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New coumarin-benzotriazole based hybrid molecules as inhibitors of acetylcholinesterase and amyloid aggregation

Atamjit Singh[a], Sahil Sharma[a], Saroj Arora[b], Shivani Attri[b], Prabhjot Kaur[b], Harmandeep Kaur Gulati[a], Kavita Bhagat[a], Nitish Kumar[a], Harbinder Singh*[,a], Jatinder Vir Singh*[,a], Preet Mohinder Singh Bedi*[,a]

[a]*Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, Punjab 143005, India*

[b]*Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab 143005, India*

Corresponding authors: 1) Dr. Harbinder Singh
Department of Pharmaceutical Sciences,
Guru Nanak Dev University,
Amritsar, Punjab 143005, India
Email id: singh.harbinder40@gmail.com

2) Jatinder Vir Singh
Department of Pharmaceutical Sciences,
Guru Nanak Dev University,
Amritsar, Punjab 143005, India
Email id: jatindervirsingh1@gmail.com

3) Dr. Preet Mohinder Singh Bedi
Department of Pharmaceutical Sciences,
Guru Nanak Dev University,
Amritsar, Punjab 143005, India
Email id: bedi_preet@yahoo.com

Abstract

A novel series of triazole tethered coumarin-benzotriazole hybrids based on donepezil skeleton has been designed and synthesized as multifunctional agents for the treatment of Alzheimer's disease (AD). Among the synthesized compounds **13b** showed most potent acetylcholinesterase (AChE) inhibition (IC_{50} = 0.059 μM) with mixed type inhibition scenario. Structure-activity relationship revealed that three-carbon alkyl chain connecting coumarin and triazole is well tolerable for inhibitory potential. Hybrids obtained from 4-hydroxycoumarin and 1-benzotriazole were most potent AChE inhibitors. The inhibitory potential of all compounds against butyrylcholinesterase was also evaluated but all showed negligible activity suggesting that the hybrid molecules are selective AChE inhibitors. **13b** (most potent AChE inhibitor) also showed copper-induced $A\beta_{1-42}$ aggregation inhibition (34.26 % at 50 μM) and chelating properties for metal ions (Cu^{2+}, $Fe^{2+,}$ and Zn^{2+}) involved in AD pathogenesis along with DNA protective potential against degenerative actions of ·OH radicals. Molecular modelling studies confirm the potential of **13b** in blocking both PAS and CAS of AChE. In addition, interactions of **13b** with $A\beta_{1-42}$ monomer are also streamlined. Therefore, hybrid **13b** can act as an effective hit lead molecule for further development of selective AChE inhibitors as multifunctional anti-Alzheimer's agents.

Keywords: Alzheimer's disease; coumarin-benzotriazole hybrids; acetylcholinesterase inhibitors; amyloid aggregation inhibitor; molecular docking.

Alzheimer's disease (AD) is the most prevalent (70 to 80 % of all dementia forms) type of dementia in elderly people (usually 65 years and above) all around the globe characterized by progressive memory loss, impaired linguistic efficacy, mood disturbances, behavioural changes and cognitive impairments which finally carries toward death. It is a neurodegenerative disorder that starts slowly and gets worse over time with lost body functions that make the life of AD patient miserable and pose heavy monetary as well as a social burden to family and society.[1] Thus, it is a global emergency to control this disease without any delay.

Though the origin of AD is still ambiguous lifestyle, genetic and environmental factors are suggested to be involved in the onset as well as the progression of this disease.[2] Generation of toxic amyloid-beta (Aβ) protein and its extracellular plaques, as well as neurofibrillary tangles, originated from hyperphosphorylated tau proteins were considered as the two major pathological hallmarks of AD and no standard treatment was available for this disease until Davies and Maloney (in 1973) proposed cholinergic hypothesis.[3] Following the same, four cholinesterases (acetylcholinesterase and butyrylcholinesterase) inhibitors tacrine, donepezil, rivastigmine and galantamine were approved by the FDA. These drugs offer only palliative care and unable to freeze or reverse disease progression.[4] One NMDA receptor antagonist (memantine) was also approved but it doesn't offer much relief similar to cholinesterase inhibitors.[5] In last 20 years, apart from cholinergic, amyloid and tau protein cascade, various novel AD mechanisms have also been explored by the researchers that include inflammation, oxidative stress and deregulation of bio-metals, etc. suggesting multi-targeted nature of this disease.[6] Along with the hydrolysis of acetylcholine (ACh), acetylcholinesterase enzyme (AChE) is found to promote Aβ aggregation that interacts with its peripheral anionic site (PAS).[7] Metal hemostasis in the brain of AD patient has been observed to be disturbed by extracellular elevation of zinc and copper along with the intracellular accumulation of iron. Copper and zinc were observed to bind with Aβ and promote reactive oxygen species (ROS) by inducing its aggregation.[8] Oxidative stress resulted from the abundance of redox-sensitive metals (Cu, Fe, and Zn) found to induce proteolysis of amyloid precursor protein via upregulating β-secretase that leads to an increase in Aβ. Iron was found to delay ordered aggregation of Aβ that promotes its toxicity. Chelation of iron was observed to provide protection from Aβ toxicity.[9]

Coumarins (benzopyran-2-ones) offer a potential class of compounds that possess a wide range of therapeutic properties (**Fig. 1**). Numerous coumarin derivatives like warfarin as an anticoagulant **(1)**, esculin as vasoprotective **(2)**, scopoletin as anti-inflammatory **(3)**, hymecromone as an antispasmodic **(4),** etc. are already in clinical practice. Besides this, coumarin derivatives are reported to have a diverse range of biological activities like antioxidant, anticancer, antidepressant, antinociceptive, hepatoprotective, antibacterial, antifungal, antiviral, antidiabetic and anti-Alzheimer's. Especially in Alzheimer's drug development, multifunctional coumarin hybrids **(5-9)** have also been reported with promising results.[10]

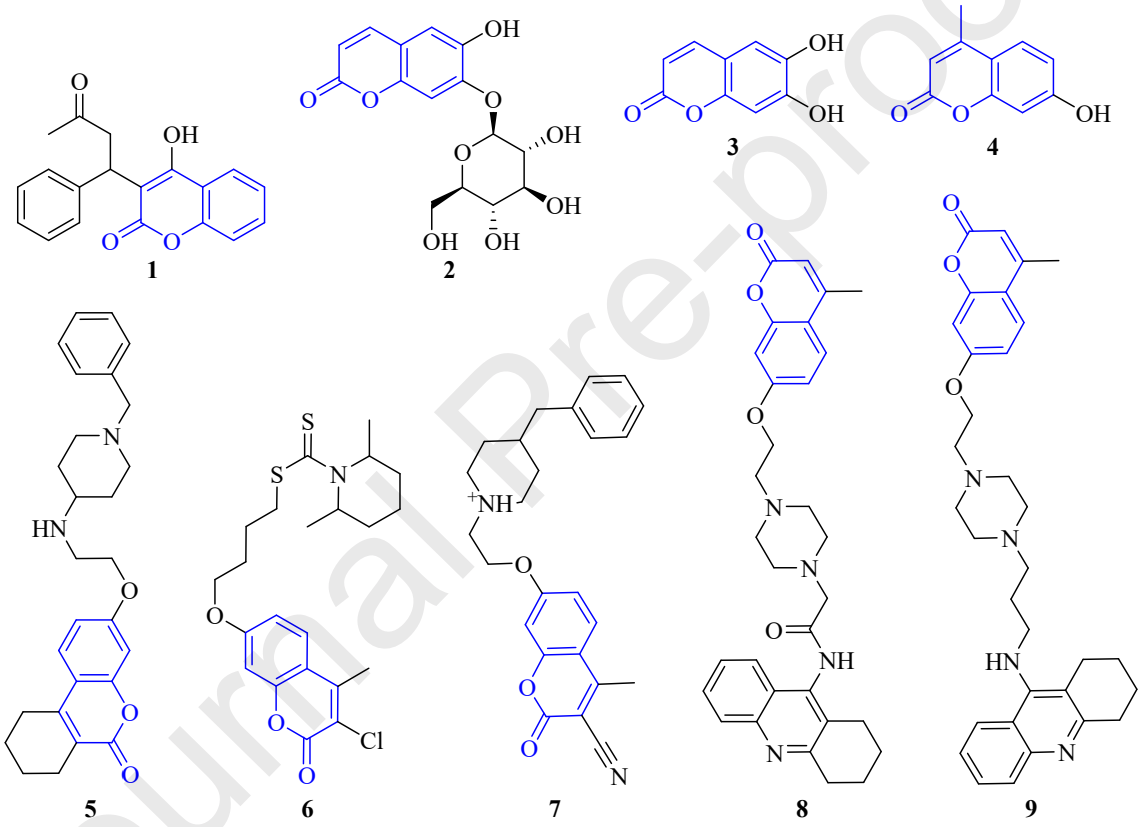


Fig. 1. Various coumarin derivatives in clinical practice **(1-4)** and multifunctional anti-Alzheimer's agents **(5-9)**.

