DESIGN, SYNTHESIS, AND IN VITRO EVALUATION OF NOVEL ACRIDINE DERIVATIVES AS MONOAMINE OXIDASE INHIBITORS

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ABSTRACT
Alzheimer's disease (AD) is a complex neurodegenerative ailment, which demands the use of multifunctional agents for its therapy. Currently, FDA-approved drugs for AD are only palliative. The current research focused on adding a flavone nucleus to the amino group of the acridine nucleus to provide it with Monoamine oxidase (MAO)-B inhibitory properties. Method: We designed and synthesized ten flavone substituted acridine derivatives and evaluated for in vitro MAO B inhibitory activity. Molecular modeling studies were conducted in AutoDock Vina with hMAO B (PDB ID: 2V60). Compounds exhibited MAO B inhibition with IC50 values in 3.24 µM to 9.48 µM concentration. Compounds AF6 and AF14 showed the highest activity with an IC50 value of 3.24 µM for MAO B. The results highlighted the MAO B inhibitory effect of the novel Acridine-Flavone hybrids and they can be promising multitarget directed ligands for AD.

Keywords: Alzheimer’s disease, Acridine, Docking, Flavone, Monoamine Oxidase, Neurological Disorder, Tacrine.

INTRODUCTION
The oxidative deamination of biogenic and xenobiotic amines is catalyzed by monoamine oxidase (MAO), which plays a vital part in amine breakdown in the central nervous system (CNS) and peripheral tissues. The enzyme targets a wide spectrum of neurotransmitters implicated in MAO's major substrates in the brain, including adrenaline, noradrenaline, dopamine, and serotonin.1,2 MAO's unique role in mood disorders3, anxiety, and depression by influencing the action of a wide range of individual neurotransmitters schizophrenia4-6, attention deficit hyperactivity disorder7-9, migraine10, sexual maturation11 and neurodegenerative disorders12 have all received considerable attention. AD is a devastating neurological illness that now affects more than 35 million people worldwide, with the number expected to climb to 66 million by 2030.13,14 AD has an etiology that is unknown due to the disease's complex pathophysiology. The pharmaceutical drugs used in the clinical research stages are only palliative. As the proportion of glial cells to neurons increases in the aging brain, MAO B levels rise. MAO B inhibition should be considered in multitarget therapy to treat Alzheimer's disease because of the higher levels of MAO B as well as other monoaminergic abnormalities in Alzheimer's brain.15 Although MAO B inhibition has been combined with AChE inhibition, no monoaminergic system-targeting medication is currently in clinical use. The ChE inhibitors accomplished cognitive functions, but lack disease-modifying effects.16 The pathological hallmarks of AD are the accumulation of intracellular neurofibrillary tangles (NFTs) and extracellular senile plaques containing amyloid-Aβ proteins, which induce neuronal death and cerebral shrinkage. Even in the early stages of Alzheimer's disease, the presence of an "inflammatory" cascade in the brain can be observed. This cascade causes oxidative stress by triggering inflammatory responses such as reactive oxygen species (ROS) and cytokines.17 MAO, a mitochondrial enzyme restricted to the outer membrane18, is essential for the metabolism of monoamine neurotransmitters and other amines.19 Oxidative deamination catalyzed by MAO produces hydrogen peroxide, an oxidative stress negotiator. MAO-A and MAO-B are two types of MAO. MAO-B, the most common isoform found in the brain20, deactivates dopamine, trace amines such as 2-phenylethylamine, and possibly other neuro-modulatory amines including polyamines.21 In AD brains,
MAO-B expression is higher in the hippocampus and cerebral cortex than in healthy brains. Overexpression of MAO-B increases monoamine metabolism and increases the production of free radicals and hydrogen peroxide (H2O2), perhaps speeding up the neurodegenerative mechanisms in AD. Based on their neuroprotective characteristics, MAO inhibitors have been proposed as prospective therapeutics for Alzheimer's disease and other neurodegenerative illnesses. MAO inhibitors limit the development of neurotoxic compounds that cause ROS to occur. Activated MAO in the brains of patients with AD is a biomarker for AD. AD is a complex disease that involves multiple biological systems. A single-protein-targeted medication will not relieve the condition. Single-target medicines for Alzheimer's disease are ineffective and hence multitarget-directed ligand methods for the disease are being developed. As a result, multifunctional compounds with two or more complementary biological functions could be a significant step forward in the disease's treatment. The FDA-approved drugs for AD are Tacrine, Donepezil, Rivastigmine, Galantamine, and Memantine. The battle is currently ongoing because of the lack of therapeutically viable drugs. The current research focused on incorporating a flavone nucleus into the acridine nucleus. 9-Amino acridine derivative (Tacrine) is a proven AChE inhibitor. The flavone nucleus is having antioxidant and hepatoprotective properties. Some of the flavones have an effect on MAO B inhibition. The purpose of the study is to incorporate a flavone nucleus into the acridine nucleus to make it an AChE and MAO B inhibitor and thus get a better multifunctional antialzheimers lead molecule.

