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Research Article

ACETYLCHOLINESTERASE INHIBITORY EFFECT OF 3-(1*H*-INDOL-3-YL)-1, 3-DIPHENYLPROPAN-1-ONE DERIVATIVES

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ABSTRACT

Objective: The objective of the study is acetylcholinesterase (AChE) inhibitory effect of 3-(1*H*-indol-3-yl)-1, 3-diphenylpropan-1-one derivatives by Ellman's method, physostigmine is used as positive control.

Method: 3-(1*H*-indol-3-yl)-1, 3-diphenylpropan-1-one derivatives were synthesized by the addition of chalcone (0.25 g, 1 mmol), indole (0.12 g, 1 mmol) in ethanol (5 ml), and concentrated hydrochloric acid (5 mmol %). These earlier synthesized compounds were screened for AChE inhibitors by modifying Ellman's method.

Results: Among the tested compounds, 3a and 3j were found to be having more potential than other compounds with half maximal inhibitory concentration values of 13.64 and 14.3 µg/ml, respectively. Whereas, compounds 3c, 3e, 3g, and 3i exhibited an average AChE inhibition of 16.4, 17.9, 17.6, and 21.1 µg/ml, respectively.

Conclusion: The compounds 3-(1*H*-indol-3-yl)-1, 3-diphenylpropan-1-one derivatives were found to be possible lead molecules in AChE inhibition and even though, the molecules were structurally dissimilar to that of the standard, still they exhibited a considerable degree of inhibition and encourage the researchers to look into the mode of action of their inhibition ability against AChE.

Keywords: Alzheimer's disease, Neurotransmitter, Dementia, Bovine serum albumin, Ellman's method, Physostigmine.

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INTRODUCTION

Alzheimer's disease (AD), is the most common form of dementia, affecting millions of elderly people accounting for about 50-60% of the overall cases of dementia among persons over 65 years of age. AD is characterized by atrophy of the cerebral cortex and loss of hippocampal and neocortical neurons. It is characterized by memory deficit and progressive impairment of cognitive nerve functions [1] leading to mental deterioration, decrease in cognitive ability and severe behavioral, and psychological abnormalities such as irritability, anxiety, and depression [2]. Many factors have been found to be implicated in AD, such as low levels of acetylcholine (ACh) availability [3], β-amyloid deposits, decrease in oxidative phosphorylation process, and metal ion exchange process, which seem to play significant roles in the disease [4].

The therapies under evaluation for the treatment of AD have disease modifying and neuroprotective approaches [5]. The behavioral abnormalities are best treated first by non-pharmacological interventions. Pharmacological agents used for treatment of neuropsychiatric illnesses include antipsychotics, antidepressants, and mood stabilizers [6]. Current treatment of AD focus on increasing cholinergic neurotransmission in the brain by inhibiting cholinesterases (ChEs).

In this regard, acetylcholinesterase inhibitors (AChEI) are approved for the treatment of mild to moderate AD [7,8]. Four AChEI are approved by food and drug administration, they are tacrine, donepezil, rivastigmine, and gallantamine [9,10]. Unfortunately, the potential effectiveness is often limited by the side effects [11]. The care should be taken during AD treatment because it is related to the vital organ brain, its disorder with central nervous system (CNS). The challenge for this disorder is rational drug design in the discovery of mechanism-based inhibitors due to its role in the hydrolysis of the neurotransmitter ACh leading to senile dementia, related with selective loss of cholinergic nerve signals in the form of neurons, and reduced frequency of ACh neurotransmitter, especially in the CNS.

Due to the multi-pathogenesis of AD, one of the current strategies is to develop novel anti-AD agents with multiple potencies and target specificity [12]. Genistein is biosynthetic compound one of the simplest flavonoid form of the leguminosae [13]. It expresses a broad spectrum of pharmacological activities, such as antioxidant, anticancer, and antimicrobial [14-16]. In recent years, it was reported that genistein showed the neuroprotective effect and ameliorated learning and memory deficits in the AD rat model [17,18].

Hence, in an attempt to develop new molecules for the treatment of AD, new indole diphenylpropan-1-one derivatives 3a-j, was screened against AChE enzyme. The indole derivatives are known to possess different biological activities [19,20]. The derivatives 3a-j was reported earlier from our laboratory [21] and their pharmacological behavior; it was found that the derivatives were not promising enough to be considered for further investigation on the cell lines tested. This might be because of lacking in specificity or the competitiveness of molecules at the binding site of enzymes involved, because of racemic behavior. The synthesized derivatives were accompanying indole nucleus as a bioisosteric substitute of the diphenylpropan-1-one moiety which is reported to be important for AChEI activity [22].

