2. RESEARCH ENVISAGED

AD progression is strongly attributed to the accumulation of toxic forms of self-induced and/or AChE-induced Aβ peptides. Accumulation of Aβ generates NFTs and further Aβ along with NFTs cause neuronal death. Moreover, Aβ initiates apoptosis in AD, a critical feature of neurodegenerative disorders. According to previous reports, AChE produces Aβ peptides leading to formation of AChE-Aβ peptide complex which is more toxic compared to the Aβ peptide alone. Recent studies indicate a link between cholinergic and amyloid hypotheses. Complex pathogeneses of AD mandates the development of small molecules that exhibit dual effects, i.e. anti-ChE as well as anti-Aβ aggregatory.

Pathogenic role of oxidative stress in the development of neurodegeneration has also been well reported in AD. Oxidative stress leads to deposition of senile plaques, NFTs, and deposition of Aβ in the brain. Several studies have established that the neurotoxic effect of aggregated Aβ is mediated through its ability to increase oxidative stress through spontaneous generation of free radicals and ROS [9]. Moreover, apoptosis and oxidative stress are closely linked physiological processes involved in various pathological conditions including AD. Apoptotic cell death comprises of a sequence of events causing activation of caspase cascade which causes fragmentation of the cellular proteins and DNA, leading to the disintegration of the cell ultimately [10]. Thus, a significant neuroprotection in AD could be achieved by employing chemical entities exhibiting antioxidant and antiapoptotic potentials.

Development of multi-target-directed lignads is considered to be the most promising approach currently in the drug discovery process for diseases with complex etiology like AD. A single-targeted drug might not always affect the complex biological system adequately even though it may totally change the behaviour of its target. In contrast, a multi-target-directed ligand interacts with lower but balanced affinity compared to a single-targeted molecule because it is unlikely that a small, drug-like molecule will bind to a variety of different targets with equal high affinity. Multi-target-directed drugs can actually have a better efficacy/safety ration than mono-targeted drugs. A balanced suboptimal activity for different therapeutic targets might have safety liability and reduce the risk of therapeutic resistance [40]. Hence, there is a need to design such compounds that can simultaneously act on different causative targets of AD with balanced affinity. This can be achieved by linking together of structurally different active moieties which

possess affinity for different targets. The resulting hybrids with relatively weak but balanced affinity for different targets could be beneficial to treat a complex disease like AD [40].

Canonical Wnt signaling pathway is important for survival and development of nervous system. Recent studies have shown the involvement of Wnt signalling pathway in various neurodegenerative diseases including AD wherein impairment of Wnt signalling pathway is associated with AChE- as well as Aβ-mediated neurotoxicity [41]. Additionally, the levels of GSK-3 and β-catenin, the two key downstream regulators of Wnt signalling pathway, are dramatically altered in the AD afflicted rat brain [42]. β-Catenin target gene - neuroD1 is a transcription factor which plays a vital role in neurogenesis [43]. Expression of neuroD1 is regulated by Wnt/β-catenin signaling activation [44]. Previous reports have demonstrated that the AChE-Aβ complex-mediated neurotoxicity inactivates Wnt pathway while activation of Wnt components prevent degradation of cholinergic neurons in AD [45].

Isoalloxazine, the basic flavin nucleus has been extensively studied for discovery of drugs for infectious diseases in humans and animals like trypanosomiasis and malaria [46]. A bis-isoalloxazine derivative linked by a piperazine moiety has been accounted for the prevention of association of prion proteins and Alzheimer-specific Aβ peptides [47]. On the basis of the knowledge about receptor active site and literature reports, a series of isoalloxazine derivatives were evaluated for *in vitro* anti-ChE and anti-Aβ aggregatory activities. The most potent test compounds so obtained were than assessed for their neuroprotective potential in AD, utilizing different *in vitro* and *in vivo* experiments. Finally, the role of the test compounds in activation of Wnt/β-catenin signaling pathway has also been established.

