SUMMARY

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Introduction

Little molecules can be influential implements in science and medicine, working as therapeutics and as investigates that can help to light up the macromolecules controlling biological processes. Certain classes of compounds that are exceedingly spoken to in the generally bioactive compound populace, have exhibited an abundance of biological activities in addition to sound 'medicine like' properties. In search of CNS acting drugs, diverse series of compounds have been synthesized in the Pharmaceutical Chemistry laboratory. These compounds were further assessed pharmacologically as CNS acting agents.

The proposed work has been divided into three main sections depending upon the pharmacological applications.

SECTION I is focused on the pharmacological evaluation of some novel triazines and cyclic guanidine derivatives as cholinesterase inhibitors and Aβ anti-aggregatory agents as potential therapeutics for Alzheimer's disease.

SECTION II of this research work focussed on the pharmacological actions of some novel benzazepine derivatives as 5-HT_{2C} receptor agonists for their potential applications as anti-obesity, antidepressants and in erectile dysfunction.

SECTION III focussed on the pharmacological evaluation of some novel benzazepines as dopamine D_1 receptor agonists as potential anti-Parkinsonian drugs.

SECTION I

AD is a progressive brain disorder that harms and inevitably obliterates neurons, prompting loss of memory and other brain capacities. It generally expands gradually and slowly deteriorates as more neurons shrivel and die. Eventually, Alzheimer's is serious disease, and right now, there is no treatment. AD is described as a progressive, neurodegenerative disorder that influences the cholinergic regions of the CNS, associated with spatial memory and cognitive function. Alzheimer's is not a regular component of aging, in spite of the fact that the best known danger variable is increasing age, and the greater part of individuals with Alzheimer's are elderly populace. Regularly, AD is diagnosed in individuals in excess of 65 years old, in spite of the fact that the less-predominant early-onset Alzheimer's can happen much prior. In 2006, there were 26.6 million sufferers around the world. Alzheimer's is anticipated to influence 1 in 85 individuals comprehensively by 2050. The trademark attributes of AD incorporate the quick loss of cholinergic neurotransmission, quickened collection of amyloid-β (Aβ) peptides and development of neurofibrillary tangles

(NFTs) of hyperphosphorylated tau protein. These qualities build the premise for the cholinergic, amyloid and tau hypothesis for AD pathology, individually.

Conversely, the amyloid hypothesis proposes that the movement of AD is credited to the accelerated aggregation of toxic forms of Aβ and/or AChE-induced toxic aggregates of Aβ peptides. In this respect, late studies show a correlation between the cholinergic and amyloid theories. Previous reports also emphasize on the therapeutic potentials of triazines and cyclic guanidines in treatment of AD. These numerous components in AD pathology command the need to generate treatments that demonstrate dual ChE inhibition and decrease the formation of neurotoxic Aβ-aggregates. Research into the cholinergic hypothesis has prompted the advancement of a few fused and non-fused ring systems as ChE inhibitors as well as some cyclic guanidines for their capacity to restrain the conglomeration of Aβ plaques. In accordance with above notes, a series of novel triazines and cyclic guanidine derivatives were synthesized in the Pharmaceutical Chemistry laboratory. The compounds were further assessed pharmacologically using different *in vitro* and *in vivo* experiments. Firstly, the compounds (**triazines** and **cyclic guanidine derivatives**) were assessed using *in vitro* cholinesterase inhibition assay described by Ellman. The compounds (**TRZ-15**, **TRZ-19** and **TRZ-20** from triazine series and **3b** from cyclic guanidine series) came out as potent cholinesterase inhibitors. They were further assessed using scopolamine induced amnesic mice models. The compounds (**TRZ-15, TRZ-20** and **3b**) have shown neuroprotective property as witnessed by increase in memory, cholinesterase levels and modification of oxidative parameters viz. MDA and catalase. **TRZ-19** did not show much promising results therefore it was excluded from further studies. The remaining compounds (**TRZ-15, TRZ-20** and **3b**) were evaluated further using rat hippocampal neuronal culture *in vitro*. Firstly, the compounds (**TRZ-15, TRZ-20** and **3b**) were found to be safe when assessed utilizing MTT assay. Secondly, the compounds (**TRZ-15, TRZ-20** and **3b**) were again assessed against $A\beta_{1-42}$ induced toxicity in rat hippocampal neuronal culture. The compounds (**TRZ-15, TRZ-20** and **3b**) have shown protection in MTT assay. Further, the compounds' (**TRZ-15, TRZ-20** and **3b**) treatments have shown decrease in apoptotic nuclei and ROS scavenging property on rat hippocampal neurons as assessed by Hoechst staining protocol and H2DCF-DA assay. Additionally, the compounds (**TRZ-15, TRZ-20** and **3b**) significantly reduced Aβ plaques and cleaved caspase-3 positive neuronal cells when assessed against $A\beta_{1-42}$ induced rat hippocampal neuronal cells utilizing DAB and fluorescent staining protocols. According to previous reports, Aβ induced Alzheimer's rat model had shown similar pathology as that of transgenic rodent models and it could also be correlated with humans suffering from AD.