Rationality

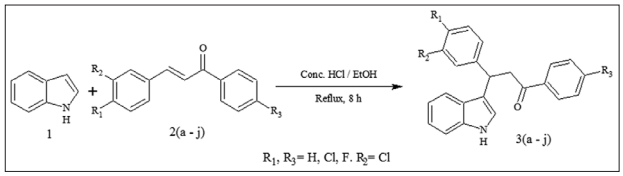
The literature survey revealed that the title compounds do possess considerable degree of biological activity [19,20]; hence, tested for antiproliferative activity [21]. The title compounds were found to be moderately active against the cell lines tested. This prompted us to

further take up the molecules in the *in vitro* AChEI activity by random screening against the enzyme. The *in silico* adsorption, distribution, metabolism, excretion, and toxicity studies also strengthen our claim that the molecules are potentially safe through oral absorption. Hence, the present investigation was envisaged to screen the 3-(1*H*-indol-3-yl)-1, 3-diphenylpropan-1-ones derivatives for their AchE inhibitory activity.

METHODS

The synthesis of 3-(1*H*-indol-3-yl)-1, 3-diphenylpropan-1-one 3a-j were reported earlier from our laboratory [21].

Schematic representation of the already synthesized 3-(1*H*-indol-3-yl)-1, 3-diphenylpropan-1-ones derivatives 3a-j [21].



CHEMICALS

Acetylthiocholine iodide (ATCl), 5,5'–dithio-bis-2-nitrobenzoic acid (DTNB), bovine serum albumin (BSA), and electric eel AChE (AChE; EC 3.1.17; lyophilized, 500 U/vial solid, 65 U/mg) were purchased from Sigma. Physostigmine was used as the positive control. Buffers and other chemical were of extra pure analytical grade. The following buffers were used: Buffer A: 50 mmol Tris–HCl, pH 8, containing 0.1% BSA; Buffer B: 50 mmol Tris- HCl, pH 8 containing 0.1 M NaCl, 0.02 M $MgCl_2 \times 6H_2O$.

ENZYME INHIBITION ASSAY

The synthesized compounds 3a-j (Table 1) were screened against AChE *in vitro* screening according to the modified Ellman's method [23,24]. Acetylthiocholine was used as a substrate and hydrolysis of acetylthiocholine was determined by monitoring the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction with DTNB with thiocholine catalyzed by enzymes at a wavelength of 412 nm. Briefly, 25 µl of 15 mm ATCI, (43 mg/10 ml in Millipore water), 125 µl of 3 mm DTNB, (11.9 mg/10 ml buffer B), 50 µl of buffer A, and 20 µl of compound at a concentration of 100 µg/ml were added to 96 well plates and the absorbance was measured at 412 nm every 13 s for five times. After adding 25 µl of 0.22 U/ml enzymes, (0.34 mg AchE dissolved in 100 ml buffer A), the absorbance was read again every 13 s for five times. The absorbance was measured using a Multi-Mode Microplate Reader. Percentage of inhibition was calculated by comparing the rates of the sample with the blank; control samples contained all components except the tested extract. Physostigmine was used as positive control. Then, the mean of three measurements for each concentration was determined (n=3). Half maximal inhibitory concentration (IC_{50} value) was calculated and listed in the (Table 2).

Statistical analysis

Statistical analysis was carried out using one-way ANOVA. The data are represented as in mean±standard deviation.

RESULTS AND DISCUSSION

Degeneration of cholinergic neurons and decrease in ACh levels in neocortex, hippocampus, and basal forebrain play a major role in the pathophysiology of AD. The pathological hallmark of AD is widespread neurotic plaques which are accumulations of amyloid beta (Aβ) protein [25]. Production and accumulation of Aβ appear to be central to the pathogenesis of AD [26]. Aβ is a short polypeptide of about 42 amino acids produced by the abnormal proteolytic cleavage of amyloid precursor protein, which involves enzymes-like gamma-secretase [27]. Production and deposition of Aβ are the central event triggering oxidation, lipid peroxidation, and excessive excitotoxicity of glutamatergic neurons, inflammation, apoptotic cell death, and formation of neurofibrillary tangles [26]. Neurofibrillary tangles are paired helical filaments composed of tau protein, which in normal cells are essential for axonal growth and development [28]. However, when hyperphosphorylated, the tau protein forms tangles that are systematically deposited within neurons located in the hippocampus and medial temporal lobe, the parietotemporal region, and the frontal association cortices leading to cell death [29-31]. The cell death in the basal forebrain (nucleus basalis of meynert) leads to deficit in neurotransmitter systems of ACh, serotonin, and norepinephrine. Studies report that deficit in cholinergic system is responsible for cognitive decline and memory loss in patients with AD [32]. The disturbances in neurotransmitter systems also lead to a variety of behavioral abnormalities, including depression, psychosis, and agitation [33] (Fig. 1).