Benzotriazole is a versatile nucleus in the field of medicinal chemistry and has long known metal chelating properties. In past two decades, a wide range of biologically active molecules has been designed by researchers across the globe in which benzotriazole is acting as a core nucleus itself or modulating the activity of other biologically active pharmacophores (**Fig. 2**). Benzotriazole derivatives designed so far has shown potential biological activities such as antibacterial **(10)**,

antituberculosis **(11)**, antifungal **(12)**, antiviral **(13)**, antiprotozoal **(14)**, anticancer **(15)**, antiemetic **(16)**, antioxidant **(17)**, and anti-inflammatory **(18),** etc.[11]

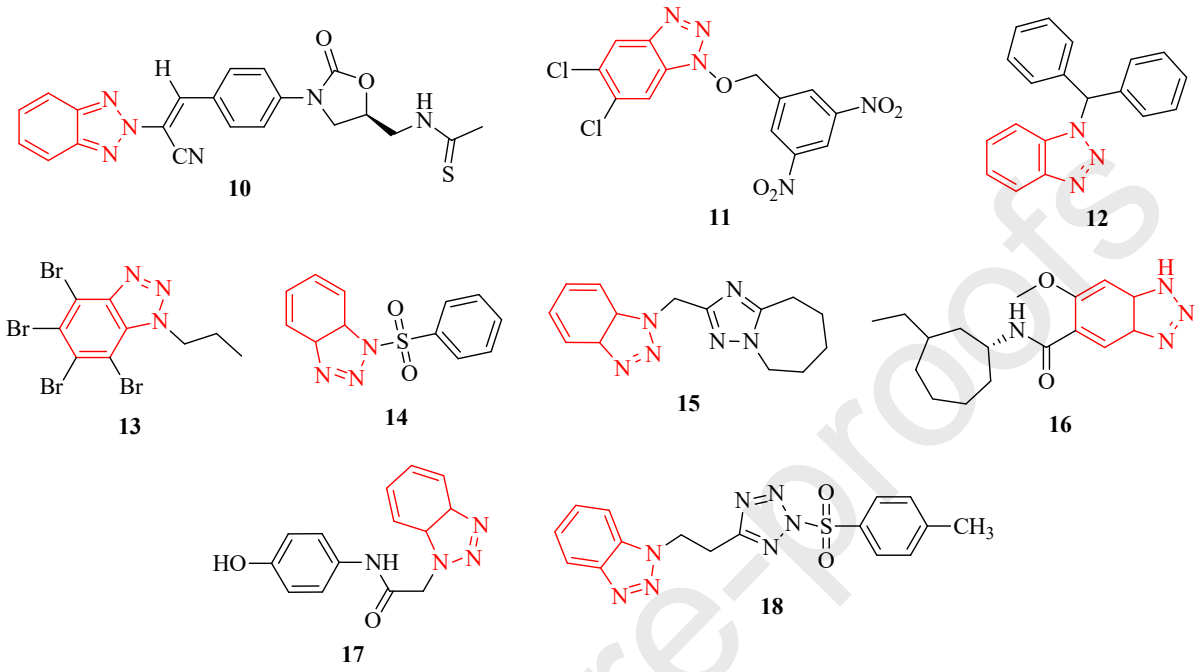


Fig. 2. Various biologically active benzotriazole derivatives **(10-18)**.

Molecular hybridization is a well-established stratagem that involves combining two or more pharmacophores with or without any linker and provides a single hybrid molecule having properties of all combined bioactive substances. Molecular hybridization remains successful in giving various potential candidates to clinical trials within the last 30 years that includes MCB3837, Ro 23-9424, CBR-2092, and TD-1792 etc.[12,13] Triazole is a well-known bioactive moiety that has been successfully utilized for the development of potential bioactive compounds (**Fig. 3**) including multifunctional anti-Alzheimer's agents **(23-28)**.[14-17] Molecular docking studies of these multifunctional agents suggest that triazole more fondly interacts with acetylcholine binding site of AChE and impart higher selectivity toward AChE over butyrylcholinesterase (BuChE).[18] Triazole linker appropriately separating two active pharmacophores, was observed for introducing anti-Aβ aggregation properties in the molecule.[19]

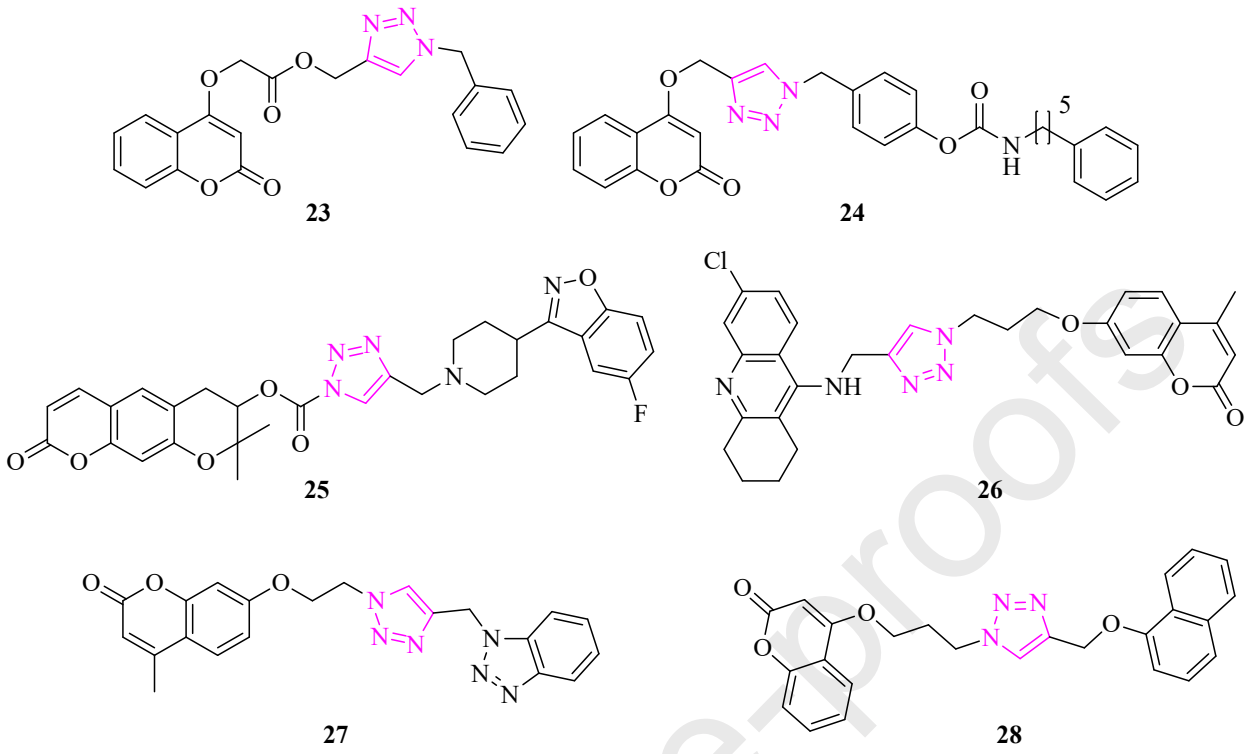


Fig. 3. Triazole linked hybrid molecules with anti-Alzheimer's activity **(23-26)**.

Peripheral anionic site (PAS) of AChE interacts with Aβ fragment and promotes its aggregation while catalytic anionic site (CAS) selectively accepts ACh. Donepezil is the only drug that interacts simultaneously with both PAS and CAS of AChE enzyme, which may be the possible reason for its dual AChE as well as Aβ aggregation inhibitory potential. These properties makes donepezil, a perfect template for Alzheimer's drug development.[20] The favourable skeleton of donepezil and strong pharmacophoric features of coumarin and benzotriazole along with the successful history of molecular hybridization with triazole linker provides a solid base for utilizing all of them in designing novel multifunctional anti-Alzheimer agents (**Fig. 4**). Thus considering the pressing need of potential multifunctional agents for the management of AD, novel triazole linked coumarin-benzotriazole hybrids based on donepezil skeleton template has been designed by taking account of Lipinski rule of 5 and ADME properties (Supplementary Table S1 and S2), synthesized via click chemistry approach and evaluated for their anti-Alzheimer potential.

