

Synthesis of Galanthamine-Indanone hybrid derivative as Anti-Alzheimer's disease (AD)

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ABSTRACT:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, memory impairment, and behavioral abnormalities. Despite the availability of symptomatic treatments, there is still no effective cure for the disease. Galanthamine, a well-known acetylcholinesterase (AChE) inhibitor, has shown clinical benefits in improving cholinergic neurotransmission; however, its therapeutic application is limited by toxicity and side effects. The present study focuses on the design and synthesis of novel peptide-galanthamine derivatives with the objective of developing safer and more potent anti-Alzheimer agents. These derivatives were synthesized in solution using the fragment condensation technique and structurally characterized by melting point determination, nuclear magnetic resonance (NMR) spectroscopy, and mass spectral analysis. Acute toxicity studies were performed in mice using standard experimental protocols to evaluate their safety profile. In the synthesized compounds, galanthamine was chemically linked to an indanone moiety through a piperazine linker, resulting in multifunctional analogues with enhanced pharmacological potential. The newly developed derivatives exhibited significant acetylcholinesterase inhibitory activity and were also expected to possess inhibitory effects against β -secretase, a key enzyme involved in amyloid- β plaque formation in Alzheimer's disease. The incorporation of the indanone ring provided a dual mechanism of action, contributing to improved anti-Alzheimer activity. Behavioral assessments demonstrated enhanced cognitive performance, while biochemical investigations revealed favorable neuroprotective effects. Furthermore, hepatic dysfunction parameters indicated reduced toxicity compared to the parent drug, galanthamine. Overall, the synthesized peptide-galanthamine derivatives displayed superior potency, efficacy, and safety, highlighting their potential as promising multifunctional therapeutic candidates for the management and treatment of Alzheimer's disease.

Keywords- Alzheimer, Acetylcholinesterase, NMR, Synthesis, Derivative.

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INTRODUCTION

Alzheimer's disease (AD) is a complex neurodegenerative condition marked by significant behavioural abnormalities, progressive cognitive function deficits, and memory loss [1-5]. The most prevalent type of dementia in the elderly, AD, is now the fourth most common cause of mortality in developed nations. AD currently affects over 35 million individuals globally and is on the rise in those over 65. If no effective therapy is found, the affected population is predicted to quadruple by 2050 [5-10]. Cholinergic neurotransmission deficit [11], impaired beta-amyloid protein metabolism and tau protein phosphorylation [12], and the involvement of inflammatory and oxidative pathways [5] have all been linked to the pathophysiology of AD. Over the past few decades, a number of logical pharmacological approaches have been developed, including both cholinergic and noncholinergic therapies [13-20]. Nevertheless, the only therapy approach that has been shown to improve central cholinergic function is presently authorised for the management of AD.

Tacrine, donepezil, rivastigmine, and galantamine were the first medications to be approved [21-25]. Nevertheless, rather than treating or preventing AD, these medications that alter such a single target would only make palliative care possible. The traditional method of modifying at a single target may not be sufficient in this complicated disease due to the multipathogenesis of AD. As a result, looking for candidates that act at several pathogenic cascade locations has emerged as a novel approach to the development of new AD medications.

Therefore, correlations between AChEIs and substances that function at various pathogenic cascade sites offer extra advantages. Novel Galanthamine-Indanone hybrids [10], dual inhibitors of AChE and monoamine oxidase (MAO) [26], dual AChEI and serotonin transporters [12], and powerful AChEI with calcium channel blocker [27, 28] are examples of the multitarget approach in drug creation for the treatment of AD. The significance of oxidative cell damage and free radical production in the pathophysiology of AD

has been the subject of numerous investigations in recent years. According to recent studies, oxidative stress is a major factor in the early stages of the pathogenic cascade by causing tau protein hyperphosphorylation and Ab aggregation [29]. As a result, oxidative stress is becoming a key target for the treatment of AD. Clinical trials have examined a number of antioxidants [30,31,32].

Galantamine (1, Fig. 1) is a naturally occurring AChEI that is authorised in numerous nations to treat AD. Its unique dual mode of action combines reversible, competitive inhibition of AChE with allosteric regulation of nicotinic receptors [33]. Additionally, compared to other AChEIs like tacrine, it is less hazardous. Galantamine has been suggested as the perfect starting structure for creating multi-target directed ligands because of these special qualities. A lot of work has been done during the last ten years, it is emphasised.

Previous studies modified the structure of galantamine primarily at the 6-position hydroxyl group and the 11-position nitrogen atom, to which a second pharmacophore was added to increase the anti-AD efficacy.[34] However, these structural changes typically used galantamine as a starting material, producing molecules that are larger and more complicated than galantamine itself, which could lead to pharmacokinetic issues. Additionally, there are reports about galantamine analogues that differ from galantamine in terms of their structural backbone. [35] Nevertheless, these investigations did not concentrate on medicinal chemistry, but rather on synthetic chemistry.