Additionally, some benzazepine derivatives and diarylthiazole-benzylpiperdine hybrids synthesized in Pharmaceutical Chemistry laboratory were also evaluated biologically to assess their multi-target-directed potential for use in the management of AD.

- The present study was mainly focussed on the following objectives:

1. To assess the anti-cholinesterase activity of the synthesized compounds using standard *in vitro* cholinesterase inhibition assay described by Ellman.
2. To assess anti-Aβ aggregation profile of the compounds selected in the previous assay using thioflavin T (ThT) and Congo red (CR) assay.

3. To evaluate the antiamnesic and antioxidant potentials of the selected compounds using scopolamine-induced amnesic mice model.
4. To assess the neuroprotective, antioxidant, antiapoptotic and reactive oxygen species (ROS) scavenging properties of the selected compounds *in vitro* using SH-SY5Y human neuroblastoma cell line and primary rat hippocampal neuronal culture.
5. To assess the *in vitro* blood brain barrier (BBB) permeability of the potent compounds.
6. To assess the selected compounds for their neuroprotective potential using $A\beta_{1-42}$-induced Alzheimer‘s rat model.
7. To confirm the neuroprotective, antioxidant and antiapoptotic potentials of the selected compounds by estimation of various protein markers using Western blot technique.
8. To elucidate the mechanistic pathway involved behind the neuroprotective role of the compounds by estimation of certain protein markers using Western blot techniques.
9. To evaluate the safety profile of the potent test compounds.
10. To assess the pharmacokinetic profile of the most potent test compound.

2. RESEARCH ENVISAGED

5-HT_{2A}, 5-HT_{2B} and 5HT_{2C} receptors possess 46–50% sequence similarity as they are homologous in nature. Selective binding to 5HT_{2C} receptor is an important criterion because 5-HT_{2A} agonists can have potentially hallucinogenic and cardiovascular (CV) side e□ects [44] while 5-HT_{2B} agonism is linked with heart valvulopathy and pulmonary hypertension [45]. To avoid side e□ects, a potential drug candidate should show its selectivity for 5-HT_{2C} receptor and should be devoid of intrinsic activity on 5-HT_{2A} and 5-HT_{2B} receptors, present predominantly in the thoracic aorta and fundus respectively [46].

In the present study a series of 1-methyl-3-benzazepin-2-one derivatives possessing the scaffold present in the lorcaserin as potentially selective 5-HT_{2C} receptor agonists were initially evaluated *in vitro* to determine their 5-HT_{2A} and 5-HT_{2B} selectivity using isolated rat thoracic aortic and fundus strips respectively. Based on the results of preliminary screening, some compounds were chosen for further evaluation in animal models for depression, anxiety, obesity and penile erection.

- The following objectives were laid down for the current study:

1. To screen the new synthesized compounds for their 5-HT_{2A} and 5-HT_{2B} potentials using isolated rat aortic strip and isolated rat fundus preparations.
2. To evaluate those compounds which were found to be inactive in the above *in vitro* experiments, for their 5-HT_{2C} agonistic activity using suitable animal models of depression (primary level of screening).
3. To assess the selected (from the primary level of screening) potent compounds for anxiety via 5-HT_{2C} receptors using suitable animal models and to evaluate their 5HT_{2C} agonistic profile using appropriate 5-HT_{2C} antagonist.
4. To evaluate the selected compounds pharmacologically for obesity via 5-HT_{2C} receptors using appropriate animal models in presence of a 5-HT_{2C} antagonist.
5. To evaluate the selected compounds pharmacologically for erectile dysfunction via 5-HT_{2C} receptors using suitable animal model for penile erection in presence of a 5-HT_{2C} antagonist.
6. To estimate neurotransmitter i.e. DA and 5-HT levels in the test compound-treated rat brains by HPLC technique to substantiate the *in vivo* results.

