REVIEW ARTICLE

PHOSPHODIESTERASE (PDE) INHIBITORS IN ALZHEIMER'S DISEASE: ROLE AND CURRENT STATUS

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ABSTRACT

Alzheimer's disease is a progressive neurodegenerative disease, usually associated with old age. With the increasing geriatric population throughout the world, it will pose a great socio-economic burden on the healthcare system and the caregivers. The clinically approved drugs for management of this disease include cholinesterase inhibitors and memantine. But these drugs modulate only the symptoms of this disease rather than addressing the underlying pathology. Therefore, there is an urgent need to discover new therapeutic agents which, coupled with effective diagnostics, can prove effective in therapeutic management of this disease. Phosphodiesterase inhibitors represent an emerging class of drugs with several isoforms reported to play a crucial role in the pathology of this disease. This review discusses various phosphodiesterase inhibitors which are in preclinical and clinical studies along with physico-chemical properties that impact CNS penetration and subsequent efficacy.

Keywords: Alzheimer's disease, therapeutics, Phosphodiesterase, PDE inhibitors, clinical trials

INTRODUCTION

Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disease, usually associated with old age. It constitutes almost 60-70 % of cases in the dementia group of disorders. Owing to the ever-increasing population worldwide[1], especially geriatric population, this disorder is reaching epidemic proportions, with a large socio-economic burden. This disease is characterized by three primary groups of symptoms namely, cognitive dysfunction, psychiatric symptoms and behavioural disturbances along with difficulties in performing day-to-day activities independently. Increasingly, the coexistence of vascular disease, in the form of neuroinflammation, has been associated with Alzheimer's disease and has been recognized clinically, pathologically and epidemiologically[2].

Literature survey indicates that there is an immense increase in efforts towards development of diagnostics and therapeutics for this debilitating disorder, particularly in the past decade[3]. This has resulted in simultaneous development in understanding the pathophysiology and biochemistry of AD leading to genesis of several hypotheses[4]. Three most prominent hypotheses are amyloid hypothesis[5], tau hypothesis and cholinergic hypothesis[6].

In the amyloid cascade, Aβ peptide is generated by the proteolytic cleavage of amyloid precursor protein (APP)[7]. In the amyloid genic pathway, toxic species such as $A\beta_{40}$ and $A\beta_{42}$ are generated via a two-step proteolytic cleavage involving β- and γ –secretases. Among these species, $A\beta_{42}$ has a greater propensity to aggregate-forming toxic oligomers that are further converted to senile plaques[8,9]. By far, the most attractive target to treat AD involves the development of molecules that affect the stability, removal or aggregation of Aβ[10,11].

Another important pathological hallmark of AD comprises of the formation of neurofibrillary tangles (NFTs), which are composed of aggregated hyper -phosphorylated tau protein[12]. Normally, tau protein is associated with the microtubule and is known to facilitate its stabilization intracellularly[13]. However, in AD, tau is highly phosphorylated by different kinases. This results in reducing its binding to microtubules and subsequent

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sequestration into NFT. The loss of tau function results in microtubule instability and reduction in axonal transport along with aggregate induced neurotoxicity.

The cholinergic hypothesis began with the systematic biochemical investigation of the brains of patients with AD in the early 1960s that revealed substantial neocortical deficits in the enzyme responsible for the synthesis of acetylcholine (ACh), namely, choline acetyltransferase (ChAT)[14]. Further, it was observed that there was reduced choline uptake, ACh release and loss of cholinergic neurons[15]. Therefore, it was proposed that degeneration of cholinergic neurons in the basal forebrain and the associated loss of cholinergic neurotransmission in the cerebral cortex and other areas contributed significantly to the deterioration in cognitive function seen in patients with AD.

Currently, the most widely used anti-AD drugs in clinical practice are donepezil[16] (Aricept), galantamine (Reminyl), rivastigmine (Exelon), and memantine (Noojerone) (Fig.1). The first three are acetylcholinesterase (AChE) inhibitors (AChEI), whereas memantine is a low-affinity non-competitive antagonist of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors (GluR). However, none of these agents address the underlying pathology of AD. An in-depth analysis conducted by Bachurin et al., indicates that over the last decade, more than 50 drug candidates have successfully passed phase II but none have passed phase III clinical trials.

