International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.1, pp 125-131, 2014-2015

Design and *Insilico* molecular prediction of flavone-fused-thiazole analogues as Acetyl Cholinesterase and β-Secretase inhibitor in the treatment of Alzheimer's disease

Pavadai Parasuraman*, Ramalingam Suresh

Department of Pharmacy, FEAT, Annamalai University, Annamalai Nagar, Chidambaram,Tamil Nadu-608002, India

Abstract: The objective of this study is to evaluate the Acetyl Cholinesterase and β-Secretase inhibitory activity of designed flavone-fused-thiazole analogues using *insilico* docking studies. *Insilico* docking studies were carried out using Schrodinger software based on GLIDE (Grid-based Ligand Docking with Energetics). Acetyl Cholinesterase inhibitors, including Donepezil, Tacrine, Galantamine and Rivastigamine are being used currently in the treatment of Alzheimer's disease and used as standards in the present study. The docking scores of flavone-fused-thiazole analogues were varied between -0.16 and -7.5 against Acetyl Cholinesterase and -1.6 and -9.3 for β-Secretase. Test compound **(PS206)** 1-(2-oxobutyl)-3-(5-(3, 6, 7-trihydroxy-5, 8-dimethoxy-4-oxo-4H-chromen-2-yl) benzo[d] thiazol-2-yl) urea was found to be more significant when compared with the standard docking score. These molecular docking analyses could lead to the further development of potent inhibitors for the treatment of Alzheimer's disease. Further investigations on the above compounds are necessary to develop potential chemical entities for the prevention and treatment of Alzheimer's disease.

Key Words: *Insilico* drug design, Acetyl Cholinesterase, β-Secretase, Alzheimers disease, Flavone-fused-thiazole, MTDL.

Introduction

Alzheimer's disease (AD), the most widespread form of dementia, is a complex neurodegenerative disorder[1,2]. According to Alzheimer's disease International, about 44.4 million peoples with dementia were estimated worldwide in 2013. This statistic will increase to a level of 75.6 million in 2030 and 135.5 million in 2050. By now, 62% of the populace with dementia survives in developing countries and expected to reach 71% by 2050. The fastest augmentation in the aged populace is taking place in China, India and their south Asian and western Pacific neighbors.

The molecular etiology of Alzheimer's is not fully understood, there exist several hypotheses surrounding its causes. The oldest is the cholinergic hypothesis, which centers on the underproduction of acetylcholine as the causative agent. It has also been postulated that it may cause what are known as plaques and tangles. These are formations that are consistently found in the brains of Alzheimer's patients upon autopsy. These tangles, specifically intraneuronal fibrillary tangles, are the results of aggregations of misfolded tau protein inside nerve cell bodies, and under the Tau hypothesis, this would be the earliest influence on the pathology of Alzheimer's. The plaques, however, consist of misfolded amyloidal beta peptide fragments, which aggregate between neurons, and under the amyloid hypotheses, it is the deposition of these peptides which is the initial influence. Although this is the dominant hypothesis, of course there are others, as well as variations and combinations of these.

Five FDA approved Alzheimer's treatments fall into two categories, based on their mechanisms of action. Considering that the cholinesterase hypothesis is the oldest etiological hypothesis; it makes sense that the oldest of these, and the majority, are targeted to this characteristic of Alzheimer's. Donepezil, Tacrine, Galantamine and Rivastigamine acts as cholinesterase inhibitors, while the fifth, memantine, is the only n-methyl-d-aspartate receptor antagonist.

Experimental evidence points to the involvement of several targets and pathways in the AD pathogenesis, but all drugs developed to date are monofunctional, hitting only a single target among the many involved. Therefore, these drugs are inherently insufficient for the treatment of complex diseases like AD, which have multiple pathogenic factors. It is critical to recognize; however, that combining two or more drugs always raise the potential for greater side effects[3]. Another therapeutic option is now emerging, based on the paradigm that a rationally designed, single molecule may possess multiple concomitant biological properties. Clearly, therapy with such a single multimodal drug would have inherent advantages over combination therapy [4]. Such therapy would prevent the challenge of administering multiple single-drug entities that could have distinctive bioavailability, pharmacokinetics, and metabolism. Furthermore, in terms of pharmacokinetic and toxicological optimization, the clinical development of a drug able to hit multiple targets should not, in principle, be different from the development of any other single lead molecule. This therapy thus offers a far simpler approach than combination therapy. In addition, the risk of possible drug-drug interactions would be reduced and the therapeutic regimen greatly simplified, with the prospect of enhanced patient compliance, which is a critical issue in AD care[5].

