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# DESIGN, SYNTHESIS, CHARACTERIZATION AND EVALUATION OF NEWER POTENT APOLIPOPROTEIN E4 INHIBITORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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**Keywords:**

Oxazole heterocycle, Docking, Apolipoprotein E4, Anti-Alzheimer

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**ABSTRACT:** A major genetic suspect for Alzheimer's disease is the pathological conformation assumed by Apolipoprotein E4(Apo E4) through intramolecular interaction. The aim of the current study is to synthesize newer potent Apo E4 inhibitors. In the present study, with specific pharmacophoric features of three Hydrogen bond donors and three hydrophobic spheres, a large library of ligands was constructed for Apo E4 inhibitors. The newly designed ligands were subjected for docking studies using Autodock®Tools 1.5.6 and optimized for Lipinski rule of five and further screened by *in-silico* toxicity studies. Out of that oxazole heterocyclic nucleus and its analogs were chosen for synthesis and characterized for spectral analysis such as IR, NMR, LC-MS. The active compound LS 4 was evaluated for cytotoxicity study through MTT Assay and neuroprotective study against Aβ- L-DOPA toxicity induced SH-5YSY cell line. Compound LS 4 showed 93.81% of neuroprotective activity at 1.567µg/ml.

**INTRODUCTION:** Alzheimer's Disease (AD) is a neurodegenerative disorder in which the death of the brain cells causes memory loss and cognitive decline *i.e.* dementia. The disease starts with mild symptoms and gradually becomes severe. AD is one of the leading causes of mortality worldwide. It is the cause of 60–70% of cases of dementia[1]. Alzheimer's is the most common cause of dementia among older adults. Dementia is the loss of cognitive functioning thinking, remembering, and reasoning, and behavioral abilities to such an extent that it interferes with a person's daily life and activities [2]. The disease is characterized by accelerated accumulation of amyloid β (Aβ) plaque around neurons and hyperphosphorylated micro-tubule-associated tau protein in the form of neurofibrillary tangles within the cells [3].

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The discovery of Aβ and its accumulation in brain resulted in the formulation of the "amyloid cascade hypothesis" which states that the deposition of Aβ subsequently leads to the formation of neuro-fibrillary tangles, neuronal cell death and dementia [4]. Amyloidogenic pathway results from a mutation and replaces the normal pathway in which α-secretase acts on the amyloid precursor protein (APP), a membrane protein, followed by Y-secretase forming a harmless peptide but the amyloidogenic pathway involves the breakdown of APP by β-secretase followed by Y -secretase, and results in the formation of Aβ plaque, whose major constituent is the 42 residues long Aβ42 [5].

Aβ oligomers and plaques are potent synaptotoxins, block proteasome function, inhibit mitochondrial activity, alter intracellular $Ca^{2+}$ levels, and stimulate inflammatory processes. The Above processes contribute to neuronal dysfunction [6]. For more than 20 years, studies of the brains of those with advanced age and AD have consistently found damage or abnormalities in the cholinergic pathway that appeared to correlate well with the level of

cognitive decline. As a result so called "cholinergic hypothesis" was developed, which essentially states that a loss of cholinergic function in the central nervous system contributes significantly to cognitive decline associated with advanced age and AD [7-8].

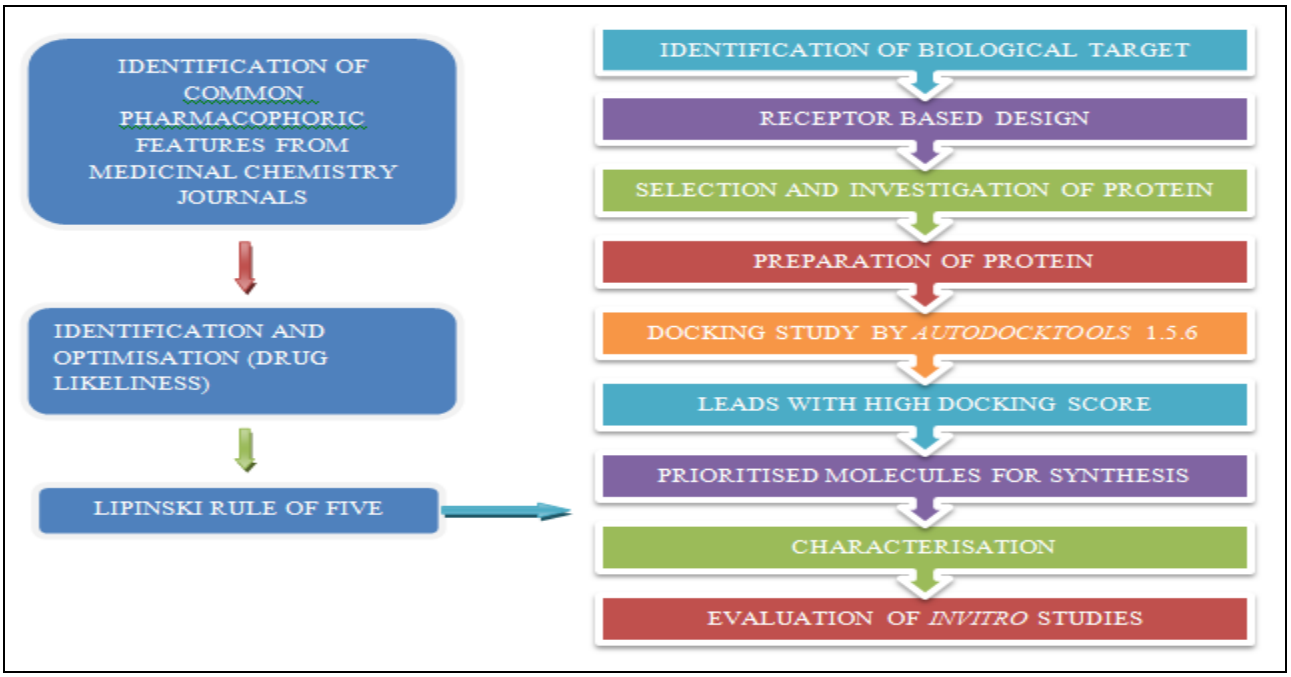
AD cases can be categorized into two main categories, the (pseudo) sporadic late-onset AD (LOAD) and early-onset familial AD (FAD). LOAD is characterized by disease manifestation ages above 65 years. Increasing age is the major risk factor for LOAD. In addition, the apolipoprotein E (ApoE) gene on chromosome 19 has been demonstrated to represent FAD cases. FAD occurs earlier, sometimes already in the twenties. FAD is caused by autosomal dominant mutations in either APP or the presenilin-1 or -2 gene/ protein.