In accordance with these reports, $A\beta_{1-42}$ induced Alzheimer's rat model was developed by streotaxic surgery. The compounds (**TRZ-15, TRZ-20** and **3b**) were further assessed using this model. The compounds (**TRZ-15, TRZ-20** and **3b**) showed neuroprotective role as there was significant increase in learning and memory in $A\beta_{1-42}$ induced Alzheimer's rat model. Further, the brain sections were used for staining and Western blot studies. The compounds (**TRZ-15, TRZ-20** and **3b**) have again validated the previous studies as significant decrease in Aβ burden and number of fluoro-jade c stained positive cells was observed in hippocampal region of $A\beta_{1-42}$ induced Alzheimer's rat brains after treatments with the compounds (**TRZ-15, TRZ-20** and **3b**). Western blot analysis showed that there was decrease in $A\beta_{1-42}$, cytochrome c and cleaved caspase-3 levels in $A\beta_{1-42}$ induced Alzheimer's rat brains treated with the compounds (**TRZ-15, TRZ-20** and **3b**). According to previous reports, the canonical Wnt/β-catenin pathway (a neuroprotective pathway) is involved in AChE-Aβ induced neurotoxicity. In accordance with the previous reports, it was explored weather the compounds were showing neuroprotection through this pathway. The results showed that the compounds (**TRZ-15, TRZ-20** and **3b**) activate canonical Wnt/β-catenin pathway. Confocal microscopic images suggested that there was significant increase in number of β-catenin positive cells in $A\beta_{1-42}$ induced Alzheimer's rat brains treated with the compounds (**TRZ-15, TRZ-20** and **3b**). Western blot results further substantiated the confocal results. The immunoblot results showed that there was significant increase in pGSK3a and pGSK3b levels in $A\beta_{1-42}$ induced Alzheimer's rat brains treated with the compounds (**TRZ-15, TRZ-20** and **3b**). Significant increase in levels of β-catenin was also observed in $A\beta_{1-42}$ induced Alzheimer's rat brains treated with the compounds (**TRZ-15, TRZ-20** and **3b**). These results indicated that the compounds (**TRZ-15, TRZ-20** and **3b**) showed neuroprotection by activating canonical Wnt/β-catenin pathway. The LD_{50} of the compounds (**TRZ-15, TRZ-20** and **3b**) were assessed using OECD guideline 423. The LD_{50} of the compounds (**TRZ-15, TRZ-20** and **3b**) is >2000 mg/kg. As there are very few drugs available to treat AD, the compounds (**TRZ-15, TRZ-20** and **3b**) could be suitable potential drug candidates to treat AD.