EXPERIMENTAL

Insilico Design
The flavone-substituted acridine derivatives were designed by adding various substituted flavone moieties to the 9-Amino acridine nucleus via the Mannich reaction. Figure-1 depicts the compound's general structure. With the help of commercially accessible and freely available software, Insilico experiments on 20 new compounds were successfully completed. ACD Lab Chemsketch was used to draw the structure of the designed compounds. Software such as Swiss ADME was used to calculate the physicochemical properties of the newly developed compounds. Pre ADMET was used to calculate the permeability of the designed chemicals across the BBB. ProTox II was used to predict the toxicity of the developed compounds.

![Fig.-1: General structure of Acridine- Flavone hybrids](image)

Molecular Docking
Docking studies were carried out for all the 20 newly designed substituted 9-Amino acridine derivatives on enzymes MAO B using AutoDock Vina version 1.1.2 with hMAO B (PDB ID: 2V60).

Synthesis and Characterization
The chemicals used in this study were obtained from Sigma Aldrich. Purification of the produced products was accomplished through recrystallization using suitable solvents. TLC plates in suitable solvents are used to verify the reaction completion. The melting point, vibrational spectra (IR), NMR spectra, and mass spectra of the compounds generated were all measured. The melting points were calculated using a Remi apparatus with an open capillary tube at one end and were given as-is. IR spectra of the generated analogs were recorded using KBr pellets in the range of 4000-500 cm\(^{-1}\) on a PerkinElmer FTIR. \(^1\)H and \(^13\)C NMR spectra were acquired at 300 and 75 MHz, respectively, on a Jeol AL300 FT-NMR spectrometer. Tetramethylsilane was employed as an internal reference for NMR studies in DMSO. On a TOF MS ES, mass spectra of the produced compounds were obtained using the FAB+ ionization mode.
Step: 1 Synthesis of Chalcone derivatives
3-equivalents of potassium hydroxide dissolved in ethanol were used to dissolve 2-hydroxy acetophenone (1 equivalent) and substituted benzaldehyde derivative (1.2 equivalent). At room temperature the reaction mixture was stirred for 6-12 hours, or until TLC indicated that the reaction was complete. The mixture was poured into crushed ice and acidified with dilute HCl when the reaction was completed. To obtain crystalline chalcone, the material was recrystallized from ethanol. 37,38,39

Step: 2 Synthesis of Flavone derivatives
Iodine (0.02 mmol) was added after the substituted chalcone (0.01mol) was dissolved in DMSO (2 ml). In a microwave oven, it is irradiated at level 5 for 2 minutes. Diethyl ether was used to dilute the mixture. The organic layer was washed with aqueous sodium thiosulphate solution (20%) and dried over anhydrous sodium sulphate. The reaction was monitored using hexane and ethyl acetate in a TLC plate for completion of the reaction. Using hexane, ethyl acetate, and silica gel, the crude solid was submitted to column chromatography (80:20). Ethyl alcohol was used to recrystallize the solid flavones. 38,39