Given the fact of an ever-increasing global population of geriatric patients, there is an urgent need to discover new, potential targets and molecules modulating these targets to supplement the advances made in diagnosis of the disease. Phosphodiesterase enzyme represents one of the important emerging targets in this respect.

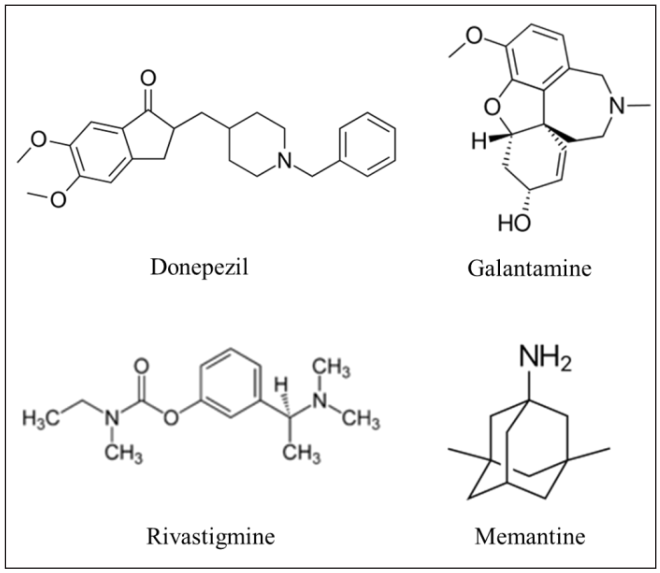

Fig. 1: Currently approved drugs for AD[16]

PHOSPHODIESTERASE FAMILY AND ITS ROLE IN AD

As shown in Fig. 2, the cyclic nucleotide phosphodiesterases (PDEs) consist of a group of enzymes that control cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) hydrolysis rates[17]. These enzymes are therefore vital signals regulators that are governed by these second messenger molecules[18]. Increase in PDE leads to decrease in cAMP[19] and cGMP, which further leads to generation of reactive oxygen species (ROS) and impaired synaptic transmission and neuronal plasticity and followed by AD[20]. Phosphodiesterases (PDEs) are a class of superenzymes which are known to degrade cAMP[21] and cGMP. The superfamily of PDEs is coded by 21 related genes and can be classified into 11 subtypes (PDE1–11). PDEs 4, 7, and 8 specifically hydrolyse cAMP among these subtypes, and PDEs 5, 6, and 9 hydrolyse GMP[22].

Both cAMP and cGMP are important second messengers in the mature brain that are directly involved in time-dependent events of memory consolidation[23]. Activation of the cAMP-protein kinase A pathway cascade triggers the activation of transcription factors such as cAMP[24] response element-binding (CREB)[21], inducing the gene transcription required to consolidate learning and memory[25]. CREB-dependent gene expression forms the basis of long-term memory formation and persistent long-term potentiation (LTP), that are the indicators of synaptic plasticity and strength.

Interestingly, specific PDE inhibitors are reported to improve memory performance in different animal models of AD. They can regulate signalling pathways by elevating levels of cAMP and/or cGMP, which can ultimately activate CREB dependent gene transcription[20,26].

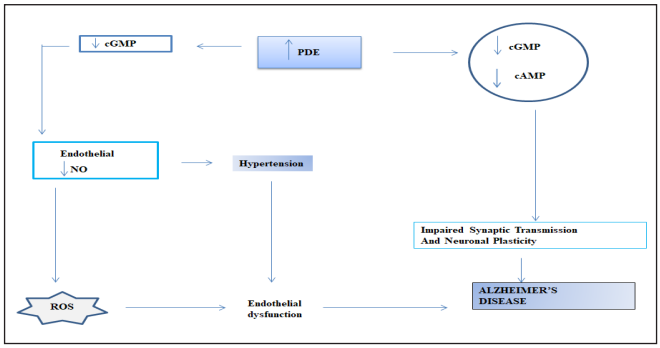

Fig. 2: Role of PDE in AD[17]

Memory enhancement of some PDE inhibitors in animal models

Vinpocetine is a specific PDE1 inhibitor and was tested as a nootropic agent[27]. It was reported to facilitate LTP, improve memory retrieval in passive avoidance in rat model and enhance cognitive performance in humans[28]. Subsequent studies indicated that vinpocetine was also involved in improving synaptic plasticity in a fetal alcohol spectrum disorder model with an impaired cortical development and sensory function[25,29].