The current study and research has been made to identify novel molecule for Apolipoprotein E4 inhibitors for Anti-Alzheimer activity. In order to identify the ApoE4 inhibitors following steps have been carried out. Identification of pharmacophoric features for designing newer ligands of ApoE4 inhibitors from the literature review. Framing and constructing a Virtual Library of compounds that selectively inhibit ApoE4 inhibitors for anti-Alzheimer activity. The current study includes the binding mechanism of ApoE4 inhibitors with the newly designed ligands through molecular docking study using using *Autodock® Tools 1.5.6*. Then, the newly designed ligands of high score value were selected and optimized using Lipinski's rule of five using *Molinspiration® software* and filtred for *Insilico*toxicity study using *OSIRIS ®Property explorer*. Based on the synthetic feasibility, chemical entities consisting of oxazole heterocycles were synthesized and purified.

The synthesized compounds were characterized by spectral analysis such as IR, NMR ($^1$H), and hyphenated techniques such as LC-MS. *In-vitro* screening has been made for the synthesized compound for neuroprotective study against amyloid β - L-DOPA induced toxicity against SH-5YSY cell line.

## EXPERIMENTAL:

### Materials and Methods:




### Drug Design:

**Selection of Target:** Human apolipoprotein (apo)E, a major component of lipoproteins plays a central role in the metabolism and redistribution of lipids such as cholesterol. *Apo*E is synthesized primarily in the liver and is also produced abundance in the brain and has significant functions in central nervous system integrity and

remodeling. ApoE transports cholesterol to neurons in the brain. In human blood circulation, *Apo*E binds lipids and makes them soluble for transporting [9]. Human ApoE has 299 aminoacids with three common isoforms (apoE2, apoE3, and ApoE4) that have an essential role in the regulation of cholesterol metabolism. They differ each at residue 112 or 158. ApoE3 contains a cysteine at residue 112 and an arginine at residue 158, whereas apoE4 has arginine at both positions, and apoE2 has cysteine. Human apoE3 is the most common isoform, while ApoE4 is hypofunctional [10].

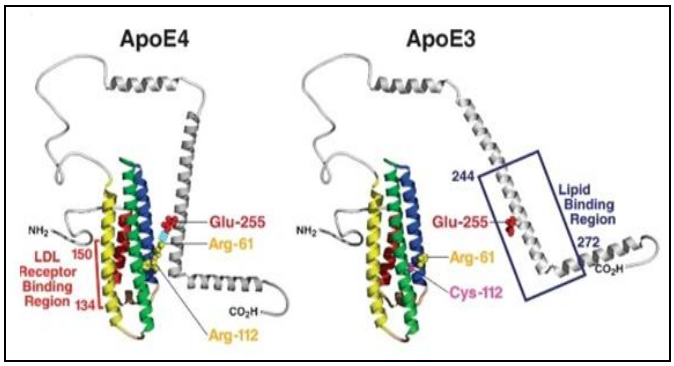



**FIG. 1: DOMAIN INTERACTION OF APOE3 AND APOE4**

ApoE4 plays an intramolecular domain interaction between its amino and carboxyl-terminal domains, leading to a compact structure. Domain interaction in apoE4 is induced by Arg112, which facilitates the formation of salt bridge between Arg-61in the amino-terminal domain and Glu 255 in the carboxyl domain. ApoE4 enhances the APP (amyloid precursor protein) and amyloid-beta production through both LRP (LDL receptor-related protein) pathway and ApoE4 domain interaction. ApoE4 with an increased risk of AD makes it a potential drug target for designing natural drug candidates for Alzheimer's disease [11].

**Selection of X-ray Crystal PDB:** The protein selection is carried out from the RCSB PDB (Protein data bank). It is a resource for studying biological macromolecules. It contains information about experimentally-determined structures of proteins, nucleic acids, and complex assemblies. Some of the recent and efficient PDB enzyme targets with low resolution were selected and further evaluated by their Resolution value, R Free, R-value, and optimized crystal ligand interaction details. Some of the efficient PDB file receptors for apoE4 with low resolution were selected (*1GS9*) from RCSB protein data bank, and their active site were identified. PDB ID for apoE4 are listed below with their resolutions

**TABLE 1: LIST OF PDB ID FOR *APOE*4**

| S. no. | Code | Resolution |
|---|---|---|
| 1 | 1GS9 | 1.7 A |
| 2 | 1B68 | 2.0 Aº |

**Pharmacophore Identification:** Pharmacophore modeling correlates the biological activity with the spatial arrangement of various features inset of active analogues. When reviewing the efficient journals and research articles, Six Pharmacophore features consisting of three hydrogen bond donor (HBD) and three hydrophobic spheres (HYP) was identified as the best model for designing ApoE4 inhibitors [10].

**Construction of a Large Virtual Scaffold Library:** The target screening library was designed by using molecular fragments from a relatively narrow and low molecular weight range (350-5000D), selected diversity at both the putative "scaffold" core. The analogue library was generated by modifying the respective functional groups with sterically and conformationally allowed substituents using the reagent database and a combinatorial design model. A library consisting of nearly new 50 lead molecules as potent ApoE4 inhibitors was generated based on the knowledge of the binding interaction of Ligand with the protein and also the common features necessary for the biological activity of the molecule. Three hydrogen bond donor (HBD) and three hydrophobic spheres (HYP) was used to screen knowledge database [12].