SECTION II

The neurotransmitter serotonin (5-HT) intercedes its actions through at least 14 distinctive receptor subtypes that have been characterized into seven major families, 5-$HT_{1–7}$. 5-HT_{2C} receptor agonists have gotten to be appealing medication focuses that have potential use in the treatment of various conditions including schizophrenia, obesity, sexual dysfunction, anxiety, depression and urinary incontinence. For these signs, selectivity over agonism at the 5-HT_{2A} and 5-HT_{2B} receptors would be a key goal in light of the fact that 5-HT_{2A} agonists can conceivably be hallucinogenic and have cardiovascular (CV) effects, while 5-HT_{2B} agonism has been related with pulmonary hypertension and heart valvulopathy. The nonselective 5-HT_{2C} receptor agonist *meta*-chlorophenylpiperazine (*m*CPP) has been indicated to reason weight reduction by decrease of food intake in rodents and humans. A nonselective 5-HT_{2C} receptor agonist dexfenfluramine is metabolized to nor-dexfenfluramine, a circulating metabolite of this weight loss drug, and the anorectic actions of nor-dexfenfluramine and dexfenfluramine are blocked by selective 5-HT_{2C} receptor antagonist, **SB-242084**. The quest for selective and potent 5-HT_{2C} agonists has distinguished lorcaserin (**APD-356**; Arena) for the treatment of obesity and vabicaserin (**SCA-136**; Wyeth) a novel anorectic and antipsychotic agent. Of late FDA board voted to suggest lorcaserin with specific limitations and patient monitoring.

Since 3-benzazepine structure contains phenethylamine skeleton which is also present in lorcaserin and dexfenfluramine, it is of great interest from medicinal chemistry and pharmacology point of view. As a component of our research endeavours to identify potential and novel 5-HT_{2C} agonist drug candidates, a series of benzazepine compounds were synthesized in the Pharmaceutical Chemistry laboratory. They were evaluated as 5-HT_{2C} receptor agonists. Firstly, the compounds (**1-59**) were evaluated *in vitro* using isolated rat aorta and rat fundus preparations to assess their ability to show their effect on 5-HT_{2A} and 5-HT_{2B} receptors as they are present on aorta and fundus respectively. Compounds (**1-6, 8, 9, 15-17, 21, 22, 24, 25, 30, 31, 42-44, 47, 49, 50, 52-56 and 59**) were eliminated from the study because of their activity on rat aorta and fundus. In search of the compounds' selectivity towards 5-HT_{2C} receptor, the remaining compounds (**7, 10-14, 18-20, 23, 26-29, 32-41, 45, 46, 48, 51, 57 and 58**) were evaluated further using TST in mice. The compounds (**7**, **19** and **28**) came out as potential 5-HT_{2C} agonists as they had shown antidepressant effect similar to the previous report of **WAY-163909**. The compounds (**7**, **19** and **28**) were further assessed using elevated plus maze test in mice. The compounds (**7**, **19** and **28**) have shown anxiogenic effect similar to *m*-CPP. The effect was reversed by selective 5-HT_{2C} antagonist

(**SB-206553**). This authenticated that compounds (**7**, **19** and **28**) are selective 5-HT_{2C} agonists. Amongst the three compounds, compounds (**19** and **28**) have shown better results as compared to compound (**7**). Furthermore, the compounds (**7**, **19** and **28**) were assessed using hypophagic rat model. As shown previously, the compounds (**7**, **19** and **28**) showed significant hypophagic response which was reversed by **SB-206553** (a 5-HT_{2C} antagonist). This again validates the results that the compounds (**7**, **19** and **28**) are selective 5-HT_{2C} agonists. Compounds (**19** and **28**) have shown hypophagic response more significantly than **7**. The three compounds (**7**, **19** and **28**) were lastly assessed on penile erection model of rat. The results validated the previous results as they have shown significant increase in number of lickings/hr. The numbers of lickings/hr were reduced when the animals were treated with the test compounds (**7, 19** and **28**) in combination with **SB-206553** (a 5-HT_{2C} antagonist). The compounds (**19** and **28**) showed more significant results as compared to compound (**7**). The LD_{50} of the compounds (**19** and **28**) were assessed using OECD guideline 423. The LD_{50} of the compounds (**19** and **28**) is >2000 mg/kg. Thus the results indicate that compounds (**19** and **28**) are more effective on 5-HT_{2C} receptor than compound (**7**) and they can be further assessed as drug candidates for different conditions associated with 5-HT_{2C} receptor viz depression, schizophrenia, erectile dysfunction, anxiety and obesity respectively.