Selective PDE2 inhibitor Bay 60-7550 has been employed to assess the role of this isoform in memory and was reported to improve memory in young and aged rats[25]. Similarly, it was shown to reverse NMDA antagonist-induced memory defects in animal models and modulate memory deficits caused by acute tryptophan depletion in rats.These studies imply that inhibition of PDE2 may augment neuronal cGMP levels[30].

PDE4 isoform has 4 subtypes, namely A, B, C and D, and these are differentially distributed in the brain[31]. The beneficial effects of PDE4 inhibitors have been exemplified with studies carried out primarily on the prototypic PDE4 inhibitor rolipram[32]. It has displayed beneficial effects on hippocampal-dependent memory, along with passive avoidance learning and in memory paradigms related to fear conditioning. Despite these, the application of rolipram in human cognition disorders has been limited by its side effects such as nausea and headache.

Several molecules such as sildenafil, tadalafil, and vardenafil, which are selective PDE5 inhibitors, are currently indicated for erectile dysfunction and for chronic human pulmonary hypertension. In the last decade, studies suggest a beneficial role of PDE5 inhibitors in cognition[33]. Memory enhancement following sildenafil treatment has been demonstrated in both rodents and primates behavioural models[34].

S14, a cell-permeable small heterocyclic molecule that can cross the blood–brain barrier, is a phosphodiesterase (PDE) 7 inhibitor. In an AD model (in APP/PS1 mice), It was previously discovered that intraperitoneal treatment with S14 provided neuroprotection. Oral administration of S14 affected amyloid (A) overload in neurons and cells. S14 improved hippocampal neurogenesis in APP/PS1 transgenic mice by targeting the cyclic adenosine monophosphate (cAMP)/cAMP-response element binding protein (CREB) pathway[26]. In APP/PS1 mice and human neuroblastoma SH-SY5Y cells co-exposed to A, S14 treatment reversed the A-induced reduction in mitochondrial mass. The restoration of mitochondrial mass was discovered to be a dual effect of S14: a rescue of mitochondrial biogenesis that had previously been slowed by A overload, and a reduction in the A-increased mitophagy mitochondrial clearance process. Therapeutic effects of the PDE7 inhibitor confirm S14 as a potential therapeutic drug for AD[35].

The next isoform of importance in memory is PDE9. Two selective PDE9 inhibitors, namely, BAY73-6691 and PF-04447943, have been reported to display enhancements in memory and synaptic plasticity along with reversal of memory deficits[21,36]. These animal model studies have highlighted the beneficial role of cGMP and/or cAMP signalling pathways in learning and memory and lead to study of some of these molecules in detail as described in the following section.

PDE inhibitors explored in preclinical and clinical studies

Cilostazol

Cilostazol, a hydro-quinolone, is a PDE 3 inhibitor which has shown encouraging memory enhancement benefits in clinical trials[37]. This drug increases cAMP levels, probably by promoting the phosphorylation of CREB[38], which is known to play a crucial role in memory enhancement and synaptic plasticity[39]. It also increases cerebral blood flow and exerts antioxidative effects. Based on these attributes, it has been tested in mice treated with Aβ, and repeated cilostazol[40] administration protected against memory impairment in these mice by preventing Aβ aggregation and accumulation of oxidative stressors[20,41–43].