The fact that complexity and manifold etiologies of AD make single-target strategy difficult to shed desirable therapeutic effect which makes the choice of Multi-Target-Directed Ligand (MTDL) to be a potentially more effective strategy[6]. MTDL goal is to enhance remedial efficacy, is rationally designed to hit several targets for a particular disease to improve pharmacological profiles[7-9].

Benzothiazole derivative KHG21834 is neuro protective against the Aβ -induced degeneration of neuronal cells *invitro* and *invivo* studies[10]. Natural flavonoids are well known anti-oxidants. In addition, various studies have reported the defensive effects of natural polyphenol, counting flavonols and flavones, against various insults, such as Aβ. It also acts as free radical scavengers and neuronal cell protectors to oxidative damage [11].

Based on the above facts, we focused on designing Multi-Target-Directed Ligands by incorporating two different heterocyclic nucleuses (Benzthiazole & Flavone) to inhibit both Acetyl Cholinesterase and β-Secretase and to assess its activity through *insilico* molecular docking studies.

Experimental

Drug Design Strategy:

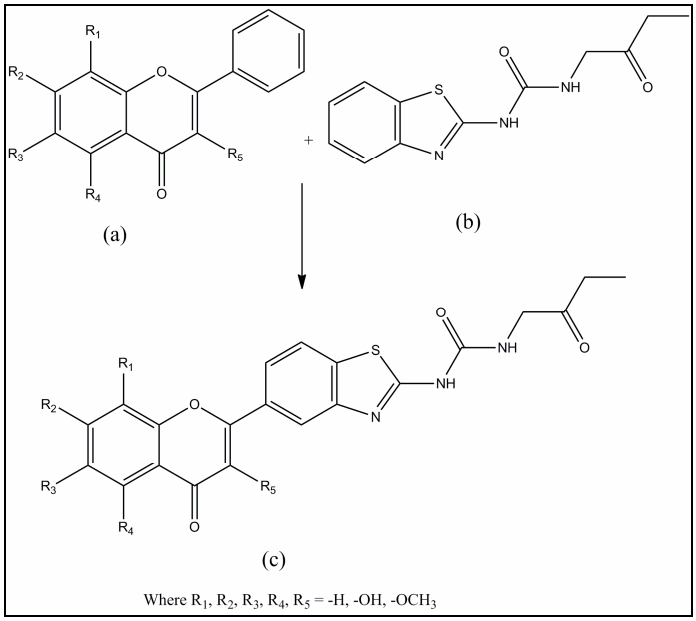


Fig 1 (a) Designed flavone, (b) Structure of KHG21834, (c) Designed MTDL for inhibiting both Acetyl Cholinesterase & β-Secretase

Natural flavones and synthetic flavones have exhibited potent activity due to the presence of hydroxy and alkoxy functional group substitution at various positions. From these criteria, we have designed ligands having three substituent's H, OH and OCH_3 at five different position 3rd, 5th, 6th, 7th and 8th respectively(**Figure 1a**) which is fused to benzothiazole nucleus KHG21834 (**Figure 1b**). By using permutation and combination, we have designed 243 molecules (**Figure1c**).

Molecular Modeling Studies:

Molecular modeling studies have been carried out using *GLIDE* (*Grid-based Ligand Docking with Energetics*) software v5.5 developed by Schrödinger running on Red Hat Enterprise Linux 5 (RHEL5) workstation. *Maestro* v9.3 Graphical User Interface (GUI) workspace was used for all the steps involved in ligand preparation, protein preparation and Extra precession docking (XP-Mode).

The X-ray crystal structures of Acetyl Cholinesterase (**PDB: 3LII**) and β-Secretase (**PDB: 3U6A**) retrieved from the RCSB Protein Data Bank. Ligand structures of 243 flavone fused thiazole analogues, and four standards were constructed using the splinter dictionary of Maestro 9.3 (Schrodinger, LLC) using the Optimized Potentials for Liquid Simulations-All Atom (OPLS-AA) force fields with the steepest descent followed by curtailed Newton conjugate gradient protocol. Partial atomic charges were computed using the OPLS-AA force field.