SECTION III

PD is a progressive neurodegenerative issue with obscure etiology. Its neuropathology incorporates degeneration of dopaminergic nigrostriatal pathway, which is a combined cause of iron deposition, increased lipid peroxidation, glutathione depletion, DNA damage through oxidation, mitochondrial dysfunction, and alterations in antioxidant enzymes activities. Diverse characteristics of PD, whether altered neurotransmitter synthesis, genetic predilection or alteration in detoxification system, all have been demonstrated to include a typical course of occasions, which are intervened by oxidative stress (OS).

PD is a neurological issue including dynamic degeneration of dopaminergic neurons that emerge from the substantia nigra and innervate the caudate and putamen. The primary approach in pharmacotherapy of PD has been dopamine (DA) substitution treatment utilizing *L*-DOPA (*L*-dihydroxyphenylalanine), a medication that can give critical palliative results for a few years. The primary impediments of the long term utilization of *L*-DOPA, nonetheless, incorporate the advancement of flighty "on-off" phenomena, and in addition dyskinesias and psychiatric side effects, for example, sleep disturbances, psychoses and hallucinations. To evade these side effects, direct acting agonists targeted for various DA receptors have been

tried. D_2-favouring agonists, such as pramipexole, ropinirole and bromocriptine, are helpful monotherapy just in the early phases of the disease, losing viability as the ailment advances. Prominently, nonetheless, their parenteral administration can have checked valuable effects for a few patients. Earlier endeavors to create D_1 agonists for PD met with constrained accomplishment as **SKF-38393** and **CY 208–243** indicated astounding effects in rat models, however these were less compelling in primates and patients. These compounds were distinguished consequently as partial agonists at D_1 receptors, and thus, the requirement for full intrinsic activity at the D_1 receptor was theorized. This speculation is backed by late studies with the D_1 receptor full agonists **ABT-431**, **A-77636** and **dihydrexidine (DHX)** which had strong effects in primate PD models and adequacy in patients.

According to previous reports, benzazepines showed protection against Parkinson's induced by MPTP in marmoset through activation of D_1 receptor (Gnanalingham et al., 1995). In accordance with these reports a series of benazepines have been synthesized and evaluated as D_1 agonists for PD. Firstly, the compounds were evaluated *in vitro* utilizing isolated rat mesenteric artery preparation to assess their D_1 agonistic potentials using selective D_1 antagonist (**R-SCH-23390**). The compounds **(3c, 3d, 3e, 3i** and **3k)** were found to be dopamine agonists. Rest of the compounds (**3a, 3b, 3f, 3g, 3h, 3j, 3l , 3m** and **5**) were found to be inactive or D_3 antagonists. The compound (**3d**) showed selectivity towards D_1 receptor as evidenced by its pD_2 value (**7.00 ± 0.23**). It was considered as potent D_1 agonists as its pD_2 value was almost equivalent to the standard **A-77636** (a selective D_1 agonist). Rest of the compounds (**3a, 3b, 3f, 3g, 3h, 3j, 3l, 3m** and **5**) were excluded from the study as they had shown antagonistic effect on dopamine receptors. The compound (**3d**) was further evaluated using different *in vitro* and *in vivo* studies.

The most potent compound (**3d**) was then evaluated further against 6-OHDA induced toxicity using SH-SY5Y neuroblastoma cells. The compound (**3d**) showed protection against 6-OHDA *in vitro*. The compound (**3d**) was assessed further in 6-OHDA induced Parkinson's rat model. The compound (**3d**) showed contralateral rotations for 7 days in 6-OHDA lesioned rats indicating its D_1 agonistic potential. Furthermore, the compound (**3d**) showed antioxidant effect in 6-OHDA lesioned rats as evidenced by improvised levels of GSH, SOD, MDA and catalase. Additionally, the compound increased the level of DA and decreased the level of cleaved caspase-3 as shown by HPLC analysis and immunohistochemical examinations. The LD_{50} of the compound (**3d**) was assessed using OECD guideline 423. The LD_{50} of the compounds (**3d**) is >2000 mg/kg. The results concluded that the compound (**3d**) is a potent dopamine D_1 receptor agonist that could be further evaluated in different pre-clinical models.