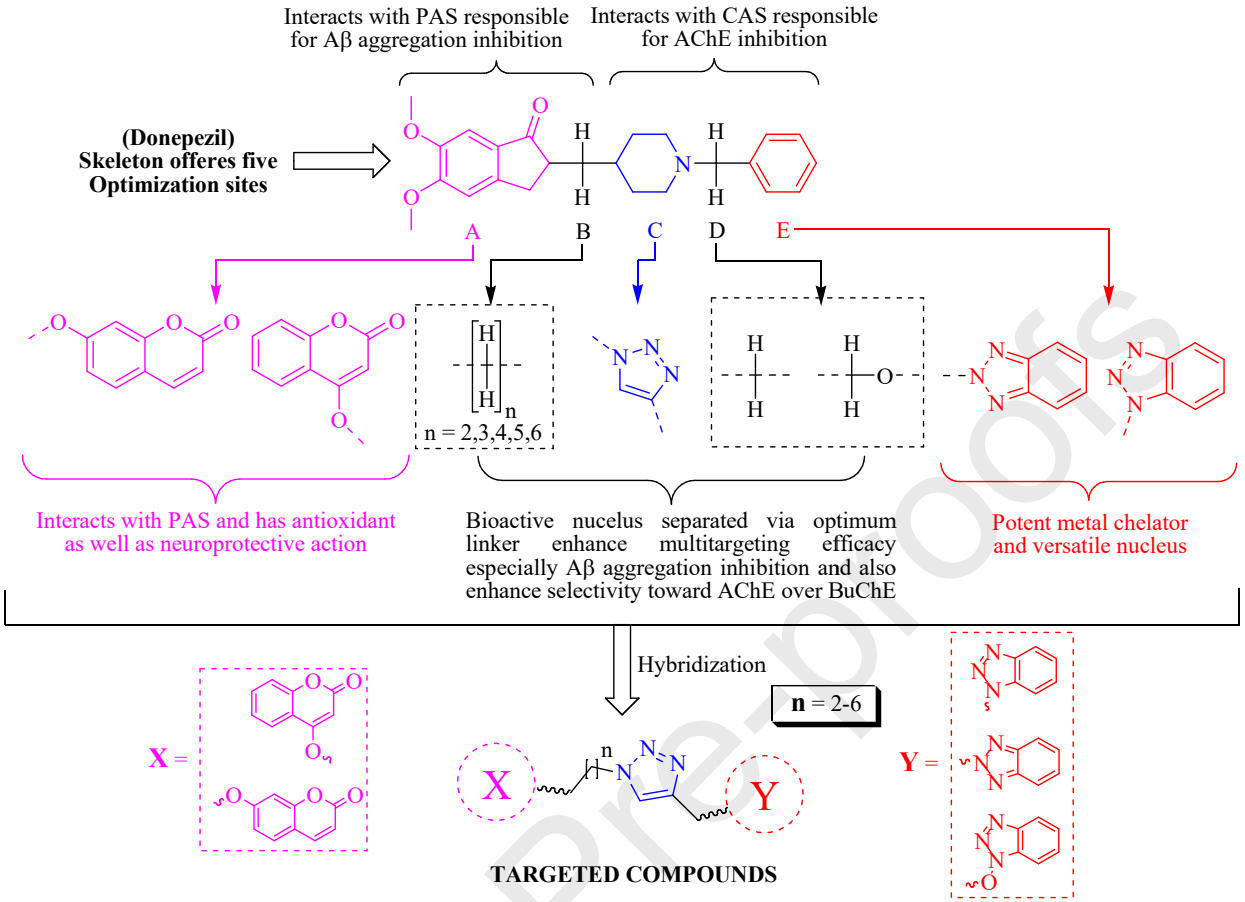
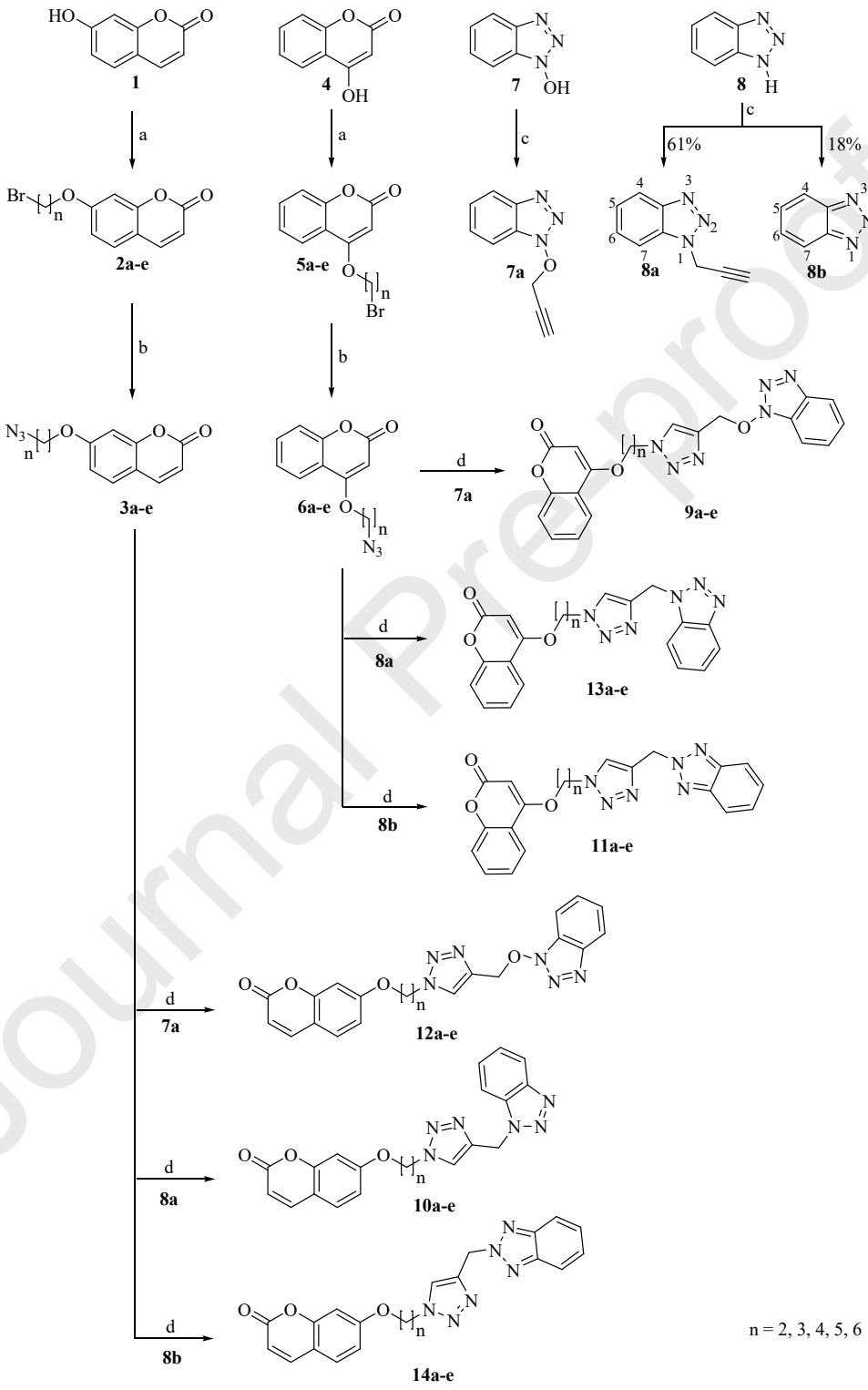


Fig. 4. Design strategy.

Designed hybrid molecules were synthesized via a series of chemical reactions (**Scheme 1**). Coumarins i.e. 4-OH coumarin (**1**) and 7-OH coumarin (**4**) were reacted with various dibromoalkanes in the presence of K_2CO_3 at 25 °C in DMF yielded alkylated coumarins (**2a-e** and **5a-e**). Alkylated coumarins were further treated with NaN_3 at 25 °C in DMF to form *N*-azidoalkyl coumarins (**3a-e** and **6a-e**). Simultaneously, OH-benzotriazole (**7**) was reacted with propargyl bromide in the presence of K_2CO_3 at 25 °C in DMF to get 1-(prop-2-yn-1-yloxy)-1H-benzotriazole (**7a**). Propargylation of 1H-benzotriazole (**8**) was done under similar conditions as used for OH-benzotriazole resulted in 1-(prop-2-yn-1-yl)-1H-benzotriazole (**8a**) with 61 % yield and 2-(prop-2-yn-1-yl)-2H-benzotriazole (**8b**) with 18 % yield. Compound **PBB (8a)** was characterized by the appearance of two doublets at 8.09-8.07 ppm and 7.73-7.71 ppm for two individual flagpole protons present on the 4th and 7th position of benzene ring (Scheme 1), while in PBC (**8b),** these flagpole protons are appeared as merged signals in a multiplet at 7.91-7.95 ppm as these protons bear in same electromagnetic environment (refer to ^{1}H NMR of **8b** in supplementary data).

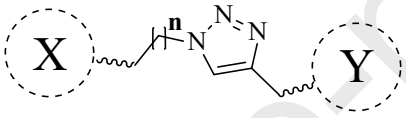
Obtained propargylated benzotriazoles were treated with prepared *N*-azidoalkyl coumarins in the presence of the catalytic amount of copper sulfate and reducing agent sodium ascorbate at 25 °C in DMF yielded various triazole linked coumarin-benzotriazole hybrids.[21]

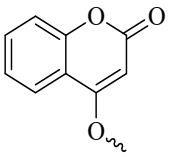
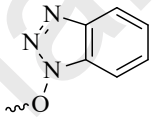
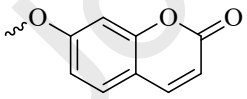
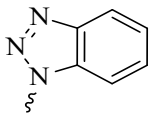


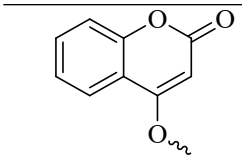
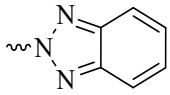
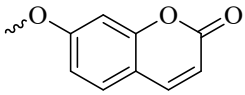
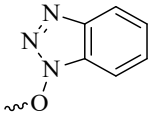
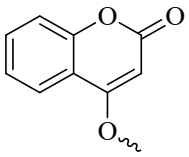
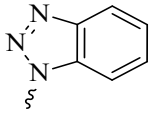
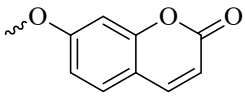
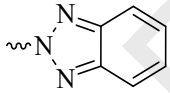
Scheme 1. Synthesis of triazole linked coumarin-benzotriazole hybrids. Reagents and conditions: (a) dibromoalkane, K_2CO_3, DMF, 0.5 to 2 h, stir, rt; (b) NaN_3, DMF, 1 to 2 h, stir, rt; (c) propargyl bromide, K_2CO_3, DMF, 0.5 to 4 h, stir, rt; (d) sodium ascorbate, $CuSO_4$, DMF, 0.5 to 8 h, rt.

As in the hydrolysis process of acetylcholine, 80 % role is performed by AChE enzyme, while BuChE performs only a secondary role. Therefore, all the synthesized compounds were screened for their inhibitory potential against both AChE and BuChE enzymes using Ellman's method and compared with standard anticholinesterase drug donepezil. Data were obtained from triplicate experiments and presented as the mean ± SD of three independent experiments (Table 1).

Table 1. AChE, BuChE and $A\beta_{1-42}$ aggregation inhibition of synthesized compounds in comparison to Donepezil.



