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Review Article

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Exploring the Applications of Phosphodiesterase Inhibitors Beyond Erectile Dysfunction

Ihab Mansoor¹, Mark Mikhail², Abigail Wiss², Diana Huynh², Monica Oakes², Heba Eassa³ and
Mohamed Ismail Nounou^{2,*}

¹Pharmaceutics Department, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt

²Department of Pharmaceutical Sciences, School of Pharmacy & Physician Assistant Studies (SOPPAS), University of Saint Joseph (USJ), Hartford, CT 06103, USA

³Pharmaceutics and Industrial Pharmacy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

***Corresponding Author:** Mohamed Ismail Nounou, Department of Pharmaceutical Sciences, School of Pharmacy & Physician Assistant Studies (SOPPAS), University of Saint Joseph (USJ), Hartford, CT 06103, USA, Email: nounou@usj.edu

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Abstract

Phosphodiesterases (PDEs) represent a class of enzymes that act mainly on cAMP and cGMP. The value of these enzymes has been known for long time. Molecules that target PDEs have been used in various therapeutic applications. This review aims to explore the potential uses of PDE inhibitors (PDEIs) as therapeutic agents to treat conditions that extend beyond erectile dysfunction (ED), as well as highlight novel delivery methods for PDEIs.

Keywords: Phosphodiesterase inhibitors; Alzheimer's disease; Cognition; Psoriasis; Sildenafil

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Phosphodiesterases (PDEs): Introduction

Phosphodiesterase (PDEs) are enzymes that hydrolyse intracellular signalling molecules, namely cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), which are produced in response to different stimuli [1]. Therapeutic actions of these enzymes were first identified by Henry Hyde Salter in 1886 who was interested in studying asthma. He noticed that his breathing

improved after drinking coffee which was later attributed to PDE inhibition by caffeine. Later on, theophylline (a caffeine analogue) was utilized as bronchodilator for treatment of asthma [2,3]. PDEs potential to hydrolyze cAMP was first identified in 1972 [4]. They are classified into 11 families and over 50 isoforms [5]. PDE4, 7, and 8 are cAMP-specific, while PDE5, 6, and 9 are cGMP-specific. Other PDEs can process both cyclic nucleotides or be slightly selective towards one of the them. Accordingly, PDEIs can selectively inhibit

certain PDE or non-selectively inhibit all or some PDEs. For example, theophylline is non selective inhibitor which inhibits PDE1 and PDE5. Other more potent and selective PDEIs were developed such as Nimodipine (PDE1 inhibitor), cilostamide (PDE3 inhibitor), Rolipram (PDE4 inhibitor) and sildenafil (PDE5 inhibitor) [6].

PDEIs as therapeutic agents

PDE inhibitors (PDEIs) increase the intracellular levels of cAMP and cGMP. In the last decades, researchers and pharmaceutical companies have been able to develop selective and potent PDEIs. Many of these inhibitors

have similar structure to the endogenous substrates [7]. Theophylline was described as PDEI in 1972 and since that time, many inhibitors have been discovered and approved for use [6]. Sildenafil citrate was developed by Pfizer in 1989 to inhibit PDE5, an enzyme that is present in platelets and vascular smooth muscle and was thought to be a good target for angina [8]. Penile erection was a common side effect of sildenafil which led to its approval as a treatment for ED in 1998. Targeting PDE isoforms has been employed in treating various disease states. Figure 1 summarizes numerous application of PDEIs beyond male enhancement.

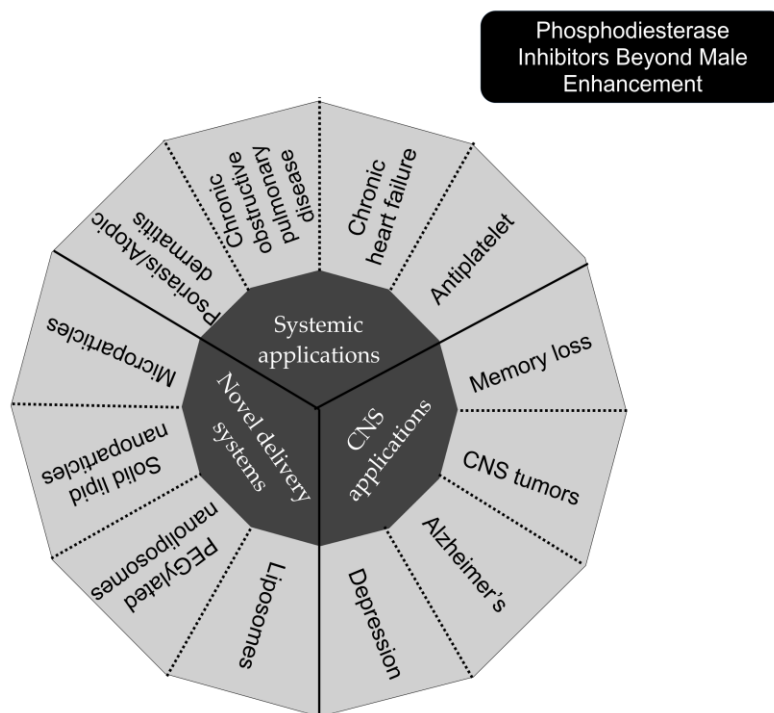


Figure 1: Different therapeutic application of PDEIs beyond male enhancement.