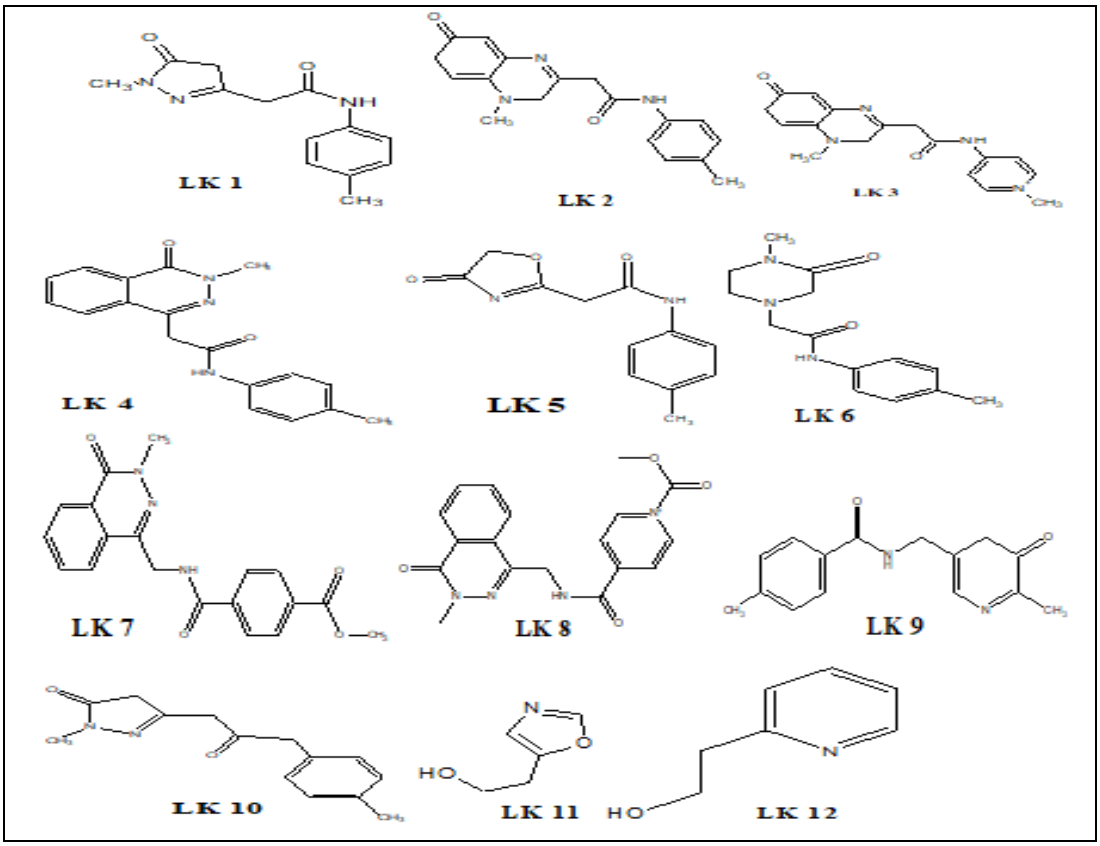
**TABLE 2: MOLECULAR FRAGMENTS USED IN CONSTRUCTION OF LIBRARY**

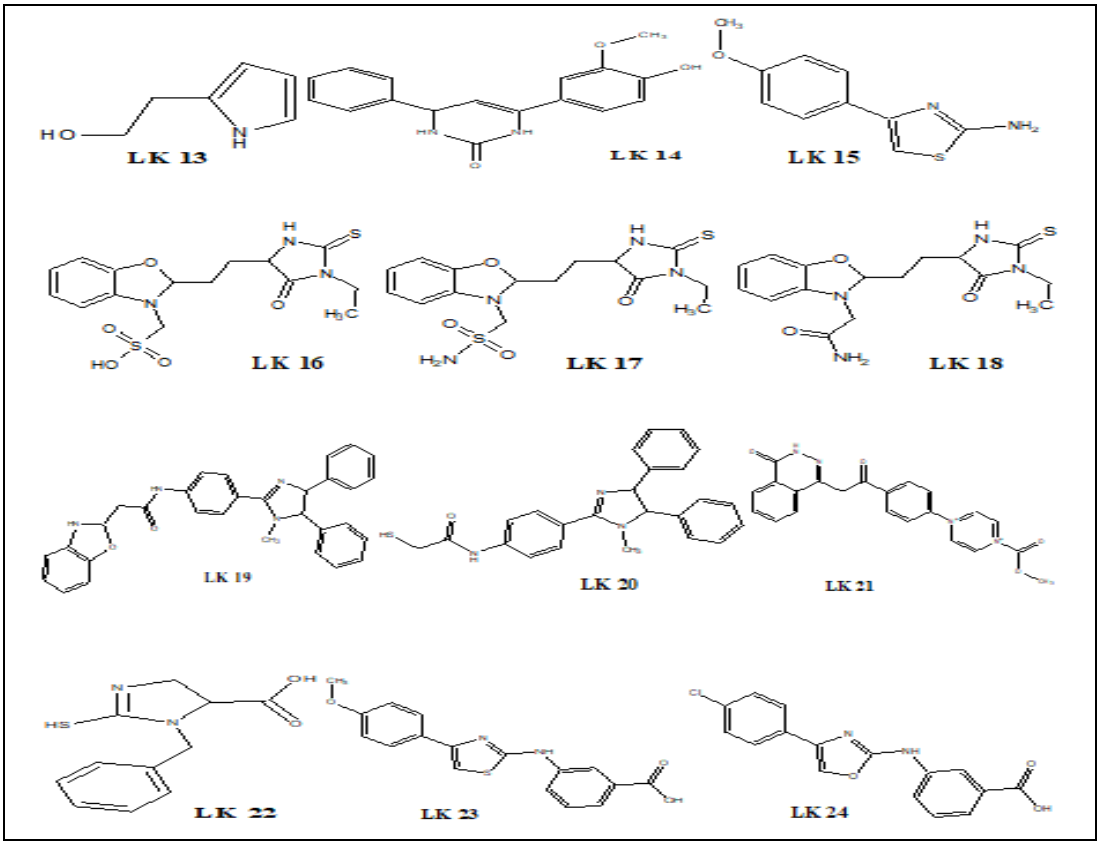
| HBA | HYP |
|---|---|
| Imines, Nitriles, | Methyl group |
| Phenolic -OH, COOH group, | Amine group |
| $CH_2$ OH, CHOH. Ether, carbonyl, | NH group |
| pyridine, Nitro, Amide. Imidazole, | Imidazole |
| isoxazole, oxazolethiazole, | Imino group |
| guanidine and sulfoxide | Hydroxyl group |

A virtual scaffold library consisting of newly designed 50 molecules has been constructed have been shown in the following **Fig. 2**.

**Lead Optimization:** All the designed ligands (50) were optimized by subjecting to Docking studies, ADMET properties, Lipinski's rule of five, Novelty prediction, and Toxicity prediction to refine further.

Using the Pharmacophore model feature and molecular feature of *Apo*E4 inhibitors obtained by reviewing the literature, a virtual scaffold library of 50 ligands were constructed and listed with structures below