Every single docking calculation have been performed using the ''Extra Precision'' (XP) mode of GLIDE program. The finest docked structure was preferred using a GLIDE score function. Glide Score (G-score) takes into account a number of parameters like hydrogen bonds (H-bond), hydrophobic contacts (Lipo), van der-Waals (vdW), columbic (Coul), polar interactions in the binding site (Site), metal binding term (Metal) and penalty for buried polar group (BuryP) and freezing rotatable bonds (RotB).

G-score = H bond + Lipo + Metal + Site + 0.130 Coul + 0.065 vdW – BuryP – RotB.

An additional scoring function utilized by GLIDE is E-model, which itself consequential from a combination of the G score, Coulombic, van der Waals and the strain energy of the ligand[12].

Results and Discussion

All the designed ligands and standards were evaluated for docking studies against Acetyl Cholinesterase and β-Secretase using GLIDE software. The docking results of the ligands with the top score when compared with standard were selected and given in the **Table I** and **Table II**. The interaction energy comprises van der Waals energy, electrostatic energy, as well as intermolecular hydrogen bonding were calculated for every minimized complex. Docking score using GLIDE varied between -0.16 Kcal/mol and -7.5 Kcal/mol against Acetyl Cholinesterase and -1.6 Kcal/mol and -8.3 Kcal/mol for β-Secretase. The GLIDE Score for the standards Donepezil, Galantamine, Rivastigmine and Tacrine docked with Acetyl Cholinesterase was -4.97 Kcal/mol, -5.1 Kcal/mol, -2.6 Kcal/mol & -4.3 Kcal/mol relatively and with β-Secretase -5.8 Kcal/mol, -5.4 Kcal/mol, -3.1 Kcal/mol & -6.1 Kcal/mol relatively. This proves that flavone fused thiazole analogues could be potential drugs for Anti-Alzheimer drug development. The GLIDE score is capable of being used as a semi-quantitative descriptor for the capability of ligands to bind to a specific conformation of the protein receptor. Generally, the ligand having low GLIDE score, will have superior kinship towards the receptor could be expected. 1-(2-oxobutyl)-3-(5-(3,6,7-trihydroxy-5,8-dimethoxy-4-oxo-4H-chromen-2-yl)benzo[d] thiazol-2-yl)urea (**PS206**) showed the best inhibition for the Acetyl Cholinesterase with -7.5 Kcal/mol glide score and β-Secretase with -7.1 Kcal/mol glide score protein receptor. We ascertain a very good concurrence between the localization of the inhibitors upon docking and from the protein structures of Acetyl Cholinesterase and β-secretase. Conformational analysis of docked complex shows that the residues GLU 313, ASN 533 and ARG 247 for **PS206 (Figure 2),** PRO 368, ARG 296 and ARG 247 for Donepezil, GLU 313 and HIS 405 for Galantamine, ASN 233, GLU 313 and HIS 405 for Rivastigmine, GLN 413 and GLU 313 for Tacrine relatively against Acetyl Cholinesterase and ASN 281, GLY 59, TYR 246, THR 280 and THR120 for **PS206 (Figure 3)**, GLU 278, ASP 80, TYR 119 for Donepezil, THR 280 for Galantamine, THR 120 and GLN 121for rivastigmine, ASP 80 for Tacrine relatively against β-Secretase plays a vital role in this receptor's activity. Docking studies performed by GLIDE has established that above inhibitors fit into the binding pocket of the Acetyl Cholinesterase and β-Secretase receptor as of the standard drug molecules. From the fallout, we could monitor that for thriving docking, intermolecular hydrogen bonding and lipophilic interactions linking the ligand, and the receptors are very important. The reason behind the rise in GLIDE score is due to close intra-