Our drug design strategy is to develop novel facile-prepared galantamine analogs by simplifying the backbone of galantamine. From the chemical point of view, galantamine contains two structural units of a universal tricyclic benzofuran core with a sterically congested quaternary carbon and a complex seven-member azepine ring. Notably, the sterical C2 allylic alcohol moiety and the quaternary carbon are very difficult to be constructed via common synthetic procedures. However, according to the classic structure activity relationship theory of AChEIs, these two fragments are not essential for the activity of galantamine, while the aromatic moiety (i.e. the benzene ring) and the nitrogen atom are. X-ray crystal structure of galantamine bound in the active site of AChE also revealed that the AChE inhibitory activity of galantamine is due to the interaction of the benzene ring with Trp84 via π - π stacking action and the interaction of the nitrogen atom with Asp72 via H bond action [36].

The binding site of AChE is deep and narrow, consisting of several domains [37]. The catalytic domain is situated at the bottom of the binding gorge.

The catalytic anionic site (CAS) binds the positively charged head of ACh. The acyl pocket restricts from binding of bulkier choline esters like butylcholinesterase. The oxyanion domain hosts one molecule of structural water involved in a dense hydrogen-bond network between substrate and enzyme.

Finally, the peripheral anionic site (PAS) lies at the entrance of the binding gorge and takes part in an interaction with A β , initiating the formation of amyloid plaques [38]. The blockade of PAS prevents from AChE-induced A β aggregation [30]. This finding prompted the design of novel AChEIs with dual-site binding: binding to the CAS and to the PAS [39]. In the present study, we describe the design of a series of galantamine linked indanone hybrid as multi-target drug[40].

MATERIALS AND METHODS

Experimental Work

The synthetic pathway of galantamine linked indanone hybrid is provided in **Scheme 1**. The target molecules consist of three parts, therefore, the synthetic strategy is firstly to prepare the galanthamine ring, and then it linked with indanone ring via piperazine show AChEI moiety. Firstly, Barton and co-workers recognized that Amaryllidaceae alkaloids, including galantamine, could be derived from a common precursor, 40-O-methylnorbelladine (1) (R)₁H, or its N-methyl Oxidative ortho-para phenol coupling of 8 forms the central C-C bond, with simultaneous generation of the quaternary center and the azepine ring.

An intramolecular oxa-Michael addition of the phenol to the quinoid system generates product 10 with the tetracyclic scaffold. Further reduction of the keto group and methylation of the amine would afford galantamine.(1) Secondly, the reaction between indanoic acid derivative (2) and piperazine (3) to form target product. Whose structures are shown in Fig. 1 the structures of all synthesized compounds were elucidated by IR spectroscopy, mass spectrometry and ¹H NMR and ¹³C NMR spectrometry.

Fig no 01- Chemical Structures of Galantamine Linked Indanone Hybrid

Procedure:

Synthesis of 9-methoxy-4-methyl-11-oxa-4-azatetracyclo[8.6.1.0¹.12.0⁶.17]heptadeca-6(17),7,9,15-tetraen-14-one

Fig no 02-Scheme 1: Synthesis of Galanthamine

Unlabelled norbelladine and N-methylnorbelladine were enzymically O-methylated in the presence of [³H]₃ SAM and a specific catechol-O-methyltransferase isolated from leaves of *L. verum*. Crude enzyme extracts were prepared by adding 50 g (fr. Wt) of frozen crushed leaves to 75 ml buffer consisting of 0.02 M Tris-HCl, pH 7.5 and containing

0.01 M mercaptoethanol. The mix. Was stirred until completely thawed. The brie was pressed through cheesecloth and centrifuged at 10,000g. The supernatant was applied to a DEAE- Sephacel column (2.3×13cm) which was equilibrated with the above mentioned buffer. The column was washed with 100 ml buffer. Elution of the enzyme was achieved with a KCl step gradient at a flow rate of 60ml h⁻¹. After eluting with 150 ml buffer containing 0.2 M KCl, the enzyme was desorbed with 150 ml 0.3 M KCl. The 4 ml-firs of major activity were collected (50 ml) and concd by ultrafiltration (10 kd). In a total Volume of 350 µl (1 M Tris-HCl, pH 7.5) norbelladine (10 nmol) and [C³H₃]SAM (62.7 µCi, 323 nmol) were incubated in the presence of the concd COMT (0.03 pkat) for 3 h at 30°. In contrast, the entire radioactivity was lost when the labelled material was diluted and crystallized, yield of 44%. m.p 198 °C to 199 °C. IR ν (cm-1) (KBr): 2950- 2800 (CH str. -NCH₃, -OCH₃), 1680 (C=O Str.), 1620 (C=C Str.) 1600-1505 (C=C Aromatic), 1280-1060 (C-O-C), 1H NMR: δ (ppm) (CDC13, TMS): 2.44 (s, 3H), 2.65,3.25(m,4H), 3.82 (s,3H), 3.95, 4.15 (d, 2H), 4.72 (m,1H), 6.04 (d,1H), 6.64,6.72 (m, 2H), 6.86 (d,1 H), MS (m/z) 285.