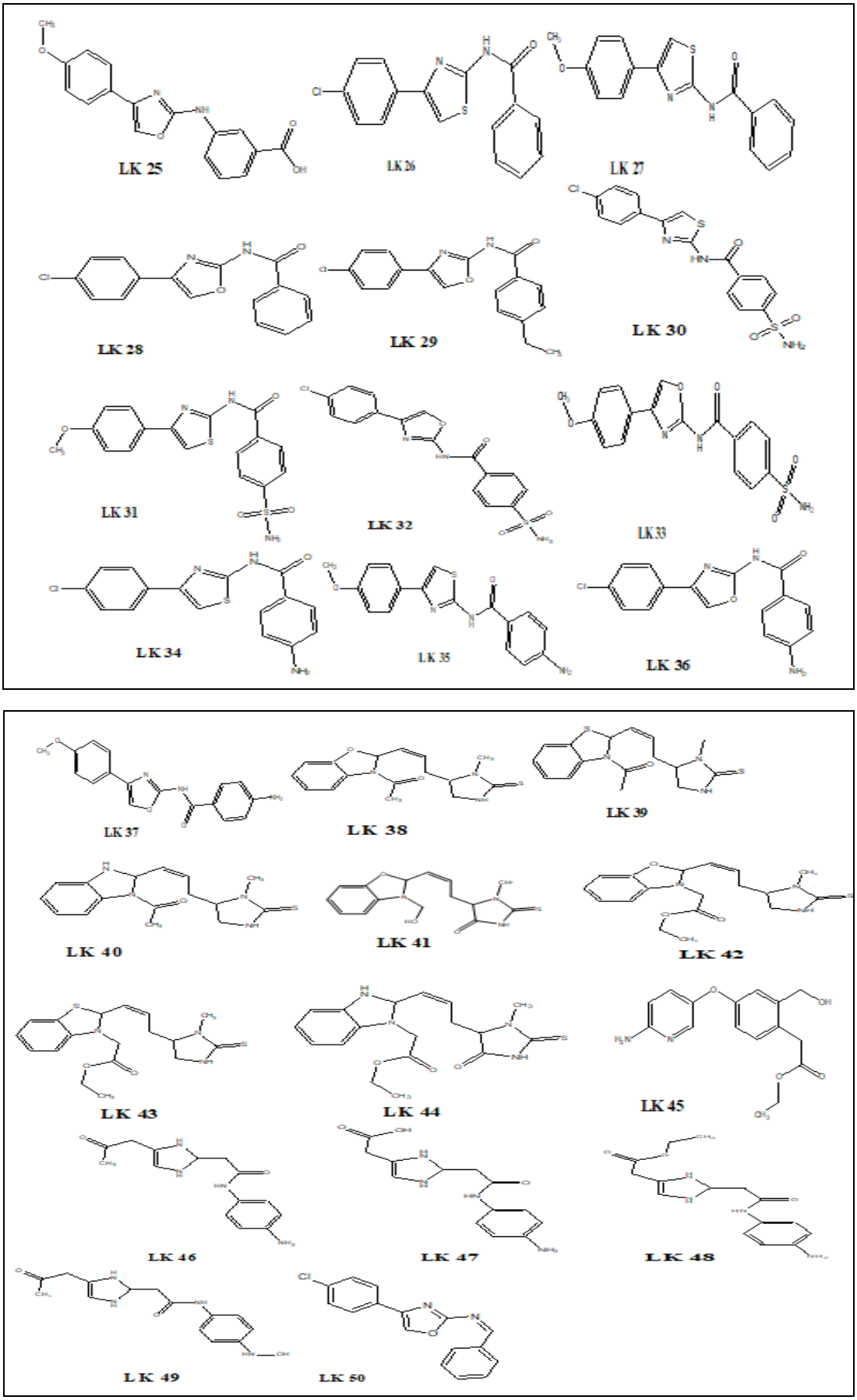



**FIG. 2: VIRTUAL LIBRARY**

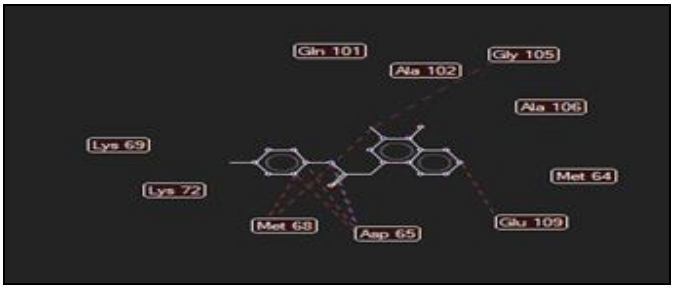
Nearly 50 new designed ligands derived from the library are docked against the enzyme 1GS9 using *AUTODOCK TOOLS 1.5.6* software. Based on the docking scores of all the 50 newly designed ligands are categorized and tabulated as follows.

- ❖ Highly active (-7.5 to -8.5 kcal/mol)
- ❖ Moderately active (-6.0 to -7.5 kcal/mol)
- ❖ Low active (below -6 kcal/mol)

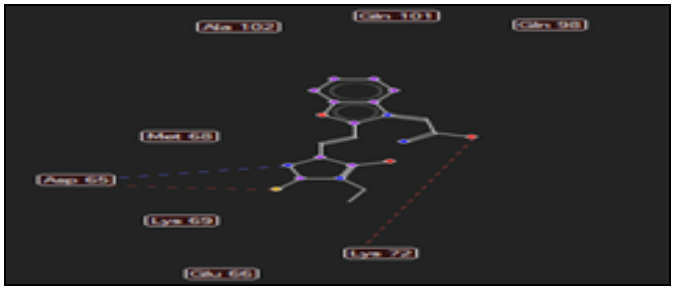
**TABLE 3: CLASSIFICATION OF DESIGNED LIGANDS BASED ON DOCKING SCORES**

| Highly Active (10) | Moderate (24) | Least Active (16) |
|---|---|---|
| LK 4, LK 18, LK 30, LK 38, LK 39, LK 40, LK 42, LK 43, LK 44, LK 50 | LK 1, LK 2, LK 5, LK 7, LK 8, LK 9, LK 10, LK 16, LK 19, LK 20, LK 21, LK 23, LK 25, LK 26, LK 27, LK 28, LK 29, LK 31, LK 32, LK 33, LK 34, LK 36, LK 41, LK 45. | LK 3, LK 6, LK 11, LK 12, LK 13, LK 14, LK 15, LK 17, LK 22, LK 24, LK 35, LK 37, LK 46, LK 47, LK 48, LK 49. |

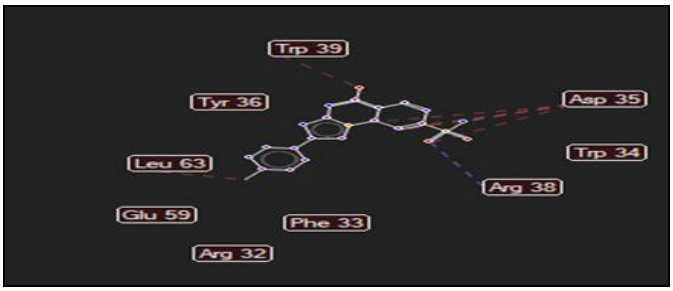
On the basis of performed docking studies, 10 designed ligands were considered as best hit molecules and their docking interaction snapshots are highlighted below.



