-(4-(Dimethylamino) phenyl)-1,3,4-thiadiazol-2-yl)-2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetamide (9j)

Yield 70%; mp 170-172 °C; IR [(KBr) υ_{max}/ cm^{-1}]: 3286 (N-H); 3042 (aromatic C–H), 1740, 1650 (C=O); 1539 (C=N); 1450 (CH_3); 1235 (C-O-C); cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.89 (s, 1H, NH), 7.86-7.80 (m, 3H, Phenyl C_2,C_6-H & Coumarin C_5-H), 7.51 (d, 1H, Coumarin-C_8-H), 7.03 (d, 2H, Coumarin-C_6-H), 6.91-6.84 (m, 2H, Phenyl C_3,C_5-H), 6.11 (q, 1H, Coumarin-C_3-H), 4.71 (s, 2H, CH_2O),), 2.43 (s, 9H, CH_3).

***In vitro* anti-Alzheimer activity**

The enzyme acetylcholinesterase effectively catalyzes the hydrolysis of acetylthiocholine (AcSCh)-which is the sulfur analogs of their respective natural substrate, acetylcholine. These substrate analogs produce acetate and thiocholine on hydrolysis. Thiocholine reacts to form the yellow 5-thio-2-nitrobenzoate anion in presence of highly reactive dithiobisnitri-benzoate (DTNB). The yellow colour, quantified by its absorbance at 405 nm[23]. We performed the Ellman assay in 96-well microtiter plates in a final reaction volume of 200 mL. Substrate hydrolysis was monitored by repeated spectrophotometric readings at 2 min. intervals by a computer-controlled microtiter plate reader which automatically computes mA_{405}/min for the best fit straight line through the data points. Then converted our data into the standardized units of nanomoles substrate hydrolyzed/min x mL, using the extinction coefficient for the yellow product (ε = 13,600 $M^{-1}cm^{-1}$) to find the concentration, c, from the equation c = A/εxl. The light path, l, is 0.5 cm.

Results and Discussion

ADMET

Table 2 summarizes the method used to establish the therapeutic compatibility of the drug and its toxicity prediction using computer assisted technology (TOPKAT). TOPKAT is a valuable technique for quantitative *in-silico* toxicity prediction, and it is used in quantitative structure-activity relationship (QSTR) models. Moreover, it estimates probability values and analyses toxicity using these QSTR models. It uses the criterion of examining the components in the optimum prediction space (OPS), and if they are outside, the findings are considered unreliable, i.e., false positives. Mutagenicity was computed. It also includes carcinogenic potency values for the TD50 mouse, rat oral LD50, rat inhalational LC50, and Daphina EC50. Increased TD50, LD50, LC50, and EC50 values imply a decrease in toxicity and a rise in the drug's safety index, making it more potent.

Molecular docking

For the inhibition of AChE molecule must bind PAS first then to CAS binding sites of receptor.

Table 2 — Toxicity properties for 9a-j and reference drug

Comp. Name		TD50 value (mg/kg)		LD50 value (g/kg)	EC50 Value (mg/L)
		Carcinogenicity (Mouse)	Carcinogenicity (Rat)	Acute oral toxicity (Rat)	Daphnia
9a	0.105	73	40.7	1.34	2.92
9b	0.0636	22.8	4.13	1.22	2.67
9c	0.0367	22.6	4.24	1.66	1.47
9d	0.257	35.3	3.27	2.26	1.47
9e	0.165	6.72	1.67	0.665	4.13
9f	0.0234	77.3	34.1	1.61	5.2
9g	0.00965	24.2	3.47	1.47	4.75
9h	0.00574	24.1	3.58	2.01	2.63
9i	0.0725	37.6	2.75	2.73	2.62
9j	0.0381	7.15	1.41	0.803	7.37
Ensaculin	0.481	197	0.308	2.44	0.207
Columbianetin	0.62	92.5	17	0.371	122