Step: 3 Synthesis of Mannich Bases
In 10 mL of glacial acetic acid, a ketone molecule, paraformaldehyde, and appropriate amine were heated at 120°C at 70 Watt in 1:1.2:1 mol ratios. TLC was used to monitor the reactions, which used a CHCl3: MeOH (9:1 or 8:2) solvent solution. Each reaction took a different amount of time to complete, as shown in Table 1. The solid was filtered, dried, and recrystallized using a suitable solvent after reaction completion. 40, 41,42

Pharmacological Screening

Inhibitory activity against MAO B
Commercial supplier Sigma-Aldrich provided the monoamine oxidases (hMAO-A, hMAO-B, E.C. 1.4.3.4), p-tyramine, Amplex Red, and horseradish peroxidase (E.C. 1.11.1.7). The chemicals were dissolved in DMSO (10 µM) and diluted to the appropriate final concentration in 0.05 µM phosphate buffer (pH-7.4). At the concentrations used, all of the chemicals are soluble. In a flat black bottom 96-well micro test plate in the dark, test medicines (20 µL) and MAO (80 µL) were incubated for 15 minutes at 37 °C. At 37 °C for 20 minutes, 200 µM Amplex Red reagent, 2 U/µL horseradish peroxidase, and 2µM p-tyramine for hMAO were added. Based on the fluorescence created, the synthesis of H2O2 and, as a result, resorufin, was measured at 37 °C in a Jasco FP 8550 spectrofluorometer based on the fluorescence generated (excitation, 545 nm; emission, 590 nm). By subtracting the background activity, the specific fluorescence emission was determined. The background activity was calculated using wells that contained all of the components except the MAO isoforms, which were substituted with a sodium phosphate buffer solution (0.05 µM, pH 7.4). The % inhibition was computed using the formula: (1-IFi/IFc) x 100, where IFi and IFc are the Fluorescence intensities for hMAO in the presence and absence of inhibitors, respectively, after subtracting the respective background. The study used Tacrine as a reference. Selegiline was used as a standard in the study. 43-45

RESULTS AND DISCUSSION

Insilico Studies and Molecular Modeling
Insilico analyses revealed that the compounds follow the Lipinski rule of five and had a topological polar surface area of less than 90 Å², indicating that they have good blood-brain barrier permeability. Predicting BBB penetration entails determining whether or not substances will flow through the blood-brain barrier. This is critical in the pharmaceutical industry to avoid CNS toxicity and increase CNS activity. In PreADMET experiments, nearly all of the newly developed drugs showed BBB penetration above 0.1. This suggests that the newly developed chemicals will be able to cross BBB and function on the CNS. ProTox II was used to predict the toxicity of the developed compounds. ProTox II calculates LD50 values and several toxicities that are detrimental, such as hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. The compounds had a very good dock score with hMAOB, according to the molecular docking experiments in AutoDockVina (PDB ID: 2V60). Figure-2 depicts the binding interaction of the most active compound. The binding interactions revealed that the chemical binds to the amino acids THR 478 by hydrogen bonding HIS 115 and TRP 119 via –alkyl contact with PRO 102.
Synthesis and Characterization
The general structure of the newly synthesized compound is as shown in Fig.-1. The ‘R’ substituents and the physical characteristics of the newly synthesized compounds are given in (Table-1).