Synthesis of (5, 6-dimethoxy-3-oxo-2, 3-dihydro-1H-inden-1-yl) acetic acid

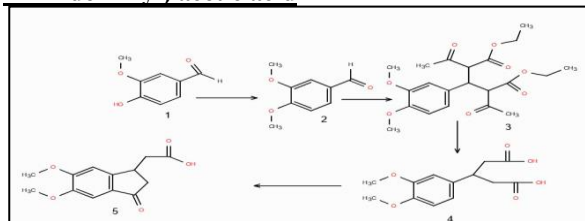


Fig no 03- Scheme 2: Synthesis of (5, 6-dimethoxy-3-oxo-2, 3-dihydro-1H-inden-1-yl) acetic acid

Synthesis of (5, 6-dimethoxy-3-oxo-2, 3-dihydro-1H-inden-1-yl) acetic acid is perform in four steps:

- Synthesis of 3,4-Dimethoxybenzaldehyde (2)
: A 250 ml three-necked flask with a magnetic stirrer, two dropping funnels, and a reflux condenser was filled with commercially available vanillin (1) 15.2 g (0.1 mol). Potassium hydroxide (KOH) (8.2 g in 12 ml of water) was added to one funnel, while pure dimethyl sulfate (12 ml, 0.104 mol) was added to the other. After melting the vanillin in a water bath, two drops of KOH were added every second. Dimethyl sulfate was added at the same rate after 20 seconds. After a few minutes, the heating was stopped because the mixture was still gently refluxing due to the reaction's heat. Throughout, the reaction mixture was vigorously agitated. The reaction liquid turned turbid and split into two layers once all the ingredients were added. The reaction mixture's pale reddish

brown color indicates that the alkaline state was maintained during the reaction. A porcelain bowl was filled with the yellow reaction mixture, which was then left overnight. In order to obtain veratraldehyde in 70–75% yield at mp 43–44°C, the hard crystalline mass was crushed, extensively cleaned with cold water to remove basicity, filtered, and dried in a vacuum desiccators.[22]

- Synthesis of diethyl 2,4-diacetyl-3-(3,4-dimethoxyphenyl)pentanedionate (3): In a dry conical flask, veratraldehyde (2) (34 g, 0.2 mol) was dissolved in ethylacetate (60 g, 0.43 mol). Piperidine (2.5 ml) was added dropwise at room temperature with continuous swirling, and the mixture was left for three days or longer (up to seven days, depending on room temperature) with the mouth stoppered. The resulting solid product was crushed, cleaned with solvent ether to produce product 4, and then recrystallized from diluted alcohol to produce the analytical product [23]. Yield 80–85%, mp 129–131 °C. IR ν (cm-1) (KBr): 1720 (C=O stretching), 1045, 1253 (C-O stretch of OCH₃). 1H NMR: δ (ppm) (CDC13, TMS): 0.82 (t, CH₃, 3H), 0.90 (t, CH₃, 3H), 1.28 (s, CH₃, 3H), 1.34 s, CH₃, 3H), 2.50, 2.71 (d, CH, H), 3.02, 3.64 (d, CH, H), 3.85 (s, OCH₃, 3H), 3.87 (s, OCH₃, 3H), 3.93 qr, CH₂, 2H), 3.96 (qr, CH₂, 2H), 4.03 (m, CH, 1H), 6.59-6.81 (m, ArH, 3H).
- Synthesis of 3-(3,4-dimethoxyphenyl)pentanedioic acid (4): A freshly made hot solution of KOH (160 g in 120 ml of water) was used to dissolve Compound 4 (40 g, 0.089 mol), and 80 ml of 90% ethanol was added. For one hour, the heated reaction mixture was refluxed in a water bath. The reaction mixture was diluted with water, distilled under low pressure to remove as much alcohol as possible, chilled, and then extracted using the solvent ether. After acidification with cold concentrated HCl and cooling, the aqueous layer produced crude 6, which was filtered and recrystallized from hot water at mp 189–191 °C [23]. Yield 65–70%, IR: ν (cm-1)(KBr): 3300–2500 (br OH stretch), 1710 (C=O stretching), 1010, 1226 (C-O stretch of OCH₃), 1260 (C-O stretch of COOH), 1H NMR: δ (ppm) (CDC13' TMS): 2.59, 2.70 (dd, CH₂, 4H), 3.59 (qn, CH, 1H), 3.84 (s, OCH₃, 3H), 3.86(s, OCH₃, 3H), 6.81 (m, ArH, 3H), 10.08 (brs, COOH, 1H), 10.19 (brs, COOH, 1H)