Vinpocetine

Vinpocetine, a derivative of vincamine[44], an alkaloid extracted from leaves of *Vinca minor*, is a PDE 1 inhibitor reported to increase cAMP and cGMP levels[45]. Three potential benefits of this natural product include blockage of sodium channels, reduction of cellular calcium influx and antioxidant activities[46,47]. Preclinical data demonstrates that vinpocetine can rescue cognitive deficits in rodent AD model, by increase in CREB phosphorylation and brain-derived neurotrophic factor (BDNF) expression, decrease oxidative stress, mitochondrial dysfunction and apoptosis[48]. Additionally, it can inhibit GSK3β, NFκB, and the NLPR3 inflammasome, decrease levels of pro-inflammatory cytokines IL-1β, IL-6, and TNFα, and arrest the cell cycle in the G1 phase by downregulating cyclin D1 and upregulating p27.Despite these promising preclinical findings, there is very little evidence about vinpocetine for the treatment of AD specifically till date[26,49].

Nicergoline

Nicergoline, an ergot derivative, inhibits Ca^{2+}/calmodulin-dependent PDE1 and cGMP-stimulated PDE2 activity[50]. In addition, it non-competitively inhibits Ca^{2+}/Mg^{2+}-dependent brain adenosine triphosphatase (ATPase)[51]. It has a complex effect on Na^{+}/K^{+} ATPase in the brain, activating Na^{+}/K^{+} ATPase at low levels, while inhibiting it at high concentrations. It also functions as a potent antagonist of the α_{1A}adrenergic receptor[52].In preclinical studies, nicergoline[36] is reported to restore cognitive deficits in AD mice[53] along with upregulating BDNF, reversing the age-related cholinergic deficiency and increasing the release of K^{+}-induced acetylcholine. In addition to this, it reduces oxidative stress[54], inflammation and apoptosis, along with decreased levels of IL-1β, IL-6, and TNF alpha[55]. A 2019 study of 22 early AD patients concluded that combination of nicergoline[56] with AChE inhibitors such as donepezil maintained cerebral blood flow to the left temporal pole and middle cingulate gyrus relative to acetylcholine esterase inhibitors alone[54]. However, it did not result in any substantial difference in dementia severity[50]. Nicergoline was found to have a good safety and lower adverse effects[57,58].

Deprenyl/selegiline

The calmodulin-dependent PDE1A2 and monoamine oxidase (MAO)[59] are inhibited by deprenyl, also known as selegiline. Deprenyl, which was discovered by Zoltan Ecseri at the Hungarian drug company Chinoin in the 1960s, is clinically used to treat Parkinson's disease, major depressive disorder, and attention-deficit/hyperactivity disorder. Deprenyl has been found in preclinical studies to increase CREB phosphorylation[60], activate Nrf2 and catalase, decrease oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis, and potentiate activation of PARP1 induced by UV radiation while down regulating expression of PARP1 protein. A meta-analysis of 27 clinical trials showed that 14 trials met the requirement for inclusion[61–63]. It was observed that there was a statistically significant difference in cognition between selegiline and placebo at 4-6 weeks and 8-17 weeks after randomization. However, the size of the treatment difference was considered unlikely to be of clinical importance[64].The improvement in daily living behaviour was found to decrease after 6 weeks. In terms of emotional state or global reaction, there were no statistically significant variations or clinically meaningful differences between selegiline and placebo[65,66].

Denbufylline

Denbufylline inhibits PDE4, which increases cAMP[67]. There is a lack of preclinical evidence for denbufylline for unknown reasons[68], while PDE4 inhibition is arguably one of the most promising approaches for cognitive enhancement and AD treatment[69], based on the preclinical evidence and a recent analysis of preclinical evidence on PDE inhibitors for the treatment[70] of AD[71]. These preclinical effects of PDE4 inhibition[72] are also indicative of the preclinical effects of denbufylline[68].

In a randomized, multicentre, parallel, double-blind, placebo-controlled clinical trial of 45 patients with mild to moderate AD who did not take any other medicines, 100 mg of denbufylline administered twice daily for 3 months showed significant improvement in the various clinical trial scores compared to placebo. It was also shown to reduce delta activity and accelerate slow imaging activity (increased in patients with AD), indicating an increase in alertness correlated with symptomatic improvement[73], while displaying no other adverse effects.