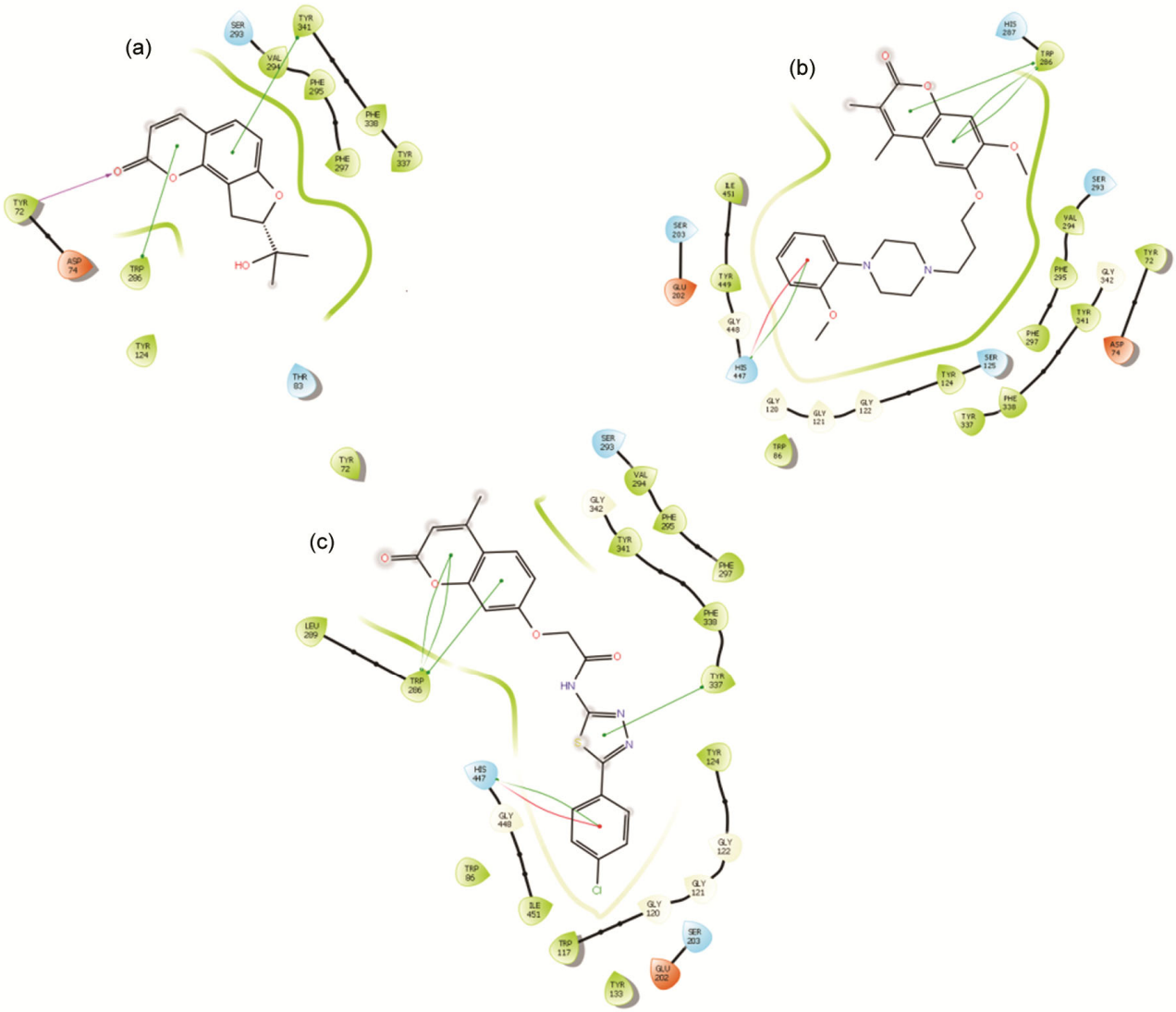


Fig. 3 — Schematic diagram of the receptor-ligand interaction of (a) Columbianetin, (b) Ensaculin and (c) 9b with 4EY7

Both the site interactions required for better inhibition. The receptor-ligand interactions represented in Fig. 3 indicate that designed molecule binds with PAS as well as CAS site. Also, the Table 3 represents that the amino acids involved in interaction with molecules which are part of CAS and PAS binding sites.