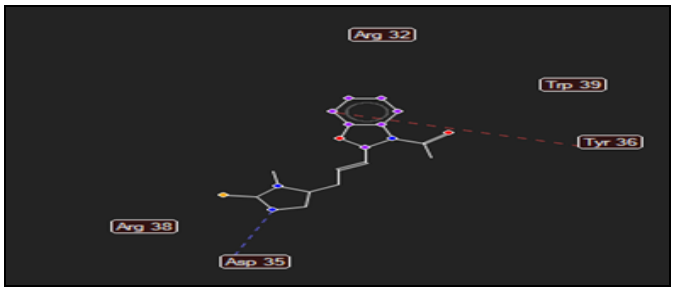

**LK 4**



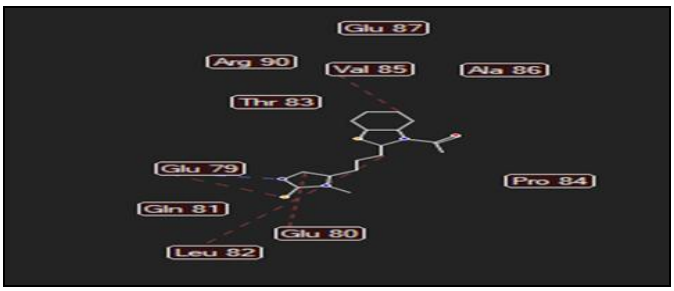

**LK 18**



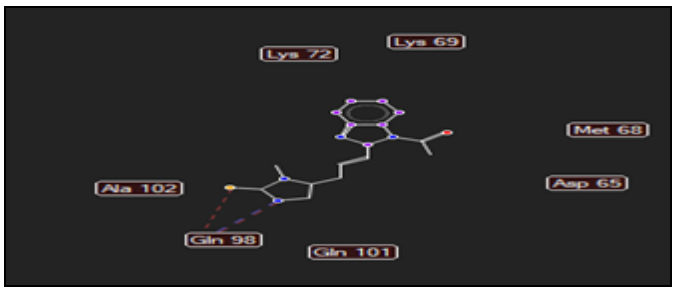

**LK 30**



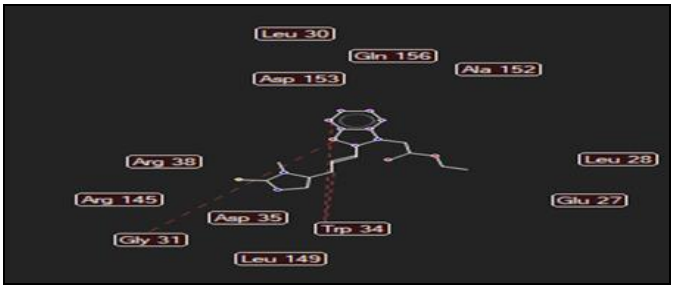

**LK 38**



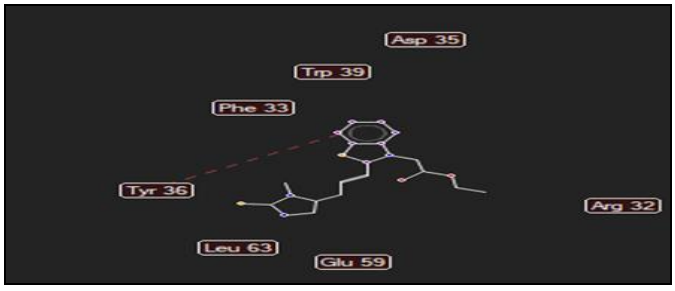

**LK 39**




**LK 40**




**LK 42**




**LK 43**


**LK 44** **LK 50**

**FIG. 3: DOCKING SNAPSHOTS OF 10 ACTIVE DESIGNED LIGANDS**

Thus, all the newly designed ligands have satisfied all the above filtering methods of good predictive activity with good docking scores and also drug-likeness properties confirming that these molecules are accepted to be orally bioavailable, and ligand containing substituted oxazole heterocyclic was selected for synthesis based on their synthetic feasibility.

**Synthesis:** Based on the synthetic feasibility, active lead compound LK 50 is chosen from the designed ligands, and its analogs are further synthesized with the described procedure.

**Procedure:**

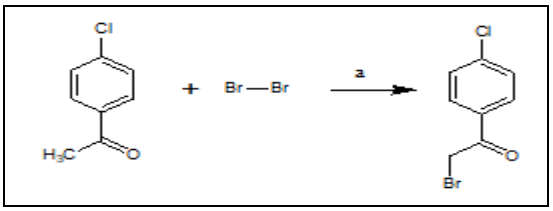
**Step 1:** To 0.01 mol of p-chloroacetophenone in 30 ml of glacial acetic acid in a 500ml flask. Add very slowly, about 30 minutes (3ml, 0.01mol) of bromine from a dropping funnel, shake the mixture vigorously during the addition and keep the temperature below 20 ºC. When the addition is complete, cool the mixture in ice-water, filter the crude product at the pump and wash it with 50% alcohol until colorless (about 50ml). Recrystalle using ethanol and the yield of pure p-chlorophenacyl bromide (M.P 96 °C) is 72%. The purity of the sample was tested by TLC using the solvent system hexane and ethyl acetate 6:4.[13]

**Step 2:** A mixture of the above obtained p-chlorophenacyl bromide (0.01 mol) and urea (0.01 mol) was dissolved in ethanol and refluxed for 8 hours. After completion of the reaction, the reaction mixture was cooled, poured into ice, and 10% NaOH solution was added. The precipitated product was filtered and dried to yield the product. Recrystallization was carried out using ethanol, and the yield of 2 amino 4-aryl oxazole was 75%. The purity of the sample was tested by TLC using the solvent system petroleum ether and ethyl acetate 8:2. [14]

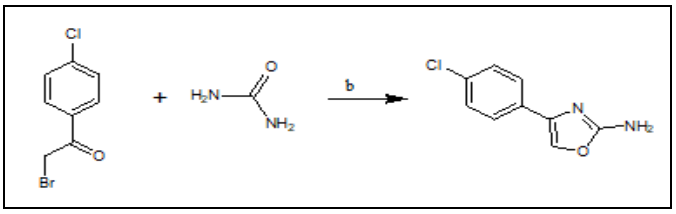
**Step 3:** The above-obtained compound 2 amino 4-(p-chlorophenyl oxazole) was dissolved in 95% ethanol and treated with different substituted aromatic aldehydes (0.01 mol). The mixture containing aldehydes was refluxed on a water bath for 3-4 hrs after the addition of 3-4 drops of glacial acetic acid. The hot mixture was poured into ice-cold water after recovery of alcohol, during which crystallization Schiff bases was obtained. The crude product was recrystallized using ethanol. The purity of the product was established by TLC solvent system used was ethylacetate: ethanol: chloroform (4:3:3). The percentage of yield was found to be 70-80%. (m.p 66° - 70 °C).[15]

**Synthetic Scheme:**

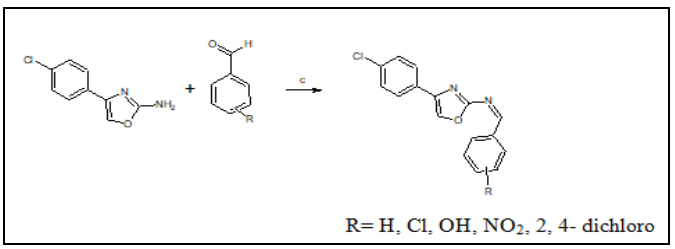
**Step 1:**




**Step 2:**




**Step 3:**



**Reagents and Conditions:**

- **a.** 50% EtOH Reflux 1hr, below 20°
- **b.** NaoH 10%, reflux 6-8 hrs RT
- **c.** Glacial $CH_3$ COOH, EtOH, reflux 3-4 hrs, RT

**Characterization:** The physical characteristic of the synthesized compounds are calculated, melting point and appearance of the lead compounds are tabulated below.