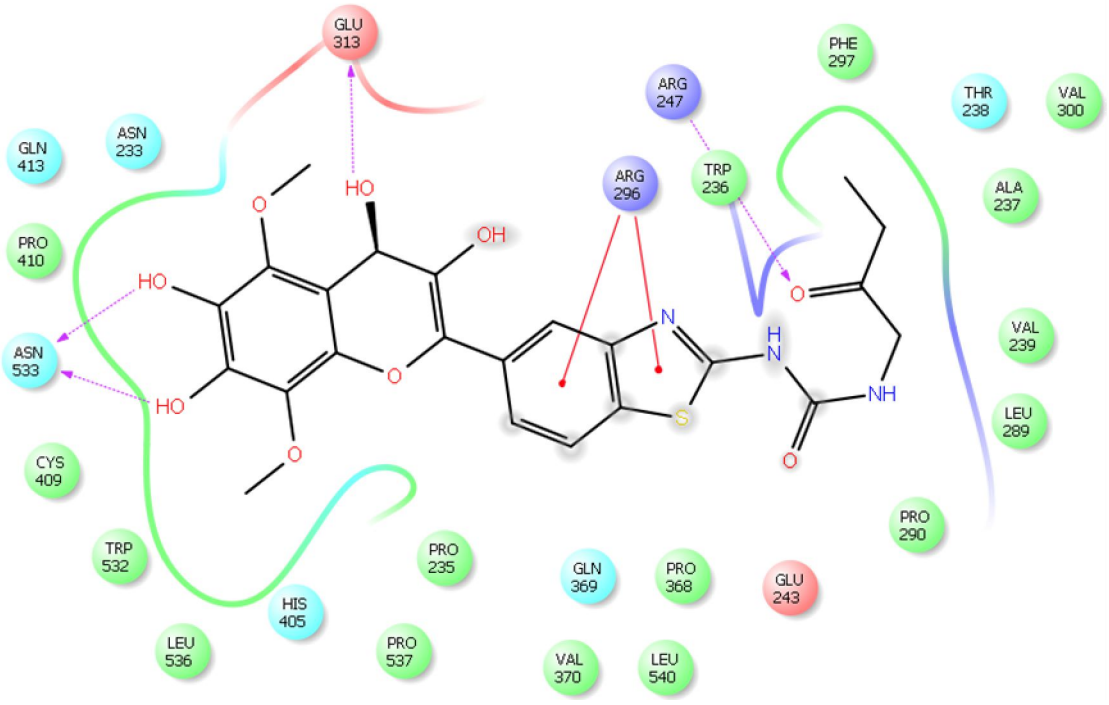
ligand contacts with the compound having tri-hydroxy substitution at 3rd, 6th, 7th position with a dimethoxy substitution at 5th and 8th position.

Table I: *Insilico* docking results against Acetyl Cholinesterase enzyme (PDB: 3LII)

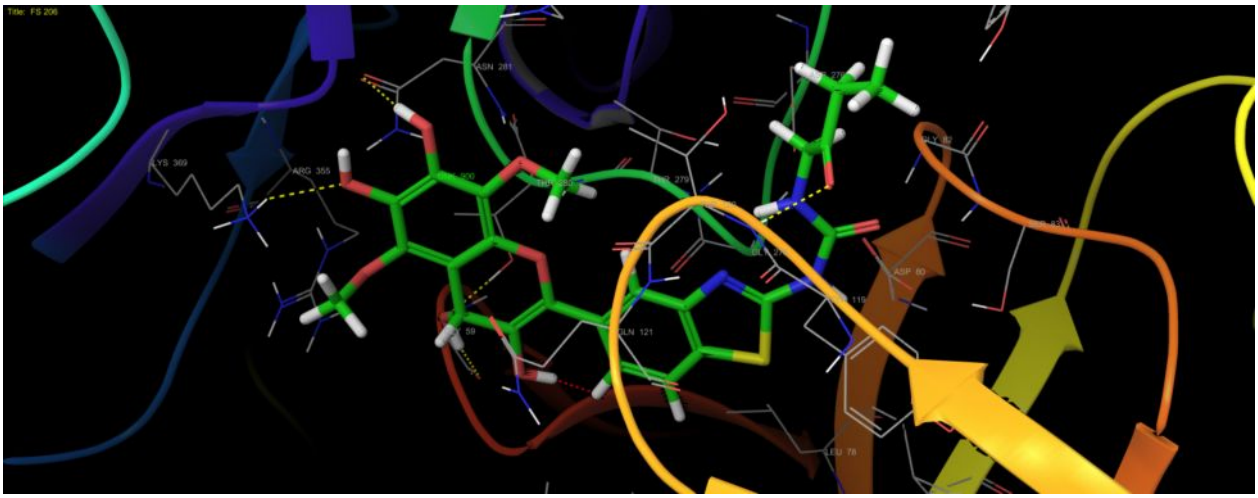
S.No	Compound	Glide Score	Glide Energy	Glide E Model
1.	PS 206	-7.5	-58.26	-89.86
2.	PS 126	-6.8	-55.78	-85.69
3.	PS 41	-6.7	-52.14	-83.30
4.	PS 230	-6.5	-48.76	-86.56
5.	PS 221	-6.1	-46.04	-75.36
6.	Donepezil	-4.9	-33.77	-43.10
7.	Galantamine	-5.1	-41.41	-61.61
8.	Tacrine	-4.3	-31.30	-38.27
9.	Rivastigmine	-2.6	-26.90	-31.84