Sildenafil

PDE5A specifically degrades guanosine cyclase induced cytoplasmic cGMP downstream of NO. Sildenafil (also known as Viagra) inhibits PDE5, thereby increasing the concentration of cytoplasmic cGMP[74].Discovered by Pfizer in the late 1980s, this drug is clinically used to treat erectile dysfunction and pulmonary arterial hypertension[75]. Additionally, it has displayed improved memory, reduction in Aβ levels and tau hyper phosphorylation along with inhibition of GSK3β and JNK. It is also involved in reduction of IL-1β, IL-6, and TNF-a secretion. It upregulates pSer133-CREB, BDNF, Arc, and Bcl2 and down regulates BACE1 AβPP, caspase-3, and Bax, along with reducing double-stranded DNA breaks and apoptotic cells in rodent models of AD[66]. In some groups of AD patients, 50 mg single dose of sildenafil showed decreased spontaneous neuronal activity in the right hippocampus, decreased cerebrovascular reactivity, and increased cerebral blood flow and oxygen metabolic rate in the brain[76]. It has been suggested that future clinical trials of sildenafil can be conducted in combination with a PDE2 inhibitor, such as propentofylline[77,78].

PF-04447943 and BI-409306

These PDE9 inhibitors have been developed by Pfizer and Boehringer Ingelheim, respectively. Pfizer's PDE9A inhibitor PF-04447943 failed to raise clinical trials scores substantially better than placebo on ADAS-Cog, the Neuropsychiatric Inventory, or the CGI-Improvement scale in a phase II multicentre, double-blind, randomized, placebo-controlled study[79]. With respect to BI-409306, the two multicenter, double-blind, parallel-group, randomized, placebo-