**TABLE 4: PHYSICAL CHARACTERISTICS OF LEAD COMPOUNDS**

| S. no. | Sample Code | Appearance | Melting Point (°c) | Molecular Weight | Percentage Yield% |
|---|---|---|---|---|---|
| 1 | LS 1 | Straw Yellow Crystals | 72 | 282.73 | 72 |
| 2 | LS 2 | Straw Yellow Crystals | 68 | 317.18 | 75 |
| 3 | LS 3 | Straw Yellow Crystals | 60 | 298.73 | 73 |
| 4 | LS 4 | Straw Yellow Crystals | 66 | 327.73 | 77 |
| 5 | LS 5 | Straw Yellow Crystals | 68 | 351.62 | 71 |

**Sample code: LS 1:** Yield: 72%; Melting point: 72ºC. Molecular formula: $C_{16}H_{11}ClN_2O$, IR (KBr, $cm^{-1}$). 671.18-C-Cl str, 1650.96-N=CH str, NMR (DMSO d6 δ ppm) 2.5-Singlet, 6.5-7.9-Multiplet, 8.0-8.2-Multiplet; Mass = 283.90 m/z

**Sample code: LS 2:** Yield: 68%; Melting point: 75ºC. Molecular formula: $C_{16}H_{10}Cl_2$ $N_2O$, IR (KBr,$cm^{-1}$).771.47-C-Cl str, 1697.28-N=CH str, NMR (DMSO d6 δ ppm) 2.5-Singlet, 7.3-8.0-Multiplet; Mass = 318.95 m/z.

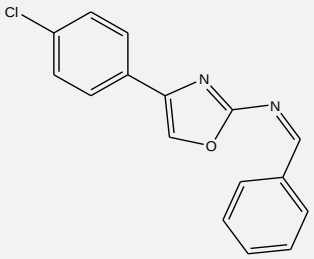
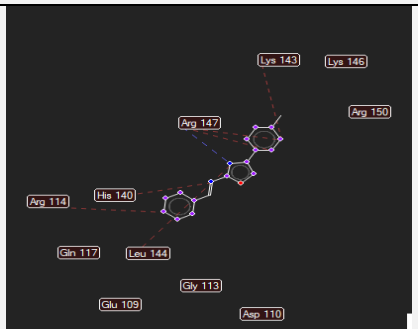
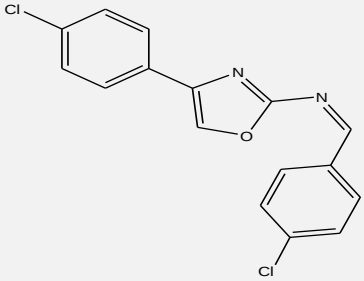
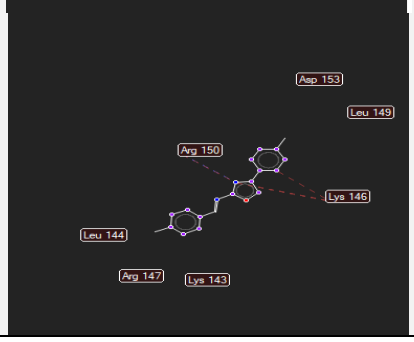
**Sample code: LS 3:** Yield: 60%; Melting point: 73ºC. Molecular formula: $C_{16}H_{11}Cl$ $N_2O_2$, IR (KBr, $cm^{-1}$). 740.61-C-Cl str, 1650.96-N=CH str, 3448.73-OH str, NMR (DMSO d6 δ ppm) 2.5-Singlet, 3.3 Singlet, 7.3-8.0-Multiplet; Mass = 294.35 m/z.

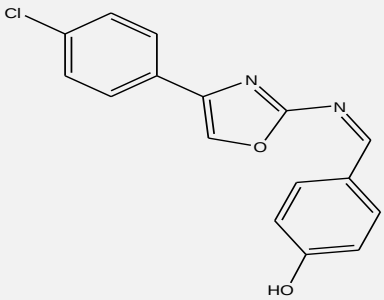
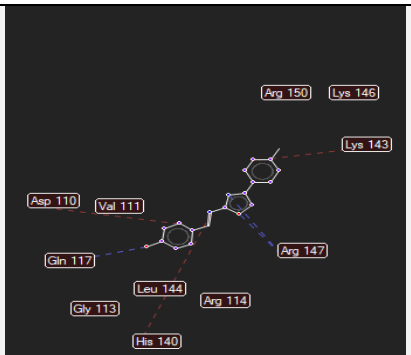
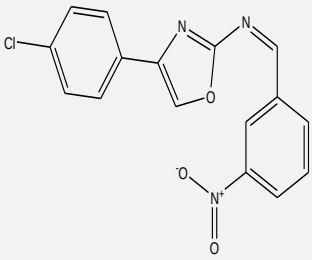
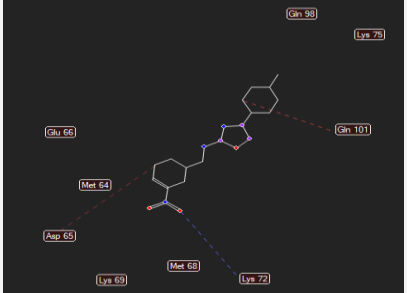
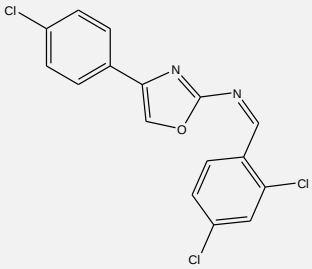
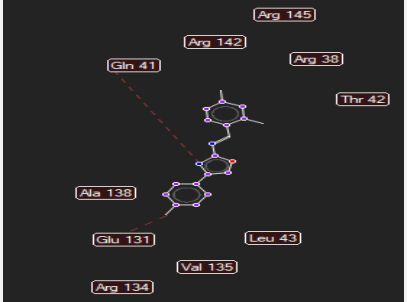
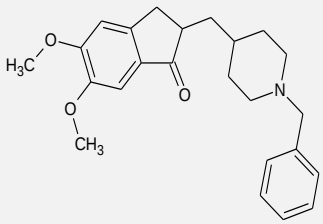
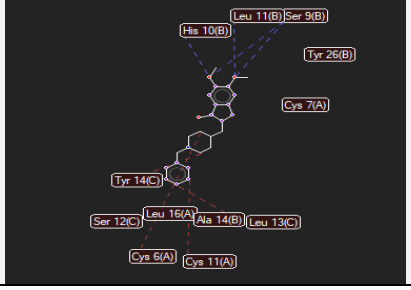
**Sample code: LS 4:** Yield: 66%; Melting point: 77ºC. Molecular formula: $C_{16}H_{10}Cl$ $N_3O_3$, IR (KBr, $cm^{-1}$). 671.18-C-Cl str, 1704.95-N=CH str, 1589.23-$NO_2$ str, NMR (DMSO d6 δ ppm) 2.2-2.5-Singlet, 7.4-8.1-Multiplet; Mass = 326.75 m/z.