Table II: *Insilico* docking results against β-Secretase enzyme (PDB: 3U6A)

S.No	Compound	Glide Score against	Glide Energy	Glide E Model
1.	PS 120	-8.3	-55.08	-67.82
2.	PS 228	-7.6	-56.50	-69.06
3.	PS 43	-7.2	-51.23	-62.96
4.	PS 206	-7.1	-51.38	-68.08
5.	PS 131	-6.9	-47.24	-62.79
6.	Donepezil	-5.8	-39.05	-57.89
7.	Galantamine	-5.4	-36.91	-41.90
8.	Tacrine	-6.1	-44.70	-65.76
9.	Rivastigmine	-3.1	-38.90	-42.27

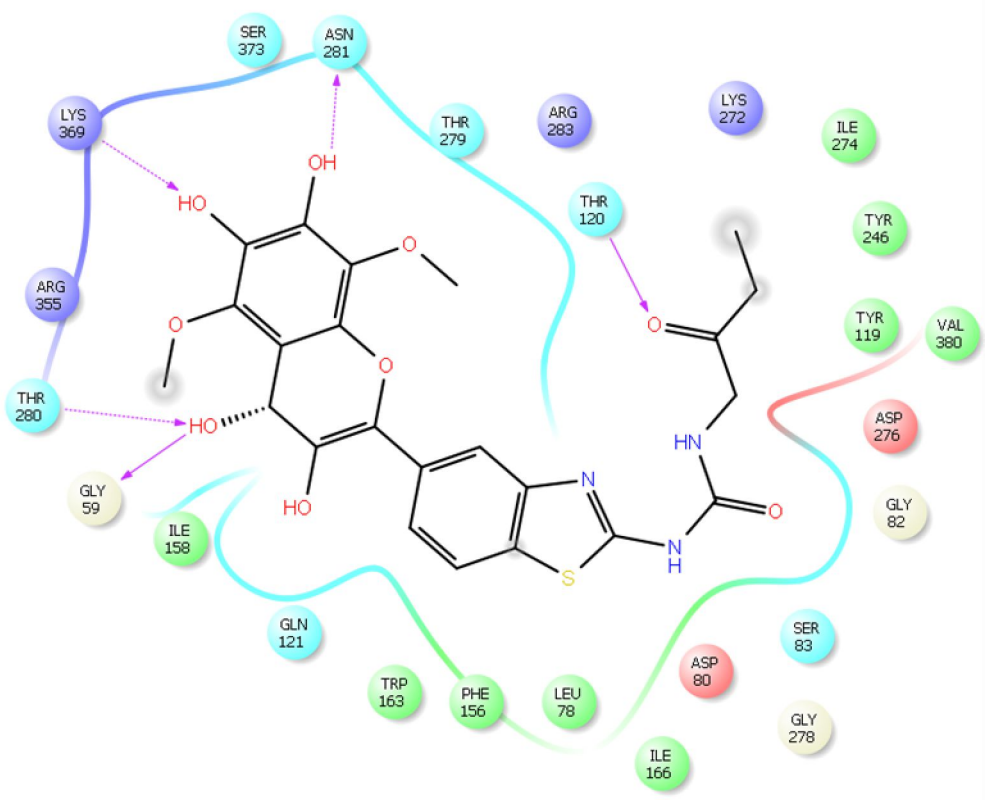


(a)