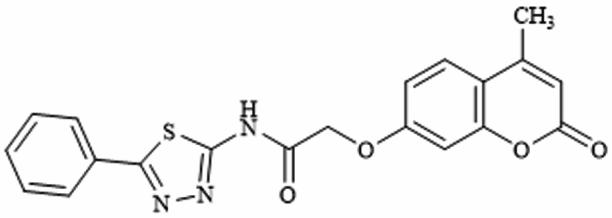
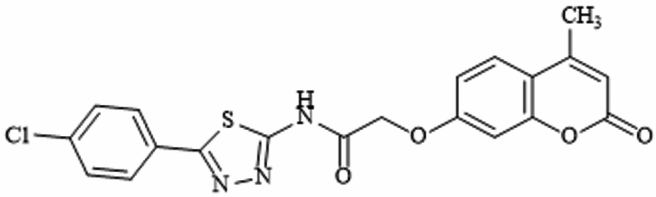
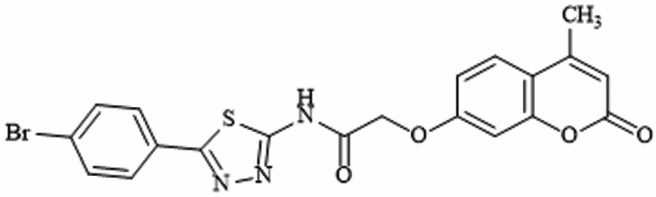
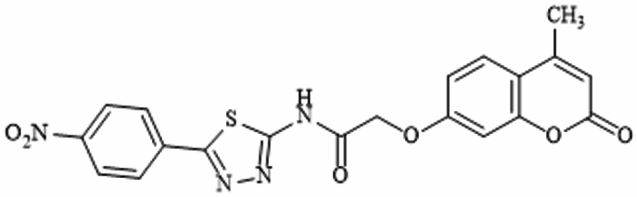
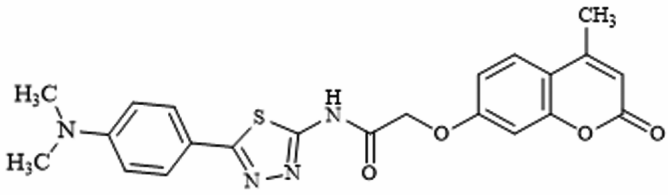
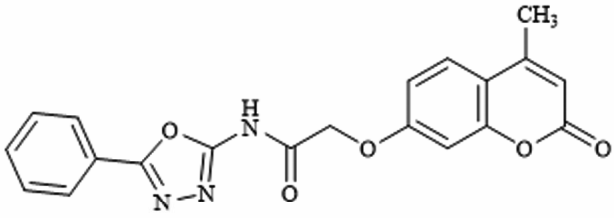
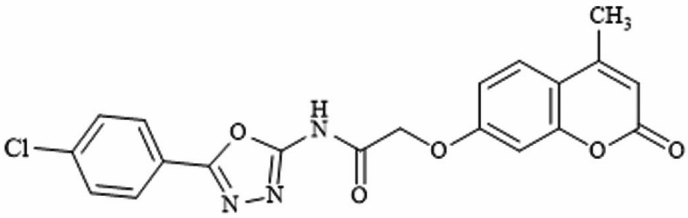
Biological activity

The Table 4 represents the inhibitory potency of compounds **9a-j** and donepezil against AChE. Compounds showed IC_{50} values ranging from 0.75 to 5.95 µM that of donepezil showed 0.023 µM. The thiadiazole series molecules showed better activity as compared to oxadiazole series. Halogen substituted derivatives showed better activity specifically chlorine containing then dimethyl amino substituted derivatives showed better activity.

Table 3 — Receptor-ligand binding interactions for compounds 9a-j and interaction of molecule