- d) Synthesis of (5,6-dimethoxy-3-oxo-2,3-dihydro-1H-inden-1-yl)acetic acid (5): Compound 4 was cyclized by mixing polyphosphoric acid (PPA) (225 g) with powdered diacid (15 g, 0.056 mol) in a steam bath for four hours while stirring. Pouring the heated reaction mixture onto crushed ice and stirring it until the greasiness was gone allowed it to break down. The keto acid (5) was extracted using chloroform following the breakdown of the heated reaction mixture. The crude keto acid was obtained by distilling out the solvent. Acetone was used to recrystallize the crude product [24]. Yield 75–80%; mp 177–178 °C; Calc. Log P 0.71; IR: ν (cm⁻¹)(KBr): 3400–2400 (br OH stretch), 1724 (C=O stretching), 1045, 1255 (C–O stretch of OCH₃), ¹H NMR: δ (ppm) (CDCl₃, TMS): 2.46, 2.52 (dd, CH₂, 2H), 2.79, 2.91 (dd, CH₂, 2H), 3.79 (qn, CH, 1H), 3.91 (s, OCH₃, 3H), 3.98 (s, OCH₃, 3H), 7.00 (s, ArH, 1H), 7.15 (s, ArH, 1H), 10.12 (brs, COOH, 1H). MS (m/z): 250(M⁺).

Synthesis of 5,6-dimethoxy-3-[2-oxo-2-(piperazin-1-yl)ethyl]-2,3-dihydro-1H-inden-1-one

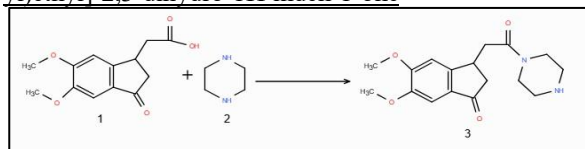


Fig no -04 Scheme 3: Synthesis of 5,6-dimethoxy-3-[2-oxo-2-(piperazin-1-yl)ethyl]-2,3-dihydro-1H-inden-1-one

Synthesize the piperazine amide derivative (3), charge a clean, dry round-bottom flask with 1.0 g of 1-indanone acetic acid (1) dissolved in 10ml of dry dichloromethane (DCM). Add 0.39 ml of thionyl chloride (SOCl₂) dropwise while stirring, attach a reflux condenser with a drying tube or gas trap to capture off-gassed SO₂ and HCl, and heat the mixture at reflux for 1-2 hours to form the acid chloride intermediate. Once reaction completion is indicated, cool the flask and remove the solvent and excess, unreacted thionyl chloride under reduced pressure or mild heating. Next, dissolve 0.45g of piperazine in 10 ml of ethanol. Cool the crude acid chloride solution to 0-5°C using an ice bath, then add the piperazine solution dropwise while maintaining vigorous stirring and strict cold temperature control. Allow the reaction mixture to warm to room temperature and stir continuously for 3- 4 hours. Monitor the reaction progress by Thin Layer Chromatography (TLC) using a mobile phase of Ethyl Acetate: Hexane (3:7) with 1% acetic acid. Upon completion, pour the reaction mixture into ice-cold water to precipitate the solid product. Collect the precipitate via vacuum filtration

using a Buchner funnel; rinse the filter cake thoroughly with cold water, and suction-dry the solid. Finally, recrystallize the crude material from an appropriate solvent system (such as ethanol) and dry the final product completely. Ensure all steps involving thionyl chloride and volatile solvents are conducted inside a functioning chemical fume hood with appropriate personal protective equipment. Yield 56-70% mp 145-155°C, IR ν (cm⁻¹)(KBr): 3320-3400 (NH sec.amine), 2930-2850 (CH Str), 1705 (C=O Str), 1645 (C=O str. amide), 1595-1500 (C=C), 1260-1120 (C–O Str.) ¹H NMR: δ (ppm) (CDCl₃, TMS): 1.95 (bs, 1H), 2.45 (dd, 1H), 2.60, 2.78(m, 2H), 2.85 (t, 4H), 2.92 (dd, 1H), 3.42, 3.58 (m, 4H), 3.82 (m, 1H), 3.91(s, 3H), 6.91(s, 1H), 7.14(s, 1H) MS (m/z) 318.