Comp.	X	Y	n	*ee*AChE[b] IC$_{50}$ (μM)[a]	*eq*BuChE[c] IC$_{50}$ (μM)[a]	$A\beta_{1-42}$ aggregation inhibition (%)[d]
9a			2	4.322 ± 0.056	≥10	16.22 ± 1.28
9b			3	1.324 ± 0.012	≥10	≤10
9c			4	3.233 ± 0.027	≥10	≤10
9d			5	≥10	≥10	≤10
9e			6	≥10	≥10	≤10
10a			2	2.112 ± 0.029	≥10	29.36 ± 1.69
10b			3	2.924 ± 0.016	≥10	15.86 ± 1.48
10c			4	4.329 ± 0.031	≥10	11.36 ± 1.89
10d			5	5.429 ± 0.038	≥10	≤10
10e			6	≥10	≥10	≤10
11a			2	1.890 ± 0.023	≥10	≤10
11b			3	1.670 ± 0.014	≥10	≤10

11c			4	2.093 ± 0.016	≥10	≤10
11d			5	4.810 ± 0.045	≥10	≤10
11e			6	6.233 ± 0.019	≥10	≤10
12a			2	2.012 ± 0.016	≥10	11.38 ± 1.23
12b			3	1.978 ± 0.026	≥10	≤10
12c			4	≥10	≥10	≤10
12d			5	≥10	≥10	≤10
12e			6	≥10	≥10	≤10
13a			2	0.097 ± 0.045	≥10	36.68 ± 2.17
13b			3	0.059 ± 0.006	≥10	34.26 ± 1.97
13c			4	0.234 ± 0.061	≥10	22.36 ± 1.81
13d			5	1.141 ± 0.012	≥10	11.36 ± 0.77
13e			6	5.422 ± 0.032	≥10	≤10
14a			2	1.176 ± 0.023	≥10	≤10
14b			3	1.156 ± 0.013	≥10	≤10
14c			4	5.436 ± 0.056	≥10	≤10
14d			5	≥10	≥10	≤10
14e			6	≥10	≥10	≤10
Donepezil				0.039 ± 0.097	8.416 ± 0.628	
Curcumin						62.26 ± 2.92

[a]IC_{50}: 50 % inhibitory concentration (mean ± SD of three individual experiments). [b]AChE obtained from electric eel. [c]BuChE obtained from equine serum. [d]Percentage $A\beta_{1\text{-}42}$ inhibition estimated by using Thioflavin T based fluorescence assay at a sample concentration of 50 μM.

In the case of anti-AChE activity, compound **13b** emerged as the most potent one making 4-hydroxycoumarin linked with 1-benzotriazole via 1,2,3-triazole, the most suitable architect for AChE inhibition. Data from biological activity suggest that chain length of three carbons (**13b**) is most suitable for AChE inhibition and as the number of carbons between triazole and coumarin moiety increases, anti-AChE activity decreases accordingly (IC_{50} = 0.059 to 5.422 μM from **13b** to **13e** respectively). A several-fold decrease in the anti-AChE activity was observed when 4-

hydroxycoumarin was replaced with 7-hydroxycoumarin (as X) (compare **13b** with **10b**, **11b** with **14b**) suggesting that carbonyl group of 4-hydroxycoumarin lies toward triazole linker is suitable for anti-AChE activity as in donepezil (carbonyl group of indanone lies towards the piperidine moiety: **Fig. 4**). The chain length between triazole (linker) and coumarin moieties greatly influence the inhibitory potential of the compounds. As the chain length increases, the inhibitory potential decreases. The chain length of three carbons was found most preferable for inhibitory activity. Most of the compounds with long-chain (n > 4) were found inactive against the enzyme (see **9d**, **9e**, and **10e**, **12c** to **12e**, **14d** and **14e**). 1-benzotriazole as Y and 4-hydroxycoumarin as X was found best combination that exhibited potent AChE inhibitory potential (**13a**-**13e**) amongst which compound **13b** was found to be endowed with most prominent AChE inhibition with the IC_{50} value of 0.059 μM which is comparable to that of donepezil (IC_{50} = 0.039 μM). While 1-hydroxy benzotriazole as Y and 7-hydroxycoumarin as X was an unsuitable combination for AChE inhibitory activity (**12a**-**12e**). Compounds consisting of 2-benzotriazole as Y and 4-hydroxycoumarin as X showed moderate inhibition of the enzyme (**11a**-**11e**). Therefore, overall preference of AChE inhibitory activity for coumarins is 4-hydroxycoumarin > 7-hydroxycoumarin, for benzotriazoles it is 1-benzotriazole > 2-benzotriazole > 1-hydroxy benzotriazole, and for carbon chain length it is 3 > 2 > 4 > 5 > 6. All the compounds were also evaluated for their inhibitory potential against butyrylcholinesterase (BuChE) enzyme in a similar manner. None of the synthetic compounds showed inhibitory activity against the BuChE (IC_{50} above 10 μM). The inhibitory potential of most potent AChE inhibitor **13e** was also tested at two more concentrations (at 50 & 100 μM) against BuChE enzyme while no significant enhancement was observed in the inhibition against BuChE which suggests the significance of these hybrid compounds towards the selective inhibition of AChE enzyme. The compound showed most potent anti-AChE inhibition (**13b**) was further subjected to enzyme kinetic studies and reciprocal Lineweaver-Burk plot was plotted with three concentration levels. In reciprocal Lineweaver-Burk plot, increase in the slope (deacreased V_{max}) as well as intercept (higher Km) was observed with increasing concentrations of **13b** (**Fig. 5**). Intersection in 2nd quadrant of the reciprocal Lineweaver-Burk plot suggest that **13b** followed a mixed-type inhibitory pattern against AChE. The inhibition constant (K_i) for **13b** was calculated as 0.04167 nM.[22]

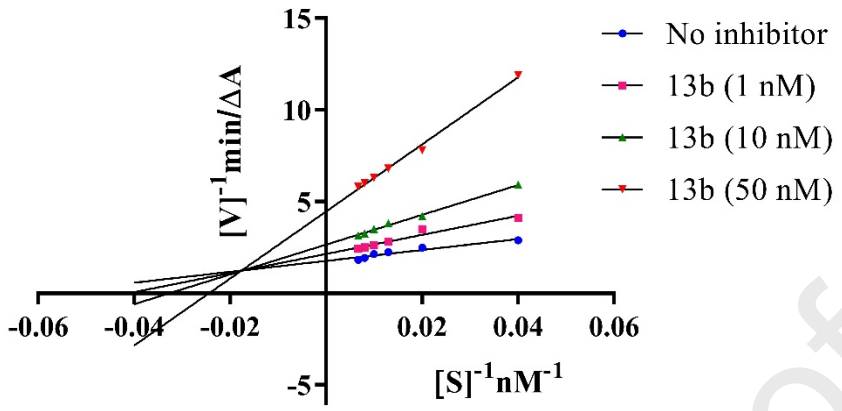


Fig. 5. Overlaid Lineweaver-Burk plot derived from the kinetic study by using no and three concentrations of **13b** with varied substrate concentrations (0.025 - 0.0150 mM) depicting a mixed type of inhibition by compound **13b**.

Amyloid beta ($A\beta_{1-40}$ and $A\beta_{1-42}$) peptides originated from amyloid precursor protein plays an important role in the pathogenesis of Alzheimer's disease. $A\beta_{1-42}$ has higher pathogenicity as compare to $A\beta_{1-40}$ due to its higher tendency to form fibrillar aggregates that promote neuronal degeneration. In addition, Copper is well known to promote amyloid-beta aggregation in amyloid pathogenesis.[23] Thus, inhibition of $A\beta_{1-42}$ by an anti-Alzheimer's agent is a potential advantage. Considering this intention synthesized compounds were screened for their inhibitory action on copper-mediated $A\beta_{1-42}$ aggregation by employing Thioflavin T based fluorescence assay using curcumin as reference anti-amyloidogenic agent. At the concentration of 50 μM, most of the compounds showed percentage inhibition below 10 % except compounds bearing 1-benzotriazole moiety either it was attached with 4-hydroxycoumarin or with 7-hydroxycoumarin respectively. Percentage inhibition was up to 36.68 % (**Table 1**) which was much lower as compare standard curcumin (62.26 %). **13b** was able to inhibit $A\beta_{1-42}$ up to 34.26 %.

Amyloid plaques in AD brains are generally designated as 'metallic sinks' due to higher concentrations of Fe, Cu and Zn ions in them. These metal ions trigger Fenton-type process and are attributed to higher oxidative stress in AD.[24] *In vitro* studies demonstrated the encouraging effect of these metals ions in Aβ toxicity through the generation of ROS in the presence of dioxygen.[25] Thus anti-Alzheimer's agents with metal chelating properties will be a significant advantage. UV-Vis spectra of **13b** showed an increase in absorption intensity and detectable blue

shifts on the addition of Cu^{2+}, Fe^{2+} and Zn^{2+} while minor changes with Mg^{2+} were observed (**Fig. 6A**), proving the complex formation ablility of **13b** with Cu^{2+}, Fe^{2+} and Zn^{2+}.[26] Stoichiometry of **13b**-Cu^{2+} complex was determined by employing a molar ratio method by titrating a solution of **13b** with gradually increasing amounts of $CuCl_2$. Absorption vs Cu^{2+} mole fraction plot (Supplementary Fig. S1: 6B) indicates the 2:1 stoichiometry of the **13b**-Cu^{2+} complex suggesting the possible efficacy of **13b** in the reduction of Cu^{2+} mediated Aβ aggregation and ROS generation.