Table-1 Physical and chemical characteristics of Acridine-Flavone Hybrids

| Sample Code | Substituents (R) | Molecular Formula | Molecular weight | Colour             | Yield (%) | Rf     | MP (°C) |
|-------------|------------------|-------------------|------------------|--------------------|-----------|--------|---------|
| AF2         | -C                | C_{29}H_{26}N_{2}O_{2} | 432.51           | Brownish yellow    | 72        | 0.64   | 155     |
| AF4         | -Cl -N            | C_{29}H_{25}C_{l}N_{2}O_{2} | 466.95           | Orange             | 70        | 0.65   | 168     |
| AF6         | -Cl -N            | C_{29}H_{25}C_{l}N_{2}O_{2} | 466.95           | Yellowish red      | 70        | 0.66   | 169     |
| AF8         | -C                | C_{29}H_{25}C_{l}N_{2}O_{2} | 466.95           | Bright Red         | 65        | 0.66   | 167     |
| AF10        | -C                | C_{29}H_{23}N_{3}O_{4} | 477.51           | Yellowish brown    | 78        | 0.72   | 164     |
| AF12        | -C                | C_{30}H_{26}N_{2}O_{4} | 478.53           | Greyish white      | 70        | 0.68   | 165     |
| AF14        | -C                | C_{29}H_{23}N_{3}O_{4} | 477.51           | Yellowish white    | 74        | 0.70   | 166     |
| AF16        | -C                | C_{30}H_{26}N_{2}O_{3} | 462.53           | Light Brown        | 78        | 0.69   | 167     |
| AF18        | -C                | C_{30}H_{26}N_{2}O_{3} | 462.53           | Brown             | 76        | 0.67   | 168     |
| AF20        | -C                | C_{30}H_{26}N_{2}O_{4} | 478.53           | Grey               | 68        | 0.68   | 169     |

Spectral Characterization
2-(2-chlorophenyl) 7-hydroxy-8-((acridin-9-yl amino) methyl)-4H-chromen-4-one (AF4)
IR peaks 3429.62 (Ar- secondary amine NH-Stretching), 3176.31 (OH stretching) 2954.66 (Ar-C-H stretching), 2917.08 (C-H) 2866.19 (Ali C-H), 1738.51 (C-C stretching), 1655.37 (Conjugated ketone.) 1643.21 (C=N) 1592.40 (Ar C=N), 1491.93 (Ar C=C stretching), 1372.59 (C-N stretching tertiary amine) 1033.77 (vibration of C-O in alcohol hydroxyl group) 755.35 (C-Cl) $^1$HNMR δ= 13.748, 8.739, 8.717, 8.233, 8.225, 8.203, 8.198, 8.188, 8.173, 8.150, 8.120, 8.081, 8.060, 8.044, 8.040, 8.023, 8.023, 8.001, 7.999, 7.985, 7.968, 7.965, 7.947, 7.923, 7.885, 7.862, 7.792, 7.744, 7.731, 7.723, 7.677, 7.673, 7.655, 7.650, 7.591,
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In-vitro Inhibitory Activity Against MAO B

The invitro MAO B activity of the newly designed compounds was conducted against MAO B. All the compounds showed very good IC50 values in µM concentration. The results are shown in (Table-2) Graphical representation of activity shown in Fig.-3.
Table-2 *In vitro* MAO B inhibitory activity

| Sample Code | MAO B inhibitory activity (µM) |
|-------------|---------------------------------|
| AF2         | 4.70 ± 0.32                     |
| AF4         | 4.20 ± 0.45                     |
| AF6         | 3.24 ± 0.35                     |
| AF8         | 5.27 ± 0.12                     |
| AF10        | 4.54 ± 0.25                     |
| AF12        | 5.42 ± 0.32                     |
| AF14        | 3.24 ± 0.25                     |
| AF16        | 6.72 ± 0.32                     |
| AF18        | 9.48 ± 0.25                     |
| AF20        | 6.27 ± 0.35                     |
| Tacrine     | 7.85 ± 0.12                     |
| Selegiline  | 0.037 ± 0.01                    |

**MAO B INHIBITION**

![Graphical representation of *in vitro* MAO-B inhibitory activity plotted in GraphPad Prism](image)

Fig.-3 Graphical representation of *in vitro* MAO-B inhibitory activity plotted in GraphPad Prism

**CONCLUSION**

Acridine-Flavone hybrids were designed as inhibitors of MAO B and the compounds were found to show good MAO B Inhibition in micromolar concentration. Among the compounds compound AF6 and AF14 which are 3-chloro and 3-nitro substituted benzaldehyde flavone hybrid of 9-amino acridine shows the highest MAO B inhibition. Substitution at the 3\textsuperscript{rd} position with an electron donating or electron withdrawing functional group increases the MAO B inhibitory activity. Molecular modeling studies and *In silico* studies on the novel derivatives also proved the compounds to be good candidates for further study and can be good lead compounds for anti-Alzheimer’s research.

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