Synthesis of 3-[2-(4-hydroxypiperazin-1-yl)-2-oxoethyl]-5,6-dimethoxy-2,3-dihydro-1H-inden-1-ol

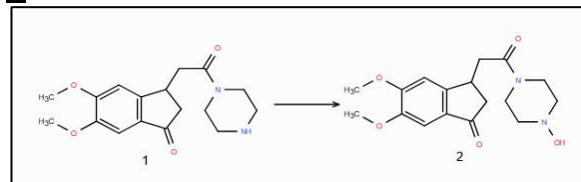


Fig no – 05 Scheme 4: Synthesis of 3-[2-(4-hydroxypiperazin-1-yl)-2-oxoethyl]-5,6-dimethoxy-2,3-dihydro-1H-inden-1-ol

About 0.1 g of the secondary amine (1) is dissolved in a dry organic solvent such as acetone or dichloromethane. The reaction vessel is cooled in an ice bath to approximately 0 °C. A suitable peroxy oxidizing agent, such as a UHP solution, is then added slowly to the reaction mixture with continuous stirring. The reaction mixture is stirred at 0 °C or room temperature for a short period, usually 15–60 minutes. The progress of the reaction is monitored by thin-layer chromatography (TLC) using a mobile phase of Ethyl Acetate: Methanol: Ammonia (8:2:0.1). After completion of the reaction, the mixture is subjected to appropriate work-up and purification to obtain the corresponding **N-oxide product**. (2) Yield 60-70% [29]. m.p 145-165 °C, IR ν (cm⁻¹)(KBr): 3200-3450 (O–H Str.), 2935-2840 (C–H Str. –OCH₃), 1705 (C=O Str.), 1640 (C=O Str. amide), 1595 and 1500 (C=C aromatic), 1260 and 1120 (C–O Str.) ¹H NMR: δ (ppm) (CDCl₃, TMS): 2.45 (dd, 1H), 2.60, 2.78 (m, 2H), 2.75, 2.90 (m, 4H), 2.92(dd, 1H), 3.45, 3.65 (m, 4H), 3.82 (m, 1H), 5.50 (bs, 1H), 6.91 (two s, 1H), MS (m/z): 334 (M⁺).

Synthesis of 5,6-dimethoxy-3-[2-[4-(9-methoxy-3-methyl-11-oxa-3-azatetracyclo[8.6.1.0^{2,7}.1^{7,17}]heptadeca-6(17),7,9,15-tetraen-14-yl]oxy)piperazin-1-yl]-2-oxoethyl]-2,3-dihydro-1H-indenone

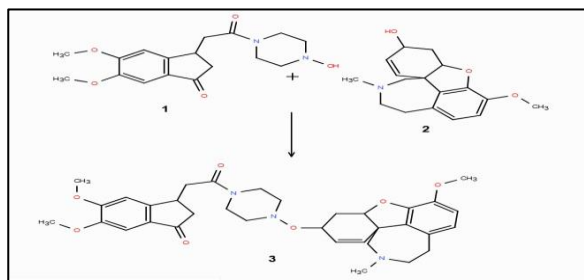


Fig no 06- Scheme 5: 5,6-dimethoxy-3-{2-[4-(9-methoxy-3-methyl-11-oxa-3-azatetracyclo[8.6.1.0^{2,6,17}] heptadeca-6(17),7,9,15-tetraen-14-yl}oxy)piperazin-1-yl]-2-oxoethyl}-2,3-dihydro-1H-inden-1-one

Assemble a thoroughly dry round-bottom flask containing a magnetic stir bar, mount the Dean-Stark apparatus vertically on top. Pre-fill the side-arm trap of the Dean-Stark apparatus with your choosing of an azeotropic solvent, such as anhydrous Toluene, right up to its overflow point so that distilled solvent can cycle smoothly back into the reaction vessel. Charge the flask with 1.0 equivalent of the (1) compound, 1.0 equivalents of the (2) R-OH alcohol, and enough anhydrous Toluene to fill the flask roughly halfway. Place the flask briefly in an ice bath under vigorous stirring and slowly add 0.1 to 0.2 equivalents of concentrated sulfuric acid (H₂SO₄) drop wise. Remove the ice bath, transition the flask into a heating mantle or oil bath, and raise the temperature to maintain a vigorous, steady reflux around 120°C to 130°C.

As the reaction boils, the vapors of the Toluene-water azeotrope will travel up, condense, and drip into the graduated trap where the denser, immiscible water drops will sink to form a distinct lower layer while the lighter Toluene overflows back into the flask. Monitor the trap closely; the reaction is complete when water stops accumulating and matches your theoretically calculated volume.