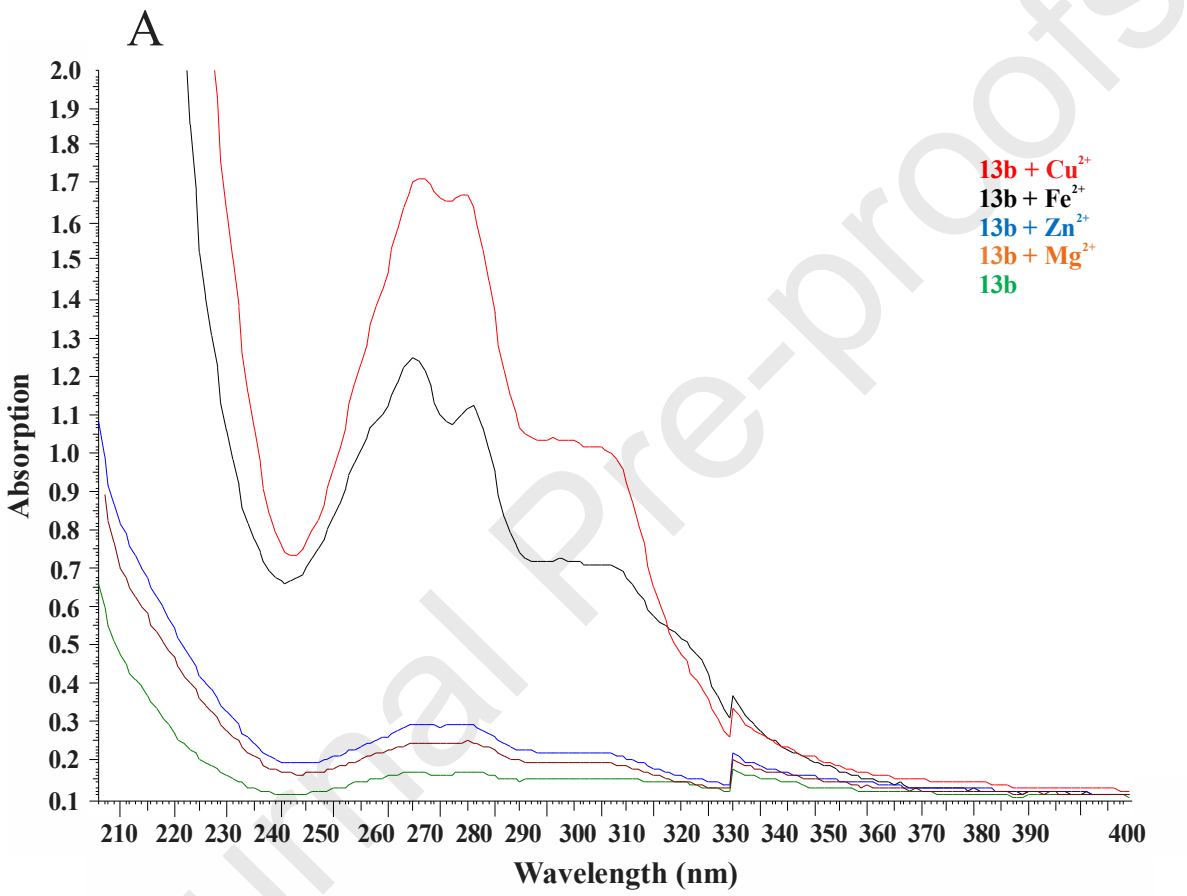
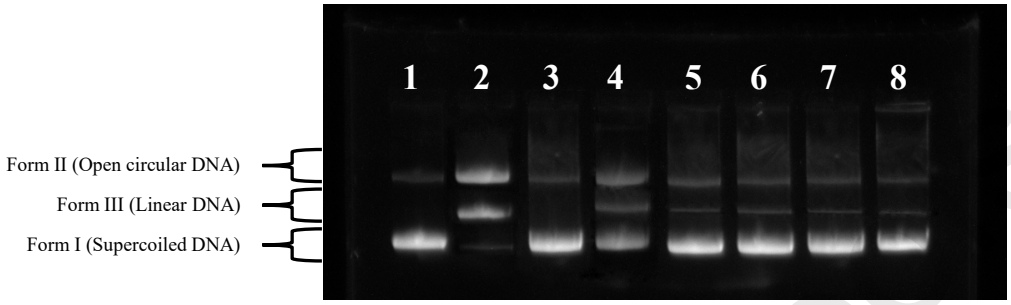


Fig. 6A. Overlaid UV spectra of **13b** (5 μM) itself alone and in the presence of Cu^{2+}, Fe^{2+}, Zn^{2+}, and Mg^{2+} (5 μM).

Ageing and Aβ induced DNA damage have significant contribution to the progression of AD.[27] Increased DNA fragmentation and nicking have been detected in AD brains. Various ROS especially H_2O_2 mediated oxidative stress trigger DNA damage in AD.[28] Thus **13b** was further evaluated for its protective effect against Fenton-type oxidative cleavage of *pBR322* plasmid DNA. *pBR322* plasmid DNA exists in three major forms namely, supercoiled circular form (I), open

circular (II), and linear form (III). In results, **13b** was able to prevent the conversion of the supercoiled circular form (I) of DNA to open circular (II) DNA and then linear form (III) DNA. Thus **13b** was able to protect DNA from degradative action of ·OH radicals (**Fig. 7**).



	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
pBR322 plasmid DNA (190 µg/mL)	3 µL	3 µL	3 µL	3 µL	3 µL	3 µL	3 µL	3 µL
Fenton's reagent	-	7.5 µL	7.5 µL	7.5 µL	7.5 µL	7.5 µL	7.5 µL	7.5 µL
Quercetin (1 mM)	-	-	15 µL	-	-	-	-	-
13b (0.2 mM)	-	-	-	15 µL	-	-	-	-
13b (0.4 mM)	-	-	-	-	15 µL	-	-	-
13b (0.6 mM)	-	-	-	-	-	15 µL	-	-
13b (0.8 mM)	-	-	-	-	-	-	15 µL	-
13b (1 mM)	-	-	-	-	-	-	-	15 µL

Fig. 7: DNA protection properties of **13b**; In lane 4 to 8 showing the capability of **13b** in preventing both open circular and linear cuts.

Cell toxicity study was performed to evaluate the safety profile of **13b** on SH-SY5Y cells using donepezil as reference. After 24 hours of incubation of **13b** or donepezil with SH-SY5Y cells, cell viability was evaluated using MTT assay. Both **13b** and donepezil showed negligible toxicity in concentrations ranging from 0.001 to 100 μM, indicating the safety of **13b** in AD treatment (**Fig. 8**).

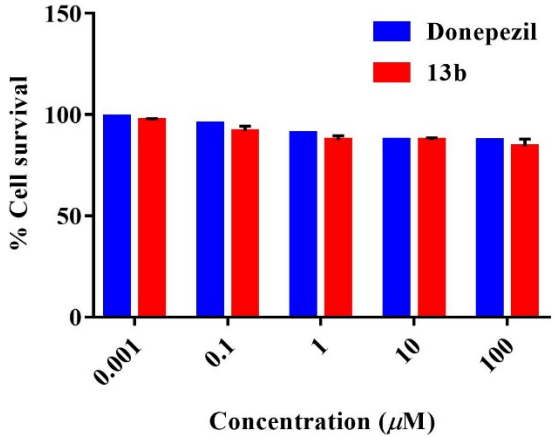


Fig. 8. Cytotoxicity study of **13b** in comparison with donepezil on SH-SY5Y cells. SH-SY5Y cells were incubated for 24 hours with increasing concentrations of both **13b** and donepezil ranging from 0.001 to 100 μM. Results are displayed as the percentage of viable cells with respect to untreated control cells. Values are expressed as the mean ± SD obtained from three individual experiments.

Molecular modelling studies were performed to get insight into various molecular interactions responsible for the modulation of inhibitory activity of the most potent AChE inhibitor (**13b**). For that purpose, the X-ray crystallographic structure of recombinant human acetylcholinesterase complexed with donepezil (PDB entry: 4EY7; resolution, 2.3509 Å), was employed.[29] Accuracy of docking protocol was validated by docking co-crystallized ligand donepezil into its binding site. The program was capable to reproduce best fit confirmation of donepezil in chain B with root mean square deviation (RMSD) value of 0.965, indicating the reliability of docking protocol. After that **13b** was docked into donepezil binding site, and best pose with -18.5818 score having ΔG value of -38 kJ/mol was selected for discussion (**Fig. 9**). The overall binding mode of **13b** with residues of donepezil binding site suggest that compound is properly positioned in the cavity and well stabilized by various electrostatic interactions. Major interactions with AChE include van der Waals, π-σ, π-π stacked, π-π T-shaped and conventional hydrogen bond interaction. The coumarin moiety has perfectly arranged itself in a cavity formed by two polar (Tyr72 and Tyr341) and two hydrophobic (Trp286 and Phe295) residues. The backbone -NH of Phe295 make conventional hydrogen bond interaction with the carbonyl oxygen atom of coumarin (H-bond acceptor; d = 2.802 Å). Similar interaction of -NH in Phe295 backbone has been observed with ketonic oxygen

of indanone moiety in donepezil but with little higher distance (H-bond acceptor; *d* = 2.986 Å) which highlight the stronghold of coumarin over indanone in the peripheral active site (PAS)