From Table 2 results, all compounds 3a-j were tested against AChE and related IC_{50}±SD were calculated. Compound 3a without substitute on

Table 1: Molecular details of target compounds 3a-j [21]

Entry code	R^1	R^2	R^3	Molecular formula
3a	H	H	H	$C_{23}H_{19}NO$
3b	Cl	H	H	$C_{23}H_{18}ClNO$
3c	F	H	H	$C_{23}H_{18}FNO$
3d	H	Cl	H	$C_{23}H_{18}ClNO$
3e	H	Cl	Cl	$C_{23}H_{17}Cl_2NO$
3f	Cl	H	Cl	$C_{23}H_{17}Cl_2NO$
3g	F	H	Cl	$C_{23}H_{17}ClNO$
3h	H	Cl	F	$C_{23}H_{17}ClFNO$
3i	Cl	H	F	$C_{23}H_{17}ClFNO$
3j	F	H	F	$C_{23}H_{17}F_2NO$

Table 2: Acetyl cholinesterase inhibitory activity of the compounds 3a-j with IC_{50} values

S. No.	Name of compounds	Percent of inhibition (IC_{50}); µg/ml (Mean±SD)
1	3a	13.64±0.012
2	3b	ND
3	3c	16.48±0.14
4	3d	ND
5	3e	17.9±0.23
6	3f	ND
7	3g	17.6±1.31
8	3h	ND
9	3i	21.1±0.31
10	3j	14.3±0.146

Data presented as mean±SD (n=3). ND: Not determined, SD: Standard deviation, IC_{50}: Half maximal inhibitory concentration

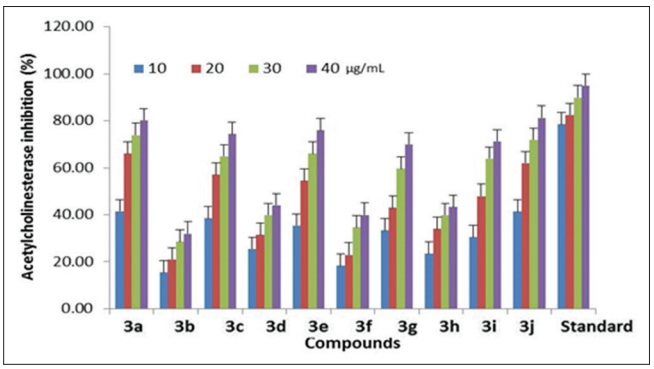
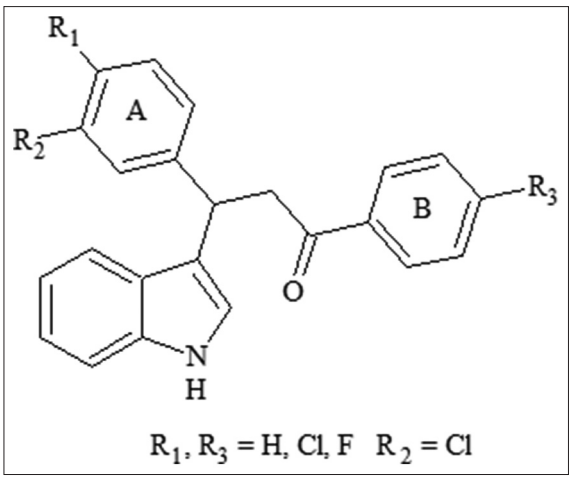


Fig. 1: Acetylcholinesterase inhibitory activity of the compounds 3a-j with half maximal inhibitory concentration values

phenyl ring was screened and found to be active. To explore, the effect of electron withdrawing halogen substituents along with the propan-1-one active site, the compound 3j having para fluorine substitution on both phenyl ring A and B are the most potent one in the series this might be because of bioisosteric replacement of hydrogen by fluorine.



Both para substituted fluorine (3j) was essential for effective anticholinesterase activity, but on the other hand only para and meta substitution of chlorine on ring A (compound 3b, 3d, 3f, and 3h) it does not lead to inhibitory effect even though, the compound 3h having para fluorine on ring B compared to other position for this moiety. The change in the size by the incorporation of fluorine might alter the probability of binding to the enzyme hence lower in activity. Among the compounds, 3c, 3g, and 3j with fluorine moiety on ring A exhibited anticholinesterase activity. Whereas, compound 3j with fluorine at para substitution on the phenyl rings (A and B) exhibited inhibition potency when compared with other positions. The activity exhibited by the compounds might be due to inhibition of hydrolysis of ACh by AChE by binding effectively.

CONCLUSION

A series of 3-(1*H*-indol-3-yl)-1, 3-diphenylpropan-1-one derivatives 3a-j were screened for AchE inhibition. Among ten derivatives tested, compound 3a, 3c, and 3j with varying substituents on phenyl ring A and B exhibited the highest potency in this series (IC_{50} values of 13.64 16.48 and 14.3 µg/ml) when compared with physostigmine. The results of the enzyme inhibition test (Ellman test) showed that electron withdrawing groups such as fluorine can render the best enzyme inhibitory effect on para position of the phenyl rings. Synthesized compounds 3a-j could be proposed as potential anticholinesterase hits, with further alterations, and modifications the molecules might become effective leads in the treatment of AD.

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