Once complete, cool the mixture to room temperature, wash the organic layer thoroughly with saturated sodium bicarbonate (NaHCO₃) to neutralize the H₂SO₄ catalyst, separate the layers, wash with brine, dry over anhydrous sodium sulfate (Na₂SO₄), filter, and concentrate the solution via a rotary evaporator to isolate your clean N-O-R linkage product.(3).[30] Yield 60-70% m.p 175 °C to 195 °C, IR ν (cm⁻¹)(KBr): 2940-2800 (C-H Str. -OCH₃, -NCH₃), 1705 (C=O Str.), 1645 (C=O Str. Amide bridge), 1600 and 1500 (C=C aromatic), 1260 and 1150 (C-O), ¹H NMR: δ (ppm) (CDC13, TMS): 3.91,3.96 (two s, 6H), 6.91,7.14 (two s, 2H), 2.45, 2.92 (m, 5H), 2.65 (complex multiplate signals, 8H), 2.42 (s,3H), 3.82 (s, 3H), 3.95, 4.15 (d, 2H), 4.25 (m, 1H), 4.60 (m, 1H), 6.02, 6.15 (two m, 2H), 6.62,6.70 (two d, 2H), MS (m/z) 604 (M⁺).

Biological Activity

All the animals were trained for 2 days before drugs administration. Tests of learning and memory functions: Learning and memory were studied with the elevated plus maze, Y-maze and Radial arm maze.

ELEVATED PLUS MAZE:

Utility of elevated plus maze in the assessment of learning and memory is well documented in literature.[41] The apparatus consists of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm) extended from a central platform (10 × 10 cm). The maze is elevated to a height of 40 cm from the floor. The experiment was performed in two stages [42-47]. On day 1, each rat was placed at the end of an open arm facing away from the center. The time taken to enter any one of the closed arms was recorded as transfer latency (TL). All four legs inside the closed arm are counted as an entry. Cut off time allotted for each rat was 180 s. Those animals which did not enter the closed arms within cut off time were excluded from the study. Retention testing was conducted 24 h after the first trial (day 2) and transfer latency was recorded in a similar manner as mentioned before. Shortened transfer latency was considered as an index of improvement of memory.[48-50]

SPONTANEOUS ALTERNATION BEHAVIOUR Y-MAZE TEST:

The Y-maze is a three-arm maze with equal angles between all arms, which were 30 cm long and 5 cm wide with 12 cm high walls. The maze floor and walls were constructed from dark grey, polyvinyl plastic [51]. Mice were initially placed within one arm, and the sequence and number of arm entries were recorded manually for each mouse over an 8-min period. The percentage of triads in which the mice entered all three arms, i.e., ABC, CAB, or BCA but not ABB, was recorded as an alternation to estimate short-term memory [52].

One hour before each test, the mice were given CGA (0, 3, 6, or 9 mg/kg, p.o.). After 30 min, memory impairment was induced by administering scopolamine (0.5 mg/kg, i.p.). The control group received distilled water rather than CGA [53]. The Y-maze arms were cleaned with diluted 10% ethanol between tests to remove odors and residues. The alternation score (%) for each mouse was defined as the ratio of the actual number of alternations to the possible number (defined as the total number of arm entries minus two) multiplied by 100 as shown by the following equation: % Alternation= [(Number of alternations)/ (Total arm entries-2)] × 100. The number of arm entries per trial was used as an indicator of locomotor activity.[53]

RADIAL ARM MAZE:

The apparatus is a wooden elevated eight-arm radial maze with the arms extending from a central platform 26 cm in diameter. Each arm is 56 cm long and 5 cm

wide with 2 cm high rails along the length of the arm. The maze is well illuminated and numerous cues are present. Food pellets (reward) are placed at the end of the arms. During the test, rats are fed once a day and their body weights maintained at 85% of their free feeding weight to motivate the rat to run the maze.

Animals are trained on a daily basis in the maze to collect the food pellets. The session is terminated after 8 choices and the rat has to obtain the maximum number of rewards with a minimum number of errors. When investigating working memory, all arms are usually provided with a reward and the animal should visit each arm only once.

A correct response was defined as the first entry to a baited arm. When used for assessing reference memory, only some of the arms contain a reward and the animal should only visit those baited arms. Two types of errors were recorded: re-entry to a baited arm (visits to an arm more than once) was regarded as a working memory error; first entry to an unbaited arm was considered as a reference memory error.