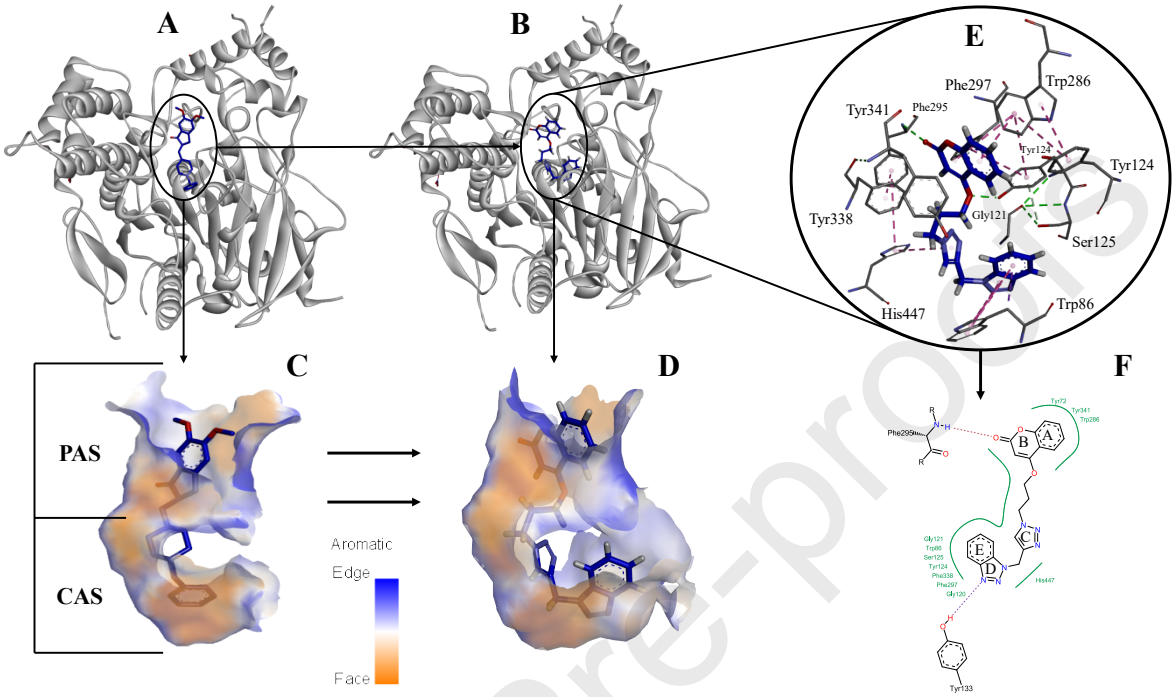


Fig. 9. (A) Acetylcholinesterase (AChE) enzyme complexed with donepezil (PDB entry: 4EY7; Resolution: 2.3509 Å); (B) **13b** docked on the binding site of donepezil; (C) Donepezil positioned in the pocket made by active site residues shown using aromatic surface interactions; (D) **13b** positioned in the pocket made by active site residues shown using aromatic surface interactions; (E) 3D view of interactions of **13b** with residues of donepezil binding site in AChE; (f) 2D view of interactions of **13b** with residues of donepezil binding site in AChE.

Both rings A and B of coumarin are showing π-π stacked interactions with indole ring in Trp286 backbone. Tyr72 and Tyr341 make van der Waals interactions with rings A and B of coumarin. OH on Tyr124 backbone also showing conventional hydrogen bond interaction (H-bond acceptor; *d* = 2.042 Å) with oxygen present between coumarin and three-carbon alkyl chain seems to promote the efficiency of **13b** for PAS. Triazole nucleus (Ring C) which acts as a linker between coumarin and benzotriazole makes π-π T-shaped interaction with hydroxyphenyl backbone of Tyr337 (π-Orbitals to π-Orbitals; *d* = 5.634 Å) whereas donepezil which makes π-Cation interaction with hydroxyphenyl backbone of Tyr337 with little shorter distance (π-Cation; *d* = 4.665 Å). Additional π-π T-shaped interaction of triazole nucleus has been observed with

imidazole nucleus of His447 (π-Orbitals to π-Orbitals; *d* = 5.349 Å) which is absent on donepezil making triazole linker an interesting replacement of piperidine moiety in donepezil skeleton for AChE inhibition. The benzotriazole moiety is well-positioned in the cavity formed by five hydrophobic (Gly121, Trp86, Phe338, Phe297, and Gly120) and three polar residues (Ser125, Tyr124 and Tyr133). Nitrogen from ring D makes a conventional hydrogen bond with -OH in Tyr133 backbone. Indole nucleus in Trp86 backbone makes similar but little closer crisscross type π-π stacked interaction (To ring D; π-Orbitals to π-Orbitals; *d* = 4.435 Å) with benzotriazole nucleus as the donepezil makes with its phenyl (To ring D; π-Orbitals; *d* = 5.051 Å). Additional π-σ interaction (-CH- to π-Orbital; *d* = 5.349 Å) of ring E form benzotriazole with -CH_2- of Trp86 backbone has been observed which was absent in donepezil to Trp86. Apart from this, Gly121, Ser125, Phe338, Phe297, Gly120 capture benzotriazole moiety through van der Walls interactions. The overall study suggests that **13b** is well decorated with small, rigid and planar groups making the finest scaffold which is able to satisfy the necessary pharmacophoric requirements for AChE inhibition.

$A\beta_{1\text{-}42}$ aggregation inhibition and good interaction of **13b** toward PAS observed during docking in donepezil binding site of AChE further encourage us to study the interaction of **13b** with $A\beta_{1\text{-}42}$ monomer. For that purpose, the NMR elucidated structure of amyloid-beta ($A\beta_{1\text{-}42}$) monomer (PDB entry: 1IYT) was employed.[30] Sphere module of LeadIT was employed for defining binding site using a radius of 6.50 Å. Previously prepared structure of **13b** was allowed to dock in prepared monomer amino acids. Best pose with -6.7274 was selected for discussion (**Fig. 10**).

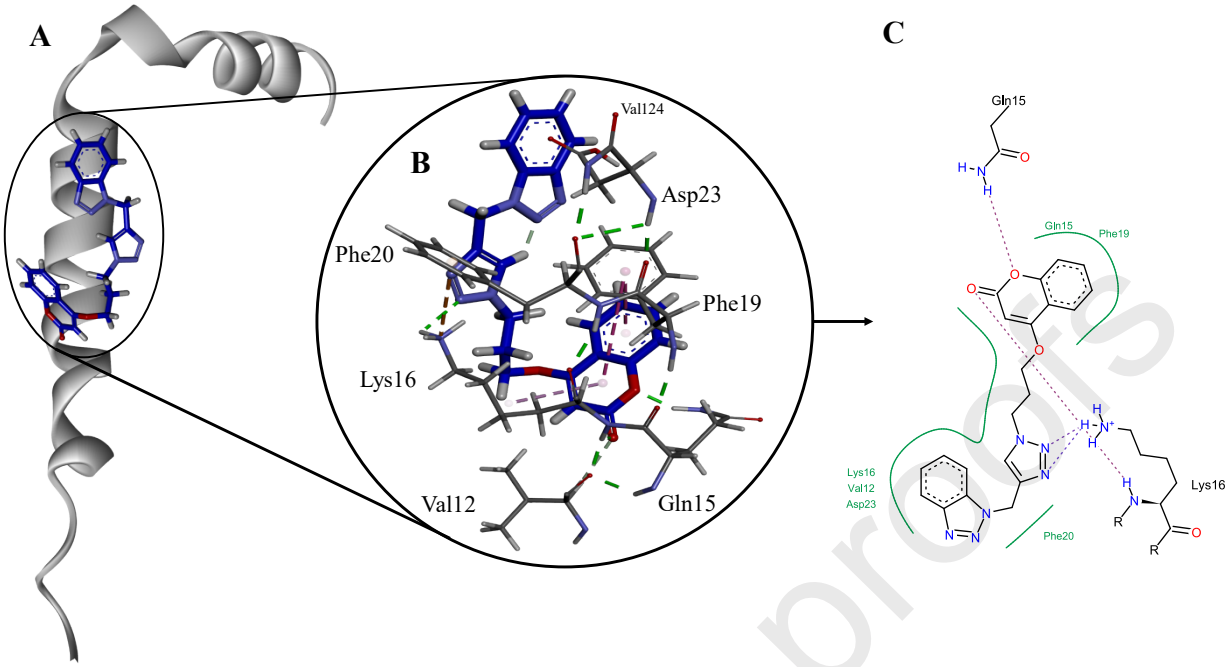


Fig. 10. (A) Amyloid beta ($A\beta_{1-42}$) monomer (PDB entry: 1IYT) complexed with **13b**; (B) 3D view of interactions of **13b** with residues of $A\beta_{1-42}$; (f) 2D view of interactions of **13b** with residues of $A\beta_{1-42}$.

The coumarin moiety of **13b** positioned itself in the cavity formed by two polar (Gln15 and Lys16) and one hydrophobic residue (Phe19). Gln15 shows conventional hydrogen bond interaction (H-bond acceptor; d = 1.633 Å) with oxygen (-O-) present in the ring B of coumarin through -NH_2 of its backbone. Another conventional hydrogen bond interaction (H-bond acceptor; d = 1.896 Å) is shown by a carbonyl oxygen atom of coumarin with core amino group of Lys16. These hydrogen bonds with very short distances showing the affinity of **13b** toward $A\beta_1$.42. Phenyl ring of Phe20 backbone showing π-π stacked interactions with both ring A and B of coumarin. Free nitrogen atoms of triazole moiety also showed conventional hydrogen bond interaction (H-bond acceptor; d = 2.376 Å) with Lys16 through amine group in its backbone. Carbon hydrogen bond interaction is also observed between Val12 and the carbonyl oxygen atom of coumarin through the core hydrogen of Val12. Nitrogen in ring D of benzotriazole makes π-lone pair interaction (lone pair to π-Orbital; d = 2.912 Å) with Phe20 through the phenyl group in its backbone. Asp23 showing van der Walls interactions with both coumarin and benzotriazole moieties via Phe19 (H-bond acceptor; d = 1.833 Å) and Phe20 (H-bond acceptor; d = 2.662 Å) by making conventional hydrogen bond

interactions with them. $A\beta_{1-42}$ are susceptible to form β-pleated "hairpin" like architects that are aggregated into fibrous form, and stabilized by hydrophobic interactions and salt bridge due to interactions between Asp23 and Lys28 residues in them. Docking study suggests the possible interaction of **13b** with Asp 23 of $A\beta_{1-42}$ monomer while strong and short distance interactions with other residues can certainly change the conformation of $A\beta_{1-42}$ monomer.