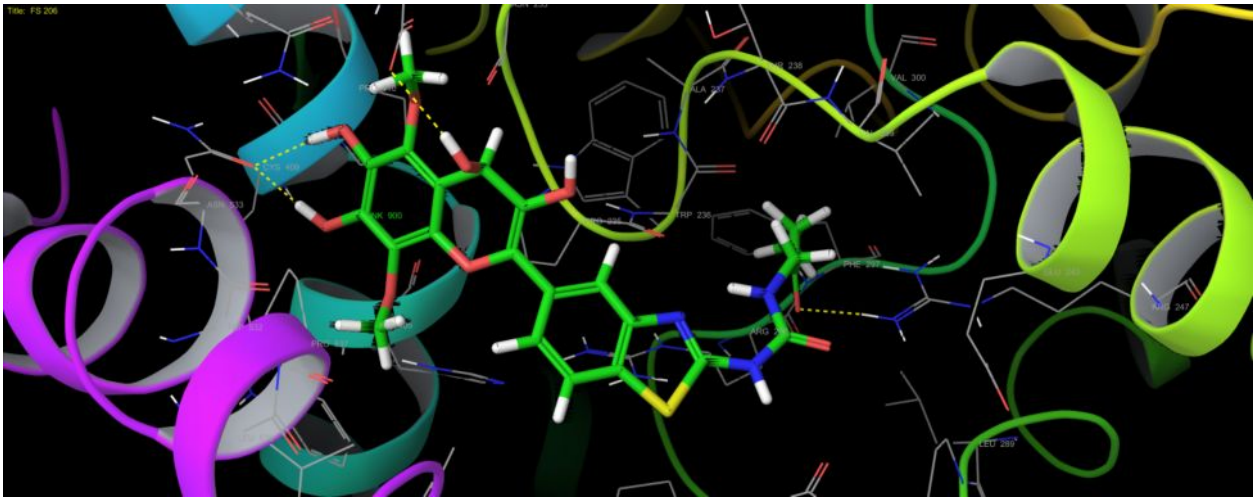


(b)

Fig 2 (a) Ligand interaction of PS 206 with Acetyl Cholinesterase enzyme **(b)** Glide docking image of PS206 with Acetyl Cholinesterase Enzyme

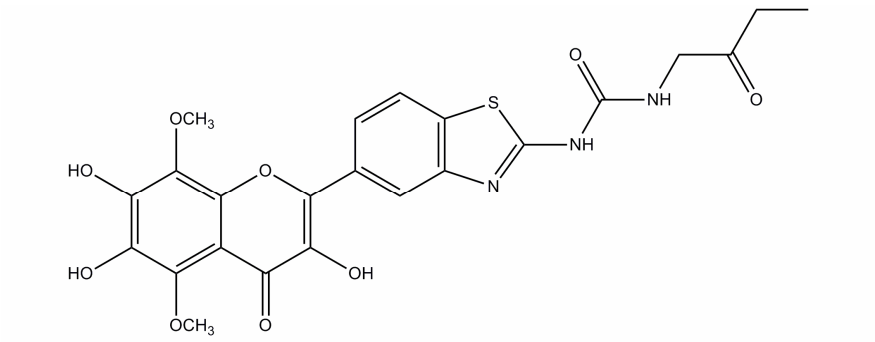


(a)



(b)

Fig 3 (a) Ligand interaction of PS206 with β-Secretase enzyme **(b)** Glide docking image of PS206 with β-Secretase.



1-(2-oxobutyl)-3-(5-(3,6,7-trihydroxy-5,8-dimethoxy-4-oxo-4*H*-chromen-2-yl)benzo[*d*]thiazol-2-yl)urea

Fig 4 Structure of potent lead **PS206**

Conclusion

In conclusion, we have identified a finest molecule of flavone-fused-thiazole analogue **PS 206 (Figure 4)** as an innovative drug candidate who was docked against Acetyl Cholinesterase and β-Secretase in a deliberate attempt to discover an MTDL, able to interfere with diverse key target points of AD Neurodegeneration. This compound **PS 206** is well thought-out as a optimal (lead) molecule, and we necessitate to synthesis and evaluate its potency against Alzheimer's disease through molecular level and *invivo* studies.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgement

The Author P.Parasuraman is grateful to University Grants Commission, New Delhi for providing UGC-BSR fellowship.

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