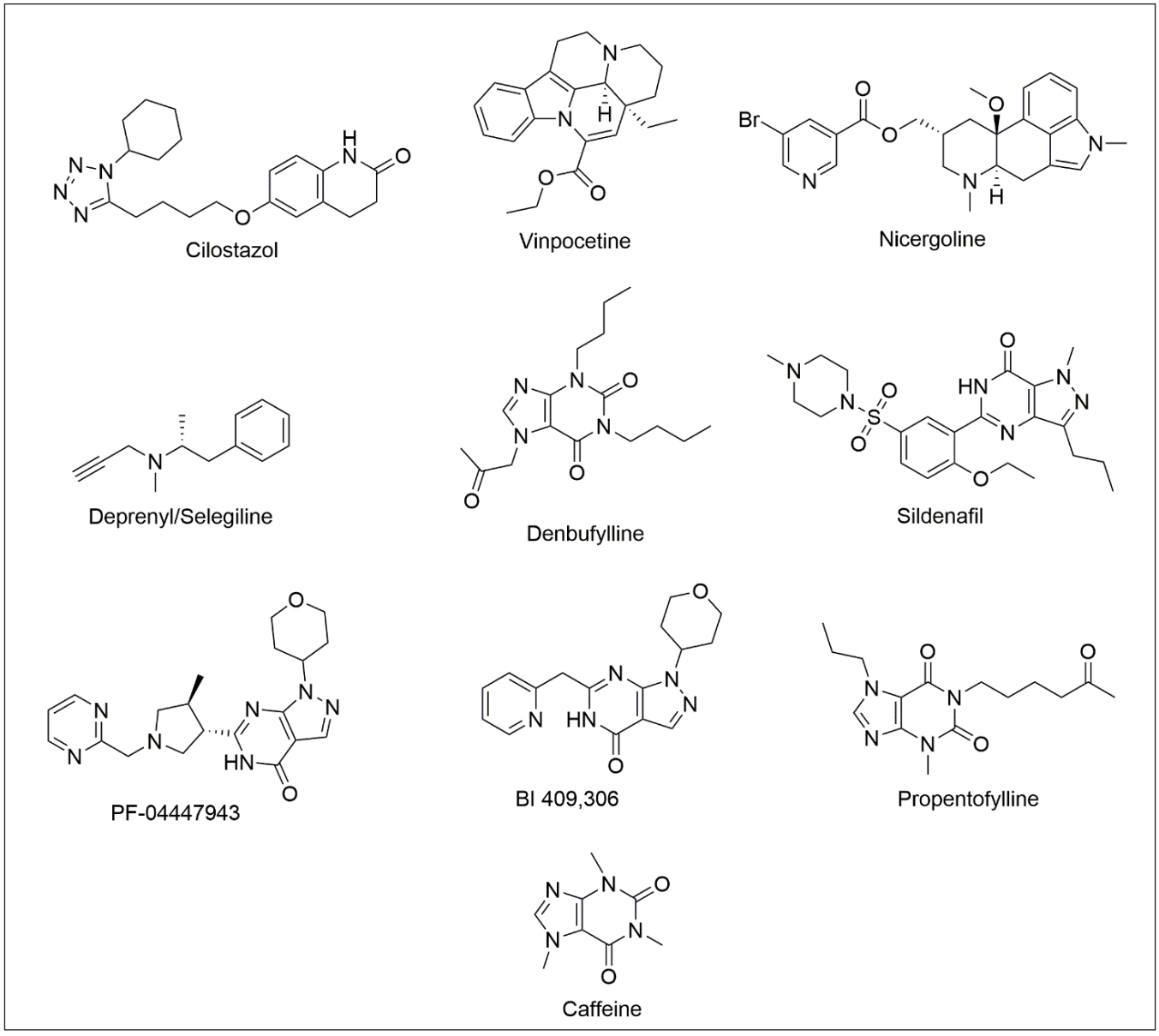

Fig. 3: Structures of PDE inhibitors[37-100]

controlled phase II studies were conducted with mild cognitive impairment (MCI) and mild to moderate AD[80] patients. This molecule did not show any significant difference on the Neuropsychological Test Battery total z-score at 12 weeks, nor on the ADAS-Cog11, the Clinical Dementia Rating scale-Sum of Boxes, or the Alzheimer's Disease Cooperative Study-Activities of Daily Living scale. PDE9 inhibition has been shown in preclinical models of AD to rescue synaptic plasticity and memory and decrease degeneration and cytotoxicity of dendritic spine density[81]. In MCI and AD patients[82] however, PDE9 inhibitors PF-04447943 and BI 409306 have been shown to be ineffective[83,84].

Caffeine

Caffeine, a derivative of methyl xanthine, is a non-specific inhibitor of PDE. In transgenic mice caffeine partially rescued protein kinase A activity in the striatum area and displayed CREB phosphorylation, reduced plasma, cortical, and hippocampal Aβ levels hand in hand with suppressing BACE1 and PS1 expression, and enhanced working memory[85]. A lower risk of incident dementia and cognitive impairment in women over the age of 65 has been associated with caffeine consumption[86,87]. Since 2016, several studies have been designed and conducted around use of caffeine in AD, some of which are described below. No significant correlation was

observed between caffeine consumption and dementia or cognitive impairment in a 2016 meta-analysis of 29,155 participants participating in 11 prospective studies, but high caffeine intake was significantly associated with a decreased risk of incident AD[88]. A J-shaped association was identified in a 2017 meta-analysis of 34, 282 individuals participating in 9 prospective cohort studies, whereby 1-2 cups of coffee daily intake was correlated with lower risk of cognitive disorders, A 2018 dose-response meta-analysis of 8 prospective studies, however, found no important correlation between the dosage of caffeine and the risk of AD or dementia[89]. A 2019 study found that in 411 cognitively-intact older adults, consuming two or more cups of coffee a day was significantly correlated with decreased Aβ plaque pressure relative to drinking less than two cups of coffee a day[90]. Therefore, a reduced risk of dementia, cognitive impairment, and/or AD may be associated with caffeine consumption[91].