In summary, this study involved the rational design of triazole tethered coumarin-benzotriazole hybrid molecules based on donepezil skeleton, their synthesis, and evaluation as multifunctional agents against AD. Compounds were synthesized using click chemistry approach and characterized by ^{1}H, ^{13}C NMR and elemental analysis. Among all synthesized compounds **13b** emerged as the most potent *ee*AChE inhibitor with mixed type of inhibitory pattern. Structure-activity relationship revealed that distance of three-carbon alkyl chain between coumarin and triazole linker is well tolerable for AChE inhibition. The combination of 4-hydroxycoumarin and 1-benzotriazole linked through triazole with three carbon chains was found the most suitable combination for AChE inhibition. The insensitivity of these hybrids towards BuChE inhibition suggesting that the compounds are selective AChE inhibitors. **13b** inhibit copper-induced $A\beta_{1-42}$ aggregation and have chelating properties for metal ions (Cu^{2+}, $Fe^{2+,}$ and Zn^{2+}) involved in AD pathogenesis. DNA nicking assay confirms the ability of **13b** to protect DNA from degenerative actions of ·OH radicals. Various binding interactions with *h*AChE justify the potential of **13b** in blocking both PAS and CAS. Interactions of **13b** with $A\beta_{1-42}$ monomer are also streamlined. Besides having such potential multifunctional actions, coumarin and benzotriazole in **13b** provide considerable space for further improving the biological potential of the molecule and thus providing a hit lead for further development of safer and potent selective AChE inhibitors as multifunctional anti-Alzheimer's agents.

Conflict of interest

The authors confirm that this article content has no conflicts of interest.

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References

1. (a) Finder VH. Alzheimer's disease: a general introduction and pathomechanism. *J. Alzheimer's Dis.* 2010;22:5-9; (b) https://www.who.int/news-room/fact-sheets/detail/dementia; 2020 Accessed 20 January 2020.
2. Oakley H, Cole SL, Logan S,E. Maus E, Shao P, Craft J, Guillozet-Bongaarts A, Ohno M, Disterhoft J, Eldik LV, Berry R, Vassar R. Intraneural β-Amyloid aggregates, neurodegeneration and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: Potential factors in amyloid plaque formation. *J. Neurosci.* 2006;26:10129-10140.
3. Davies P, Maloney AJF. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet.* 1976;308:1403.
4. (a) Romero A, Cacabelos R, Oset-Gasque MJ, Samadi A, Marco-Contelles J. Novel tacrine-related drugs as potential candidates for the treatment of Alzheimer's disease, *Bioorg. Med. Chem. Lett.* 2013;23:1916-1922; (b) Rogers SL, Doody RS, Mohs RC, Freidhoff LT. Donepezil improves cognition and global function in Alzheimer disease: a 15-week, double-blind, placebo-controlled study. Donepezil study group. *Arch Intern Med.* 1998;581:1021-1031; (c) Raskind MA, Peskind ER, Wessel T, Yuan W. Glantamine in AD: A 6-month randomized, placebo-controlled trial with a 6-month extension. The galantamine USA-1 study group. *Neurology*. 2000;54:2261-2268; (d) Saeedi M, Safavi M, Karimpour-Razkenari E, Mahdavi M, Edraki N, Moghadam FH, Khanavi M, Akbarzadeh T. Synthesis of novel chromenones linked to 1,2,3-triazole ring system: Investigation of biological activities against Alzheimer's disease. *Bioorg Chem.* 2017;70:86-93.
5. Marum RJV. Update on the use of memantine in Alzheimer's disease. *Neuropsych. Dis. Treat.* 2009;5:267-247.
6. (a) Erlanson DA, Fesik SW, Hubbard RE, Jahnke W, Jhoti H, Twenty years on: the impact of fragments on drug discovery. *Nat. Rev. Drug Discov.* 2016;15:605-619; (b) Butterfield DA, Swomley AM, Sultana R. Amyloid β-peptide (1-42) induced oxidative stress in Alzheimer's disease: Importance in disease pathogenesis and progression. *Antioxid. Redox Signal.*

2013;19:823-835; (c) Tuppo EE, Arias HR. The role of inflammation in Alzheimer's disease. *Int. J. Biochem. Cell Biol.* 2005;37:289-305; (d) https://www.medpagetoday.com/neurology/alzheimersdisease/75075; 2020_Aaccessed 18 January 2020; (e) https://sciencenordic.com/alzheimers-disease-denmark-forskerzonen/no-new-drugs-for-alzheimers-disease-in-15-years/1454636; 2020 Accessed 18 January 2020.

7. Alvarez A, Opazo C, Alarcon R, Garrido J, Inestrosa NC. Acetylcholinesterase promotes the aggregation of amyloid-beta-peptide fragments by forming a complex with the growing fibrils. *J. Mol. Biol.* 1997;272:348-361; (b) Vafadarnejad F, Mahdavi M, Karimpour-Razkenari E, Edraki N, Sameem B, Khanavi M, Saeedi M, Akbarzadeh T. Design and synthesis of novel coumarin-pyridinium hybrids: In vitro cholinesterase inhibitory activity. *Bioorg. Chem.* 2018;77:311-319.
8. Greenough MA, Camakaris J, Bush AI. Metal dyshomeostasis and oxidative stress in Alzheimer's disease. *Neurochem. Int*. 2013;62:540-555.
9. (a) Bush AI. The metal theory of Alzheimer's disease. *J. Alzheimer's Dis.* 2015;33:S277-S281; (b) Robert A, Liu Y, Nguyen M, Meunier B. Regulation of copper and iron homeostasis by metal chelators: a possible chemotherapy for Alzheimer's disease. *Acc. Chem. res.* 2015;48:1332-1339; (c) Watt NT, Whitehouse IJ, Hopper NJ. The role of zinc in Alzheimer's disease. *Int. J. Alzheimer's Dis.* 2011;971021.
10. (a) He Q, Liu J, Lan JS, Ding J, Sun Y, Fang Y, Jiang N, Yang Z, Sun L, Jin Y, Xie SS. Coumarin-dithiocarbamate hybrids as novel multitarget AChE and MAO-B inhibitors against Alzheimer's disease: Design, synthesis and biological evaluation. *Bioorg. Chem.* 2018;81: 512-528; (b) Xie SS, Wang X, Jiang N, Yu W, Wang KDG, Lan JS, Li ZR, Kong LY. Multi-target tacrin-coumarin hybrids: Cholinesterase and monoamine oxidase B inhibition properties against Alzheimer's disease. *Eur. J. Med. Chem*. 2015;95:153-165; (c) Singh A, Singh JV, Rana A, Bhagat K, Gulati HK, Kumar R, Salwan R, Bhagat K, Kaur G, Singh N, Kumar R, Singh H, Sharma S, Bedi PMS. Monocarbonyl curcumin-based molecular hybrids as potent antibacterial agents. *ACS Omega*. 2019;4:11673-11684; (d) Bhagat K, Bhagat J, Gupta MK, Singh JV, Gulati HK, Singh A, Kaur K, Kuar G, Sharma S, Rana A, Singh H, Sharma S, Bedi PMS. Design, synthesis, antimicrobial evaluation, and molecular modeling studies of novel indolinedione-coumarin molecular hybrids. *ACS Omega*. 2019;4:8720-8730; (e) Singh H, Singh JV, Bhagat K, Gulati HK, Sanduja M, Kumar N, Kinariwala N, Sharma S. Rational

approaches, design strategies, structure activity relationship and mechanistic insights for therapeutic coumarin hybrids. *Bioorg. Med. Chem*. 2019;27:3477-3510.