2. RESEARCH ENVISAGED

In spite of continuing research into the mechanism of excitotoxicity, currently no pharmacological intervention is capable of providing complete neuroprotection clinically. Thus, a compound with NMDAR antagonist, Aβ aggregation inhibitory, free radical scavenging, antioxidant and antiapoptotic activities could become an efficient tool to treat excitotxicity. In light of this, an alternative strategy is now emerging based on the assumption that a single compound could hit multiple targets, demanding the development of multi-target-directed ligands to face the complexity of excitotoxicity adequately.

Previously, some benzazepine derivatives have shown NMDAR antagonist [24], Aβ aggregation inhibitory [25], neuroprotective, free radical scavenging, antioxidant [26] and antiapoptotic activities [27]. Based upon these findings, 3-methyltetrahydro-3*H*-benzazepin-2-one derivatives having the benzazepine scaffold were synthesized in Pharmaceutical Chemistry laboratory and it was planned to perform preliminary screening for their NMDAR antagonist activity. Further, to assess their full biological activity spectrum, it was planned to study in detail $A\beta_{1-42}$ aggregation inhibitory, neuroprotective, free radical scavenging, antioxidant and antiapoptotic activities of those selected compounds which were found to possess high potency as NMDAR antagonists in the preliminary screening.

- The present study was aimed to achieve the following objectives:

1. To determine the neuroprotective potential of the synthesized compounds against NMDA-induced excitotoxicity using SH-SY5Y human neuroblastoma cell line.
2. To assess anti-$A\beta_{1-42}$ aggregation profile of the potent test compounds selected in the previous assay.
3. To assess the neuroprotective, antioxidant, antiapoptotic and reactive oxygen species (ROS) scavenging properties of the selected compounds *in vitro* using primary rat hippocampal neuronal culture.
4. To assess the *in vitro* blood brain barrier (BBB) permeability of the potent test compounds.
5. To assess the neuroprotective effect of the selected test compounds using $A\beta_{1-42}$-induced excitotoxicity rat model.

6. To confirm the neuroprotective, antioxidant and antiapoptotic potentials of the selected compounds by estimation of various protein markers using Western blot technique.
7. To estimate the levels of excitatory neurotransmitters in the hippocampal region of rat brain using high performance liquid chromatography with electrochemical detector (HPLC-ECD).
8. To check the NMDAR binding site affinity of the potent test compound using molecular docking study.

2. RESEARCH ENVISAGED

Currently, no treatment is available to slow down the disease progression or control the non-motor complications of PD. Current therapy is only for the suppression of motor symptoms for which long-term DA replacement therapy is used, i.e. *L*-dopa, which after several years of treatment develops periodic "on-off" state that makes PD treatment insurmountable. The duration of *L*-dopa treatment to remain efficacious is a severe limiting factor for PD treatment [19].

To delay the *L*-dopa associated problems, initial monotherapy is started with DA receptor agonists which act on the D_2 class receptors which mediate their actions via regulating the "indirect pathway" in the basal ganglia. Pramipaxole and ropinirol are the currently available treatments active at D_2 family receptors which have unwanted side effects like nausea, fatigue, orthostatic hypotension and hypersexuality due to localization of D_2-like family receptors in the chemoreceptor trigger zone and nucleus accumbens. The major drawback of D_2-like agonists is their ineffectiveness after some years. The number of monotherapy patients declines to 50% after three years of treatment [14].

No current treatment is available to treat the PD that targets the D_1 receptor. As stated above, D_1 receptors are constitutive and have been involved in regulating the "direct pathway" in the basal ganglia [18]. Dihydrexidine (DHX), a full D_1 receptor agonist, attenuated the motor complications arising after MPTP lesioning in monkey. Moreover, Dinapsoline, a selective D_1 agonist has shown beneficial effects against 6-hydroxydopamine (6-OHDA) lesioned animal model. A-77636 and A-86929, selective D_1 agonists have demonstrated significant improvement in rodent and primate experimental models of PD. Adrogolide, a prodrug of A-86929 [20], has shown equivalent efficacy to *L*-dopa in the preclinical as well as clinical studies. The above findings support the hypothesis that selective D_1 receptor agonists could be effective in the management of PD.