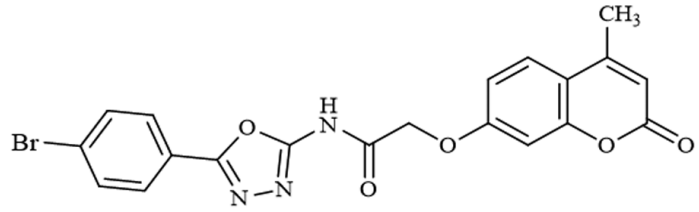
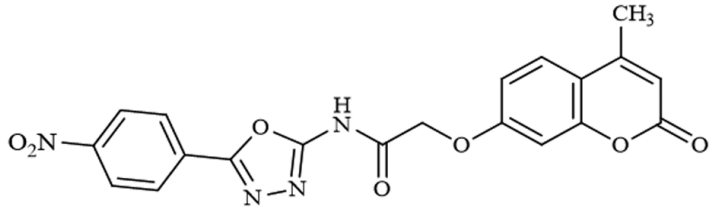
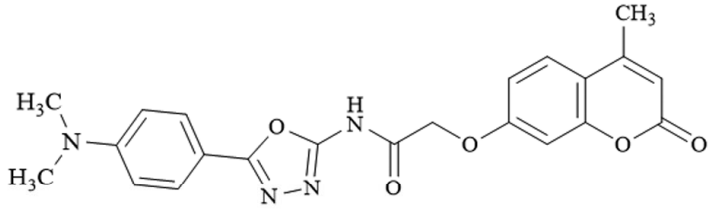
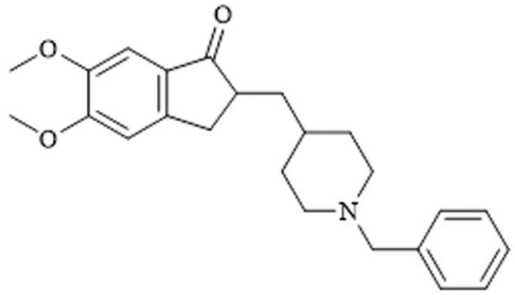
Compounds	Receptor-ligand binding interactions
9a	Trp86, Phe295, Trp286
9b	Trp286, Tyr337, His447
9c	Trp286, Tyr72, His447, Tyr337
9f	Trp86, Gly120, Tyr341
9g	Gly120, Trp86, Tyr341, Trp286
9h	Gly120, Trp86, Tyr341, Trp286
9j	Trp286, Tyr337, Phe338
Ensaculin	His447, Trp286
Columbianetin	Tyr341, Trp286

Table 4 — Anti-Alzheimer activity of compounds 9a-9j

Code	Structure	IC_{50} μM±SEM
9a		1.95±0.36
9b		0.75±062
9c		2.50±0.42
9d		4.45±0.62
9e		1.06±0.31
9f		5.95±0.53
9g		1.62±0.46

(*Contd.*)

Table 4 — Anti-Alzheimer activity of compounds 9a-9j (*Contd.*)

Code	Structure	IC_{50} μM±SEM
9h		1.91±0.56
9i		3.23±0.52
9j		1.51±0.23
Donepezil		0.023.00±0.037

Conclusion

This research involved the synthesis and screening of coumarin with thiadiazole or oxadiazole derivatives for their potential to inhibit acetylcholinesterase and thereby address Alzheimer's disease. Initially, derivative design was formulated through literature review and *in silico* investigations. Subsequently, compounds were synthesized, characterized, and finally tested for anti-Alzheimer activity *in vitro*. The *in silico* molecular docking study suggested that molecules are effectively binding at CAS and PAS sites of AChE enzyme. The molecular docking score kcal/mol were observed in rang of -10.439 to -9.032 which is better indicator for inhibition of AChE, particularly molecule 9a, -9.505; 9b, -10.719; 9c, -10.439; 9d, -9.471, 9e, -9.178, 9f, -9.509, 9g, -9.354, 9h, -9.617, 9i, -9.032, 9j, -9.576. Additionally, the ADMET study profile for molecules suggested that molecules are not toxic and have capability to cross BBB. The *in vitro* anti-Alzheimer activities of synthesized compounds 9a-j were carried out by Ellman assay. The IC_{50} value ranging from 0.75 μM to 5.95 μM, from which compound 9b is more active with IC_{50} value of 0.75 μM as compared to other compounds reported. Further these studies give information that molecules from thiadiazole series are better than oxadiazole series, particularly molecules with halogen and dimethyl amino substitution are good inhibitors of AChE enzyme. A future directional approach is needed on clinical trials and commercialization.

Acknowledgements

This study is financially supported by Board of Research in Nuclear Sciences and Department of Atomic Energy (BRNS-DAE), Bhabha Atomic Research Centre, Government of India, Mumbai, India under major research grant no. 35/14/06/2017-

BRNS /35089. The authors would like to thank Dr. N. D. Jivani, President, Dr. Dinesh L. Sutaria, Secretary, Shree Dhanvantary Pharmacy College for providing necessary facilities.

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