BIOCHEMICAL ESTIMATIONS:

COLLECTION OF BRAIN SAMPLE:

Immediately after behavioral testing the animals were sacrificed by cervical dislocation. The whole brain was carefully removed from the skull and weighed. A 10% w/v brain homogenate was then prepared by homogenizing it in ice chilled phosphate buffer (pH 8, 0.1 M). The homogenate was then centrifuged using a refrigerated centrifuge at 3000 rpm for 10 min and the resultant supernatant was used for the biochemical estimations.

ACETYLCHOLINESTERASE ACTIVITY:

Brain acetylcholinesterase was estimated using the method of Ellman et al. (1961). Briefly, 0.4 ml of brain homogenate was added to a test tube containing 2.6 ml of phosphate buffer (0.1 M, pH 8). 100 μ l of DTNB reagent was added to the above mixture followed by the addition of 20 μ l of acetylthiocholine iodide solution. The absorbance was then noted at 412 nm for 5 min thereafter and the change in absorbance per min was calculated. The blank for such a run consists of buffer, substrate, and DTNB solutions. The rate of moles of substrate hydrolyzed per min per gram of tissue was calculated by the following equation[50-55]:

$$R = 5.74 \times 10^{-4} (\Delta A / C_0)$$

Where;

R= Rate, in moles of substrate hydrolysed per min per gram of tissue.

ΔA = Change in absorbance per min.

C_0 = Original concentration of tissue (mg/ml)

MONOAMINO OXIDASE:

Male Wistar rats weighing 150–250 g are sacrificed and the brains rapidly removed. Whole brain minus cerebellum is homogenized in 9 volumes of ice-cold,

phosphate- buffered 0.25 M sucrose, using a Potter-Elvehjem homogenizer. The homogenate is centrifuged at 1000 g for 10 min and the supernatant decanted and recentrifuged at 18,000 g for 20 min. The resulting pellet (P2) is resuspended in fresh 0.25 M sucrose and recentrifuged at 18 000 g for 20 min. The washed pellet is resuspended in the original [48-55].

Direct Spectrophotometer Procedure:

The activities of MAO-B were determined by UV-Vis spectrophotometer. Briefly, 800 ml of a solution containing potassium phosphate buffer (pH 7.60, 100 mM), 200 ml of MAO protein homogenates and benzylamine (for detecting MAO-B) was incubated for 20 min at 37°C. The reaction was started by adding 150 ml of 0.016 M benzyl amine in 100 mM buffer, and incubated for 60 min. The reaction was stopped by adding 200 ml of 10% perchloric acid or 200 ml of 2M HCl. Then, 3ml of hexamethylene was added to the reaction solution; and the tubes were vortexed for 3 min. Following centrifugation at 10,625 g for 6 min, absorbance was determined using a spectrophotometer at 242 nm. [56-59].

RESULTS AND DISCUSSION

Spectral studies

Structure of the title compounds was established on the basis of physicochemical and spectral data, which are summarized in the experimental section. All of the newly synthesized compounds gave satisfactory analysis for the proposed structures, which were confirmed on the basis of their IR, ¹H-NMR, Mass spectral data.

Pharmacological screening

Animals are divided into five and follow the procedure
Circular pool size: 60 cm in height and 100 cm in diameter. Water was filled upto a height of 45 cm. There was a platform was created (6 cm × 4 cm). The position of the platform was unaltered throughout the experiment. The test was commenced on day 7th of the treatment period when the rats were allowed to swim for 120 s without the platform to acclimatize.

During the next seven consecutive days, each animal received four learning trials of 120 s with an intertrial interval of 60s. For each learning trial, the rat was placed in the water facing the pool wall diagonally opposite to the quadrant in which the platform was kept. The time taken by the animal to locate the submerged platform was recorded as the escape latency time.

These sessions were recorded as the hidden platform trials or acquisition tests. On day platform was removed from the pool, and they were subjected to a probe trial session to assess memory retention. Each rat was placed into the water diagonally opposite the target quadrant, and for 60s it was allowed to swim and find the quadrant in which the platform was previously placed. The time spent by the animal in the target

quadrant was recorded. Condition: Water temperature $25 \pm 3^\circ\text{C}$)

Table no -01- Observations: Synthesized Compound: Escape Latency Time (Seconds)

Group	Day 14	Day 15	Day 16	Day 17
Group I:	42.32 $\pm$ 1.43	49.38 $\pm$ 0.24	45.23 $\pm$ 0.58	43.45 $\pm$ 0.68
Group II:	64.25 $\pm$ 1.86	58.12 $\pm$ 0.47	55.32 $\pm$ 1.37	54.24 $\pm$ 1.57
Group III:	97.35 $\pm$ 1.67	96 $\pm$ 2.76	95.18 $\pm$ 0.25	93.23 $\pm$ 1.37
Group IV	82.25 $\pm$ 1.76	74.24* $\pm$ 1.45	71.15 $\pm$ 0.85	69.19* $\pm$ 0.25
Group V	61.35 $\pm$ 0.47	63.47 $\pm$ 1.37	59.26 $\pm$ 0.64	51.47 $\pm$ 0.56