According to the previous reports, benzazepines showed protection against PD phenotypes induced by MPTP in marmoset through activation of D_1 receptors [21]. In accordance with these reports a series of benzazepines were synthesized. In the present study, we report biological evaluation of these synthesized compounds as selective D_1 agonists as potential

therapeutics for PD. Initially, the compounds were evaluated in the *in vitro* experiments utilizing isolated rat mesenteric artery preparation to assess their D_1 agonistic potentials using selective D_1 antagonist (R-SCH-23390). The most potent compound so obtained through this primary screening was then evaluated further to check for protection against 6-OHDA-induced toxicity using SH-SY5Y neuroblastoma cells. Finally, the test compound was assessed in 6-OHDA-induced Parkinson's rat model using various behavioural and biochemical experiments.

- The present study was performed mainly with the following objectives:

1. To screen the newly synthesized compounds for their D_1 receptor agonistic potentials using isolated rat mesenteric artery preparation.
2. To assess the selected potent compound(s) using 6-OHDA-induced Parkinson's rat model.
3. To evaluate the test compound(s) for various neurochemical parameters using tissue homogenates.
4. To estimate neurotransmitter (DA) levels in brains of rat administered with the test compound(s) or standard drug using high performance liquid chromatography with electrochemical detector (HPLC-ECD).
5. To evaluate the tyrosine hydroxylase (TH) and cleaved caspase-3 protein levels to assess neuroprotective and antiapoptotic potentials of the selected compound(s) using immunohistochemistry.

2. RESEARCH ENVISAGED

Several selective D_3 antagonists have been developed earlier as potential therapeutic agents for the treatment of neuropsychiatric conditions. KCH-1110, a selective D_3 antagonist was evaluated in rodent models that showed promising antipsychotic effects [15]. AVE5997, a potent D_3 antagonist was considered for the treatment of schizophrenia, but was dropped later on due to its safety issues [16]. Another D_3 antagonist, PNU-177864 was also stopped midway like AVE5997 because of associated side effect causing myopathy in animals [17]. A437203, S33138, S33084 and SB-277011-A are also potent selective D_3 antagonists, but limited animal behavioural data are available for them. A D_3 antagonist (+)-UH-232 worsened the psychosis symptoms rather than improving them in drug-free schizophrenia patients. (+)-UH-232 increased unusual thought processes, hostility, activation and anxiety in schizophrenia patients. However, (+)-UH-232 did not induce extrapyramidal side effects [18]. Thus, to understand the exact role of D_3 antagonism in neuropsychiatric conditions, there is a need to develop newer potent and selective D_3 receptor antagonists.

Different benzazepine derivatives have been reported in literature to demonstrate selective D_3 antagonist activity with promising antipsychotic effects [8-10]. As reported in Chapter 4, a series of benzazepine derivatives, synthesized in our laboratory, were evaluated for their DA receptor sensibilities. The most potent and relatively more selective test compounds showing D_3 antagonist activity were evaluated further using different *in vivo* experimental rodent models for their antipsychotic activity. The results revealed selective D_3 antagonist potential of the test compounds which could prove to be a lead for antipsychotic therapy with low incidents of extrapyramidal side effects.

- The present study aimed at the following points.

1. To select those compounds which exhibited potent D_3 receptor antagonistic activity in the screening on isolated rat mesenteric artery preparation described in earlier chapter (Chapter 4).
2. To assess the antipsychotic potential of the selected potent compounds using different rodent models.

3. To estimate neurotransmitter (DA) levels in the brains of test animals administered with the test compounds using high performance liquid chromatography with electrochemical detector (HPLC-ECD).
4. To assess the safety profile of the selected potent compounds.