**Sample code: LS 5:** Yield: 68%; Melting point: 71ºC. Molecular formula: $C_{16}H_9Cl_3N_2O$, IR (KBr, $cm^{-1}$). 771.47-C-Cl str, 1650.95-N=CH str, NMR (DMSO d6 δ ppm) 2.5-Singlet, 7.3-8.0-Multiplet; Mass = 351 m/z.

**RESULTS AND DISCUSSION OF MOLECULAR DOCKING:** The synthesis compounds are also docked against the enzyme 1GS9 using AUTODOCK TOOLS 1.5.6 software.

**TABLE 5: DOCKING SCORE AND INTERACTION OF LEAD COMPOUNDS WITH STANDARD**

| Sample Code | Docking Score Kcal/mol | Structure | Interaction with aminoacid |
|---|---|---|---|
| LS 1 | -6.74 |  |  |
| LS 2 | -6.96 |  |  |

| | | | |
|---|---|---|---|
| LS 3 | -6.77 |  |  |
| LS 4 | -7.08 |  |  |
| LS 5 | -6.23 |  |  |
| Standard (Donepezil) (PDB ID : 4EY6 ) | -7.3 |  |  |

All the synthesized compounds were screened for *in-silico* toxicity prediction studies using OSIRIS property explorer, and none of the compounds found to be toxic. Docking results of all the synthesized compounds were analyzed and compared with the standard drug Donezepil (-7.3 kcal/mol). Compound LS 4 (-7.08 kcal/mol) was found to nearly effectively inhibit ApoE4 genotype as that of standard Donezepil, a potent drug used for the treatment of AD. Hence the synthesized compound LS-4 was selected further for screening its *in-vitro* evaluation study by Cytotoxicity study through MTT assay and *in-vitro* cell line studies for neuroprotective effect.

**Evaluation Studies:**

**A. Cytotoxicity Study through MTT Assay:** SH-SY5Y Cell line was used to determine the cell Cytotoxicity activity. The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100µg/ml) in a 5% $CO_2$ at 37 °C. Cells were seeded at 5000 cells/ well in 96-well plates, and both were incubated for 48h.Various concentrations of the sample for 24 h incubation. After the medium is removed, it was washed with the phosphate saline solution. Then the sample was placed in a new medium containing 50µL of MTT solution (5mg/ml), to each well incubated for 4h.

After the incubation, DMSO was added. The experiments were performed in triplicate, and the viability of the cell was expressed as percentages of survival relative to the control sample. The viable cells were determined by the absorbance at 570nm by microplate reader [16].

**Data Interpretation:** % Cytotoxicity of the compound is obtained.

**B. *In-vitro* Cell Line Studies for Neuroprotective Effect**.

**Aβ42-L-DOPA Induced SH-SY5Y Cell Toxicity Study:** In order to determine the toxicity of Aβ42-L-DOPA combination in the SH-SY5Y cells, freshly prepared L-DOPA in various concentrations (0–2000 µM) was incubated with (0–40 µM) Aβ42 fibrils in the sterile, clear 96-well plates containing SH-SY5Y cells ($5\times10^3$ cells/well) followed by incubation over 24 h at 37 °C under 5% $CO_2$ 95% humidified air. Different concentration of freshly prepared LS 4 (10–1000 µM/µg) was incubated with SH-SY5Y cells (5×103 cells/well) for 24 h at 37 °C under 5% $CO_2$, 95% humidified air in an incubator. To access the neuroprotective effects of newly designed Apo E4 inhibitor against Aβ42-L-DOPA-induced toxicity, the pre-treated SH-SY5Y cells were exposed to 20 µM of Aβ42 fibrils and 200 µM of L-DOPA followed by further incubation for 24 h [17].

**RESULTS AND DISCUSSION OF *IN-VITRO* STUDIES:** The percentage of cell viability was calculated for the synthesized compound LS 4, which was treated with SH-5YSY cell line from various concentrations ranging from ( 1.567 – 250 µg/ml). Results are tabulated below.

**TABLE 6: PERCENTAGE VIABILITY OF LS 4 ALONE IN SHSY - 5Y CELL LINE**

| Concentaration (µg/ml) LK-4 SHSY -5Y Cell line | OD 1 | OD 2 | OD 3 | % of Cell death | | | Mean | SD | SEM | %Live Cell |
|---|---|---|---|---|---|---|---|---|---|---|
| 250 | 0.255 | 0.267 | 0.266 | 61.01 | 59.17 | 59.33 | 59.84 | 1.02 | 0.59 | 40.16 |
| 100 | 0.345 | 0.356 | 0.321 | 47.25 | 45.57 | 50.92 | 47.91 | 2.74 | 1.58 | 52.09 |
| 50 | 0.389 | 0.39 | 0.412 | 40.53 | 40.37 | 30.00 | 39.30 | 1.99 | 1.15 | 60.70 |
| 25 | 0.465 | 0.456 | 0.467 | 28.90 | 30.28 | 28.59 | 29.26 | 0.90 | 0.52 | 70.74 |
| 12.5 | 0.521 | 0.532 | 0.521 | 20.34 | 18.65 | 20.34 | 19.78 | 0.97 | 0.56 | 80.22 |
| 6.25 | 0.588 | 0.59 | 0.588 | 10.09 | 9.79 | 10.09 | 9.99 | 0.18 | 0.10 | 90.01 |
| 3.12 | 0.612 | 0.622 | 0.614 | 6.42 | 4.89 | 6.12 | 5.81 | 0.81 | 0.47 | 94.19 |
| 1.567 | 0.654 | 0.622 | 0.644 | 0.00 | 4.89 | 1.53 | 2.14 | 2.50 | 1.45 | 97.86 |
| Control | 0.675 | 0.655 | 0.632 | | | | 0.654 | 0.022 | 0.012 | |

At a higher concentration 250µg/ml concentration, compound LS 4 showed 40.16% cell viability. At a lower concentration of 1.567, the compound showed 97.86% cell viability.

The percentage viability of LS 4 -SH-5YSY at 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.12 µg/ml are 52.09%, 60.70%, 70.74%, 80.22%, 90.01%, 94.19% respectively. The synthesized compound LS 4 has found to exhibit maximum cell viability at lower concentrations than the higher concentration.

The neuroprotective effect against $A\beta_{42}$ - L-DOPA-induced toxicity at different concentrations of pretreated LS4 into SH-SY5Y cell line was assessed. Results showed compound LS 4 showed strongest neuroprotective potential against $A\beta_{42}$ - L-Dopa induced toxicity; results are tabulated.