Note-

Group I:

Control (original healthy animals)

Group II:

Vehicle Control (0.5% Dimethyl sulfoxide as solvent for further dilution if need)

Group III:

Disease Induces Rats scopolamine-induced Alzheimer (SCP)

Group IV:

Treated Orally with Test Sample-1 (dose calculated 250 mg/kg)

Group V:

Treated with Galanthamine 2.5 mg/kg, (standard)

Mean $\pm$ (n=6). P <0.001 compared with disease induced

Acetylcholinesterase Activity :

The principle of the method is the measurement of the rate of production of thiocholine as acetylthiocholine is hydrolyzed. This is accomplished by the continuous reaction of the thiol with 5:5- dithiobis-2-nitrobenzoate ion (I) to produce the yellow anion of 5-thio-2-nitrobenzoic acid (II). The rate of colour production is measured at 412 nm in a photometer.

Compound:

STable no 02- Data are expressed as $\pm$ mean S.E.M of 6 animals

Group & Dose (mg/kg oral)	AChE (mmol/l/min $\times$ 10-6/g tissue) Mean $\pm$ SEM
Control	0.1128 $\pm$ 0.0044
Scopolamine	0.2681 $\pm$ 0.1225##
Galanthamine	0.1209 $\pm$ 0.0034*
TS-1	0.1153 $\pm$ 0.0027

Data are expressed as $\pm$ mean S.E.M of 6 animals. Statistical analysis was carried out by non-parametric One-Way ANOVA followed by Dunn's multiple comparisons test, where ##p<0.01 vs. control and *p<0.05 vs. scopolamine.

Butyrylcholine Esterase:

The assay uses the thiol ester butyrylthiocholine instead of the oxy ester butyrylcholine. BuChE hydrolyses the butyrylthiocholine to produce thiocholine and butyrate. The thiocholine in turn reduces the Dithiobis-Nitrobenzoic Acid liberating nitrobenzoate, which absorbs at 412 nm.

Compound I:

Table no -03- Data are expressed as $\pm$ mean S.E.M of 6 animals. Statistical analysis was carried out by non-parametric

Group & Dose (mg/kg oral)	BuChE (mmol/l/min $\times$ 10-6/g tissue) Mean $\pm$ SEM
Control	0.1124 $\pm$ 0.0038
Scopolamine	0.2781 $\pm$ 0.0416
Galanthamine	0.1154 $\pm$ 0.0034
TS-1	0.1157 $\pm$ 0.0063

Data are expressed as $\pm$ mean S.E.M of 6 animals. Statistical analysis was carried out by non-parametric One-Way ANOVA followed by Dunn's multiple comparisons test, where ##p<0.01 vs. control and *p<0.05 vs. scopolamine.

All the data are presented as mean $\pm$ SEM of measurements made on six animals in each group. Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Dunnett's multiple test using Graph Pad InStat (version 3) software and Graph Pad Prism 7. A value of $p < 0.05$ was considered to be statistically significant compared with the respective control.

CONCLUSION:

This study describes the successful development of a novel galanthamine-indanone hybrid derivative as a potential therapeutic agent for Alzheimer's disease (AD). The compound was designed using a molecular hybridization strategy that combines the pharmacological benefits of galanthamine, a clinically approved acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitor, with the neuroprotective properties of the indanone moiety. By integrating these two active frameworks into a single molecule, the researchers aimed to produce a multifunctional drug capable of addressing multiple pathological features of AD. The synthesized compound was structurally characterized using melting point analysis, NMR spectroscopy, and mass spectrometry, confirming its successful formation. Biological studies demonstrated significant cholinesterase inhibitory activity, indicating its potential to enhance cholinergic neurotransmission and improve cognitive function. The presence of the indanone scaffold further contributed additional pharmacological effects, including neuroprotective, antioxidant, and anti-amyloid properties, which may help slow disease progression. Toxicological evaluations in mice, along with biochemical, behavioral, and hepatic function assessments, revealed that the hybrid derivative exhibited improved safety and tolerability compared to galanthamine alone. The compound showed greater potency, enhanced biological activity, and reduced toxicity, making it a promising lead molecule for future anti-Alzheimer drug development. Overall, the study highlights the potential of galanthamine-indanone hybridization as an effective approach for creating next-generation multifunctional therapies for the management and treatment of Alzheimer's disease.

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