11. (a) Abudalo RA, Abudalo MA, Hernandez MT. Stability of benzotriazole derivatives with free Cu, Zn, Co and metal-containing enzymes: Binding and interaction of methyl-benzotriazoles with superoxide dismutase and vitamin B_{12}, IOP. *Conf. Ser. Mater. Sci. Eng.* 2018;305:012024. (b) Das J, Rao CVL, Sastry TVRS, Roshaiah M, Sankar PG, Khadeer A, Kumar MS, Mallik A, Selvakumar N, Iqbal J, Trehan S. Effects of positional and geometrical isomerism on the biological activity of some novel oxazolidinones. *Bioorg. Med. Chem. Lett*. 2005;15:337-343 (2005); (c) Augustynowicz-Kopec E, Zwolska Z, Orzesko A, Kazimierczuk Z. Synthesis and antimycobacterial activity of selected nitrobenzyloxylated benzotriazoles. *Acta. Pol. Pharm.* 2008;65:435-439; (d) Rezaei Z, Khabnadideh S, Zomorodian K, Pakshir K, Kashi G, Sanagoei N, Gholami S. Design, synthesis and antifungal activity of some new imidazole and triazole derivatives. *Arch. Pharm. Chem. Life Sci.* 2011;344:658-665; (e) Bretner M, Baier A, Kopanska K, Najda A, Schoof A, Reinholz M, Lipniacki A, Poasek A, Kulikowski T, Borowski P. Synthesis and biological activity of 1*H*-benzotriazole and 1*H*-benzimidazole analogues–inhibitors of the NTPase/helicase of HCV and of some related *Flaviviridae*. *Antiviral Chem. Chemother*. 2005;16:315-326; (f) Al-Soud YA, Al-Masoudi NA, Ferwanah AES. Synthesis and properties of new substituted 1,2,4-triazoles: Potential antitumor agents. *Bioorg. Med. Chem*. 2003;11:1701-1708; (g) Rajasekaran A, Rajagopal KA. Synthesis of some novel triazole derivatives as anti-nociceptive and anti-inflammatory agents. *Acta. Pharm*. 2009;59:355-364; (h) Caliendo G, Carlo RD, Meli R, Perissutti E, Santagada V, Silipo C, Vittoria A. Synthesis and trazodone-like pharmacological profile of 1- and 2-[3-[4-(X)-1-piperazinyl]-propyl]-benzotriazoles. *Eur. J. Med. Chem*. 1993;28:969-674; (i) Briguglio I, Piras S, Corona P, Gavini E, Nieddu M, Carta BA. Benzotriazole: An overview on its versatile biological behavior. *Eur J Med Chem.* 2015;97:612-648.
12. Viegas-Junior C, Danuello A, Bolzani VDS, Barreiro EJ, Fraga CAM. Molecular hybridization: A useful tool in the design of new drug prototypes. *Curr. Med. Chem*. 2007;14: 1829-1852.
13. (a) Xu JH, Fan YL, Zhou J. Quinoline-triazole hybrids and their biological activities, *J. Heterocyclic Chem.* 2018;55:1854-1862; (b) Domaloan R, Idowu T, Zhanel GG, Schweizer F.

Antibiotic hybrids: The next generation of agents and adjuvants against Gram-negative pathogens? *Clin. Microbial. Rev*. 2018;31:e00077-17.

14. Fan YL, Ke X, Liu M. Coumarin-triazole hybrids and their biological activities, *J. Heterocyclic Chem*. 2018;5:791-802.
15. (a) Dong H, Yuede CM, Coughlan CA, Murphy KM, Csernansky JG. Effects of donepezil on amyloid-β and synapse density in the Tg2576 mouse model in Alzheimer's disease. *Brain Res*. 2009;1303:169-178; (b) Junaid M, Islam N, Hossain MK, Ullah MO. Metal based donepezil analogues designed to inhibit human acetylcholinesterase for Alzheimer's disease. *PLoS ONE* 2019;14:e0211935.
16. Torres FC, Goncalves GA, Vanzolini KL, Merlo AA, Gauer B, Holzachuh M, Andrade S, Piedade M, Garcia SC, Carvalho I, Poser GL, Kawano DF, Eifler-Lima VL, Cass QB. Combining the pharmacophore features of coumarins and 1,4-substituted 1,2,3-triazoles to design new acetylcholinesterase inhibitors: Fast and easy generation of 4-methylcoumarins/1,2,3-triazoles conjugates via click chemistry. *J. Braz. Chem. Soc.* 2016;27:1541-1550.
17. Mamgain R. Cu(I) catalyzed coumarin-1,2,3-triazole hybrids: Click chemistry. Asian Journal of chemistry. 2019;31:2543-2547.
18. Riaz N, Iftikhar M, Saleem M, Rehman A, Hussain S, Rehmat F, Afzal Z, Khawar S, Ashraf M, Rashida M. New synthetic 1,2,4-triazole derivatives: Cholinesterase inhibition and molecular docking studies. *Results in Chemistry*. 2020;2:100041.
19. Jalili-Baleh L, Forootanfar H, Kucukkilinc TT, Nadri H, Abdolahi Z, Ameri A, Jafari M, Ayazgok B, Baeeri M, Rahimifard M, Bukhari SNA, Abdollahi M, Ganjali MR, Emami S, Khoobi M, Foroumadi A. Design, synthesis and evaluation of novel multi-target-directed ligands for treatment of Alzheimer's disease based on coumarin and lipoic acid scaffolds. *Eur. J. Med. Chem.* 2018;152:600-614.
20. Sameen B, Saeedi M, Mahdavi M, Nadri H, Moghadam FH, Edraki N, Khan MI, Amini M. Synthesis, docking study and neuroprotective effects of some novel pyrano[3,2-c]chromene derivatives bearing morpholine/phenylpiperazine moiety. *Bioorg. Med. Chem.* 2017;25:3980-3988.
21. A catalytic amount of copper sulphate and sodium ascorbate was added to a solution of propargylated benzotriazoles (1 equiv) and azidated coumarins (1 equiv). The reaction mixture

was allowed to stir at room temperature until it was completed (monitored by TLC). After completion, the reaction mixture was poured on crushed ice to eliminate copper sulfate and sodium ascorbate, set aside for some time (until ice converted into the water completely), filtered and dried to get the crude product. Obtained crude product was purified by using column chromatography using hexane: ethyl acetate (6: 4) to get triazole tethered coumarin benzotriazole hybrids. All the hybrids were synthesized in similar manner. Characterization data of most potent compound 4-(3-(4-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propoxy)-2H-chr omen-2-one (13b). Yield 74%, mp 166-168° C, Yellowish powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.32 (s, 1H), 8.04-8.03 (m, 1H), 7.90-7.88 (d, J = 10 Hz, 1H), 7.74-7.72 (d, J = 10 Hz, 1H), 7.66-7.63 (m, 1H), 7.55-7.52 (m, 1H), 7.41-7.38 (m, 2H), 7.34-7.31 (m, 1H), 6.04 (s, 2H), 5.85 (s, 1H), 4.61-458 (m, 2H), 4.22-4.19 (m, 2H), 4.39-4.37 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.19, 162.78, 162.04, 153.18, 146.02, 142.13, 133.21, 127.84, 124.87, 124.54, 123.45, 119.60, 116.84, 115.54, 111.32, 91.07, 67.12, 47.21, 43.41, 36.24, 29.13. Anal. Calcd for $C_{21}H_{18}N_6O_3$: C, 62.68; H, 4.51; N, 20.88. Found: C, 62.63; H, 4.45; N, 20.81.

22. Sonmez F, Kurt BZ, Gazioglu I, Basile L, Dag A, Cappello V, Ginex T, Kucukislamoglu M, Guccione S. Design, synthesis and docking study of novel coumarin ligands as potential selective acetylcholinesterase inhibitors. *J. Enzyme Inhib. Med. Chem.* 2017;32:285-297.
23. Butterfield DA, Swomley AM, Sultana R. Amyloid β-peptide (1-42) induced oxidative stress in Alzheimer's disease: Importance in disease pathogenesis and progression. *Antioxid. Redox Signal.* 2013;19:823-835.
24. Maynard CJ, Bush A, Masters C, Cappai R, Li QX. Metals and amyloid-β in Alzheimer's disease, Int. *J. Exp. Pathol*. 2005;86:147-159; (b) V.B. Kenche, K.J. Barnaham. Alzheimer's disease & metals: therapeutic opportunities. *Br. J. Pharmacol.* 2011;163:211-219.
25. Cheignon C, Tomas M, Bonnefont-Rousselo Dt, Faller P, Hureau C, Collin F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biol.* 2018;14:450-464.
26. Friggeri L, Vita DD, Pandolfi F, Tortorella S, Costi R, Santo RD, Scipione L. Design, synthesis and evaluation of 3,4-dihydroxybenzoic acid derivatives as antioxidants, bio-metal chelating agents and acetylcholinesterase inhibitors. *J. Enzyme Inhib. Med. Chem.* 2015;30:166-172.

27. Mao P, Reddy PH. Aging and amyloid beta-induced oxidative DNA damage and mitochondrial dysfunction in Alzheimer's disease: Implications for early intervention and therapeutics. *Biochim. Biophys. Acta.* 2011;1812:1359-1370.
28. (a) Reddy VP, Perry G, Cooke MS, Sayre LM, Smith MA. Mechanisms of DNA damage and repair in Alzheimer's disease, In: *DNA repair and human disease*. Medical Intelligence Unit. Springer, Boston, MA. 2006;98-113; (b) Monte SMDL, Luong T, Neely TR, Robinson D, Wands JR. Mitochondrial DNA damage as a mechanism of cell loss in Alzheimer's disease. *Lab Invest.* 2000;80:1323-1335.
29. Cheung J, Rudolph MJ, Burshteyn F, Cassidy MS, Gary EN, Love J, Franklin MC, Height JJ, Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *J. Med. Chem.* 2012;55:10282-10286; (b) https://www.rcsb.org/structure/4EY7 (Retrieved on 2/09/2019).
30. Crescenzi O, Tomaselli S, Guerrini R, Salvadori S, D'Ursi AM, Temussi PA, Picone D. Solution structure of the Alzheimer amyloid beta-peptide (1-42) in an a polar microenvironment. Similarity with a virus fusion domain. *Eur. J Biochem*. 2002;269:5642-5648; (b) https://www.rcsb.org/structure/1iyt (Retrieved on 2/09/2019).

- Design and synthesis of new coumarin-benzotriazole based molecular hybrids
- Ellman's method of evaluation demonstrated selective acetylcholinesterase inhibition
- Most potent compound showed inhibition against copper-induced $A\beta_{1-42}$ aggregation
- Potent compound have DNA protective properties against degenerative actions of ·OH radicals
- Molecular modelling studies with acetylcholinesterase enzyme and $A\beta_{1-42}$ monomer

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