**TABLE 7: NEURO PROTECTIVE EFFECT OF LS 4 AGAINST L-DOPA – $AB_{42}$ INDUCED NEUROTOXICITY IN SH-5YSY CELLS**

| Induced toxicity | Concentration (µg/ml) LK-4 SHSY -5Y Cell line | OD 1 | OD 2 | OD 3 | % of Cell death | | | Mean | SD | SEM | %Live Cell |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Aβ42 20µM+L-DOPA 200µM | 250 | 0.388 | 0.39 | 0.388 | 56.31 | 56.08 | 56.31 | 56.23 | 0.13 | 0.08 | 43.77 |
| Aβ42 20µM+L-DOPA 200µM | 100 | 0.424 | 0.432 | 0.455 | 52.25 | 51.35 | 48.76 | 50.79 | 1.81 | 1.05 | 49.21 |
| Aβ42 20µM+L- | 50 | 0.489 | 0.478 | 0.488 | 44.93 | 46.17 | 45.05 | 45.38 | 0.68 | 0.40 | 54.62 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DOPA 200µM Aβ42 20µM+L-DOPA 200µM | 25 | 0.609 | 0.612 | 0.622 | 31.42 | 31.08 | 29.95 | 30.82 | 0.77 | 0.44 | 69.18 |
| Aβ42 20µM+L-DOPA 200µM | 12.5 | 0.69 | 0.699 | 0.687 | 22.30 | 21.28 | 22.64 | 22.07 | 0.70 | 0.41 | 77.93 |
| Aβ42 20µM+L-DOPA 200µM | 6.25 | 0.699 | 0.701 | 0.722 | 21.28 | 21.06 | 18.69 | 20.35 | 1.43 | 0.83 | 79.65 |
| Aβ42 20µM+L-DOPA 200µM | 3.12 | 0.782 | 0.789 | 0.788 | 11.94 | 11.15 | 11.26 | 11.45 | 0.43 | 0.25 | 88.55 |
| Aβ42 20µM+L-DOPA 200µM | 1.567 | 0.823 | 0.843 | 0.833 | 7.32 | 5.07 | 6.19 | 6.19 | 1.13 | 0.65 | 93.81 |
| Control | | 0.897 | 0.878 | 0.890 | | | | 0.888 | 0.010 | 0.006 | |

After SH-SY5Y cells were exposed to L-DOPA (0–200 µM) and Aβ42 (0–20 µM) for 24 hrs caused, which is almost two folds higher than the toxicity produced by individual Aβ42, thus suggesting the synergistic action of toxicity. Pre-treatment of newly designed synthetic ApoE4 inhibitor,

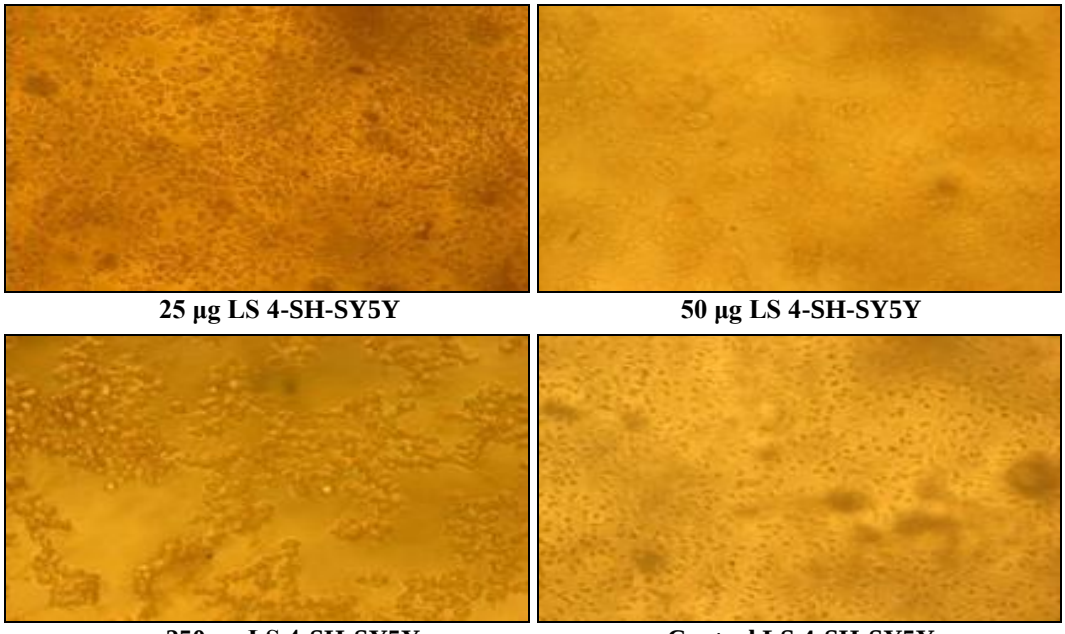
From the results, LS 4-SH-5YSY Cells at higher concentration 250 µg/m concentrations, compound LS 4 showed 43.77% cell viability. At a lower concentration 1.567µg/ml, the compound showed 93.81% cell viability against Aβ42- L-DOPA-induced toxicity.

The percentage viability of LS 4 -SH-5YSY against at 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.12 µg/ml are 49.21%, 54.62%, 69.18%, 77.93%, 79.65%, 88.55% respectively. Results showed compound LS 4 has maximum cell viability at lower concentrations than the higher concentration. The percentage viability of Aβ42- L-DOPA-induced toxicity in SH-SY5Ycells is increased with Aβ42-L-DOPA-induced toxicity in LS4-SH-SY5Y cells. Newly designed and synthesized Ligand LS 4 Apo E4 inhibitor showed the strongest protection against Aβ42- L-DOPA-induced toxicity in SH-SY5Ycells.

**TABLE 8: NEUROPROTECTIVE STUDY OF LS4 AGAINST L-DOPA-INDUCED TOXICITY IN SH-SY5Y CELLS AT 100 µg/ml**

| Treatment | % Viability |
|---|---|
| LS-4 alone (at 100µg) | 52.05 |
| Aβ42(20µM)+L-DOPA (200µM) | 42.45 |
| Aβ42(20µM)+L-DOPA (200µM)+ LS-4 (at 100µg) | 49.21 |

After the pretreatment of LS-4 with SH-5YSY cell line, the disruption of amyloid fibrils is viewed through a microscope, and their images at various concentrations are pasted below.



**25 µg LS 4-SH-SY5Y** **50 µg LS 4-SH-SY5Y**

**250 µg LS 4-SH-SY5Y** **Control LS 4-SH-SY5Y**

**FIG. 4: DISRUPTION OF AMYLOID FIBRILS AT VARIOUS DOSES ONLS 4-SH-SY5Y CELL LINE**

**CONCLUSION:** Inhibition of amyloid formation and disruption of the formed fibrillar assemblies are still one of the major therapeutic strategies proposed for the prevention and treatment of AD.

The newly designed and synthesized compound LS4 being effective in neuroprotective effect, also suggest that its multiple mechanisms is anti-amyloidogenic including Apo E4 inhibition. Taking all these findings together, we propose that the synthesized compound LS 4 (4-(4 chlorophenyl)-N-[(Z)-3-nitrophenyl methylindene]1,3 oxazole 2-amine) as a therapeutic candidate for the treatment Alzheimer's disease, which can also be subjected further for throughput screening involving animal studies in future.

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