Propentofylline

Propentofylline is the most promising PDE inhibitor amongst all the PDE inhibitors used in treatment of AD[92]. It is a caffeine-like methyl xanthine derivative that acts as a relatively potent and non-specific cAMP/cGMP PDE inhibitor and adenosine reuptake inhibitor. Propentofylline has been shown to suppress Aβ plaque deposition, tau hyper phosphorylation, GSK3β activation, microglial ROS generation, glutamate output, microglial IL-1β and TNF alpha secretion induced by LPS, IL-1β secretion induced by Aβ, and microglial proliferation in preclinical models.Other beneficial effects on aluminum-induced brain edema, hypoxia-like metabolic changes and Aβ-induced memory deficits have been reported for propentofylline[93].

Of the four PDE-inhibiting xanthine derivatives tested (i.e., pentoxifylline, propentofylline, torbafylline and albifylline)[94], propentofylline is the most effective inhibitor for all PDE isoforms that have been evaluated (i.e., PDE1, PDE2, PDE3 and PDE4), with specific effectiveness in inhibiting PDE2 and PDE4-stimulated cGMP.This has been described as a remarkable selectivity profile by various literature reports[95,96]. It has been hypothesized that propentofylline and sildenafil could have synergistic disease-modifying benefits for AD and vascular dementia patients by simultaneously inhibiting PDE2, PDE4, and PDE5. It has been suggested that the future preclinical and clinical research can make use of propentofylline[97], sildenafil, donepezil, and memantine in contrast to donepezil or memantine alone[98–100].

Fig. 3 displays structures of the currently approved drugs for AD along with drug candidates in clinical trials.

Physicochemical properties of drugs in therapeutic use and clinical trials and structural insights

The following Table I summarizes important physicochemical properties of the currently approved drugs for AD along with drug candidates in clinical trials. These properties were calculated using ChemBioDraw[101].

Table I: Physicochemical properties of currently used anti-AD drugs and PDE inhibitors[101]

Name	Molecular Weight	Log P	tPSA
Donepezil	379.50	4.01	38.77
Galantamine	287.15	1.41	41.93
Rivastigmine	250.17	2.36	32.78
Memantine	179.31	2.11	26.02
Cilostazol	369.47	3.47	78.65
Vinpocetine	350.46	2.98	32.78
Nicergoline	483.12	2.6	54.37
Deprenyl/Selegiline	187.14	2.79	3.24
Denbufylline	320.18	0.8	73.29
Sildenafil	474.20	2.12	106.91
PF-04447943	395.47	0.55	94.25
BI 409,306	311.35	0.22	78.65
Propentofylline	306.37	0.79	73.29
Caffeine	194.08	-0.8	56.22

In general, to cross the blood brain barrier, the physicochemical properties which are of primary importance include molecular weight (MW), log P and polar surface area. The analysis of these properties to assess CNS penetrating molecules indicate that molecular weight should be in the range of 200 to 400, log P ranging from 1.5 to 4 and polar surface area of <100 Å^2. Analysis of these properties indicates that most of these molecules satisfy these conditions and are likely to cross BBB, which poses a major hurdle in CNS drug discovery.

Structural analysis of these molecules indicates that presence of bicyclic nitrogen heterocycles is important for PDE inhibitory activity. Various other heterocycles that can be found in the structures include tetrazole, pyridine, piprazine and pyran. In some cases, a unique moiety like tetracyclic ring or propargylamine side chain can be observed. Alkyl groups of varying chain length have been used wither as linker or as substituent. These structural features can be considered while designing new and selective PDE inhibitors.

CONCLUSION

The present review discusses the role of PDE inhibitors in Alzheimer's disease. The complex pathological and biochemical features of this debilitating disease warrants research on newer targets and development of new drugs. PDE represents one of the attractive targets in this regard. Various PDE isoforms play a definite role in various aspects of cognition and memory and it is hoped that further studies can lead to development of therapeutics which modulate this target. Drug repurposing is another attractive possibility associated with this target. Several studies have already shown the beneficial effect of these inhibitors addressing various pathological features related to Aβ, tau hyperphosphorylation, antioxidant properties and neuroinflammation. In future, an in-depth study PDE biochemistry along with developing molecules against it might prove beneficial for AD therapeutics.

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