Systemic application

Antiplatelet effects

Platelets play an important role in the homeostasis process; they are responsible for preventing hemorrhage through triggering the coagulation cascade [9,10]. Pathological

conditions can abnormally trigger platelet aggregation leading to thrombi formation which cause high morbidity and mortality due to conditions such as myocardial infarction and pulmonary embolism [9-11]. Platelet aggregation can be inhibited via multiple mechanisms and by multiple medications such as the blockage of membrane-bound receptors by clopidogrel and prasugrel [12-13]. PDE2, 3,

and 5 are present in platelets and are responsible for catalyzing the hydrolysis of cAMP and cGMP which act as secondary messengers for platelet modulation; thus, allowing agents such as cilostazol which is a PDE3 inhibitor to be utilized as an antiplatelet medication for patients with peripheral arterial disease and following thromboembolic stroke [12-16]. PDE5 inhibitors (PDE5is), such as sildenafil, potentiate nitric oxide (NO) activity; this synergistic effect has been shown to influence in-vitro platelet aggregation. Gudmundsdottir *et al* [17]. measured platelet aggregation in human peripheral blood in the presence of sildenafil and 2 NO donors and found that both NO donors caused concentration-dependent inhibition of platelet aggregation, and that sildenafil potentiated the effects of both donors in various proportions [17]. In another study, Berkels *et al* [18]. found that sildenafil administered to healthy men may inhibit platelet aggregation. Moreover, the addition of a NO donor to sildenafil resulted in further inhibition of platelet aggregation [18]. These studies provide evidence of the potential antiplatelet applications of PDE5is.

Chronic obstructive pulmonary disease (COPD)

COPD is an inflammatory disease of the lungs that is characterized by reduced and obstructed airflow [19]. Exposure to smoke and environmental pollution can lead to pulmonary hypertension (PH) [20]. PH, a possible complication of COPD, reduces lung function, exercise ability, and quality of life. As the disease progresses, symptom exacerbation increases until death [21]. COPD is an irreversible condition and the goals of treatment are to reduce inflammation and induce smooth muscle relaxation [19]. PDEIs have been investigated as potential treatments for COPD and PH. Inhibition of PDE4 and 5 in pulmonary arteries has been shown to produce vasodilation. Inhibitors of PDE4 and 5 have been proven to reduce airway reactivity to histamine, therefore inflammation and subsequently PH [22]. A randomized trial

conducted on 278 patients with PH found that patients who received sildenafil displayed a 6-minute increase in walking distance; this trial led to the FDA approval of sildenafil in treating pulmonary arterial hypertension (PAH). A similar study found that tadalafil had similar efficacy, but the effect was transient when compared to sildenafil [23]. Sildenafil has also been examined for effect on stroke volume during exercise. The results did not show significant improvement after 3 months of treatment [24]. Combining tadalafil with roflumilast, a PDE4 inhibitor (PDE4i) has demonstrated a significant decrease in airway resistance and reactivity in animal studies [22].

Chronic heart failure (CHF)

CHF can be defined as a clinical defect in the structure or function of the myocardium [25]. PDE5 expression in the myocardium stimulates the heart [26]. A study conducted on 34 patients suffering from CHF found that compared to placebo, sildenafil reduced changes in VO₂ from baseline and reduced pulmonary vascular resistance while increasing cardiac output with exercise, which translated into improved exercise capacity and quality of life (QoL) [26-28]. Another study that was conducted on 106 patients demonstrated that sildenafil improved heart failure without decreasing blood pressure [29]. Overall, PDE5is show promising results in symptoms management and improvement of QoL in patients suffering from CHF.

Psoriasis, psoriatic arthritis, and atopic dermatitis

Psoriasis is an inflammatory condition that affects the skin and joints and is characterized by plaque formation. A significant proportion of psoriasis patients also have psoriatic arthritis [30]. PDE4, present in immune cell, is an effective target in treating psoriasis, therefore, medications that target it have been approved to treat the condition. PDE4 regulates the inflammatory pathway in several types of cells; thus, its inhibition reduces inflammation and slows down plaque formation [31,32]. Atopic

dermatitis (AD) is a relapsing-remitting inflammatory skin disease with pruritic lesions. There are several hypotheses relating to the origin of the condition such as water loss or imbalanced immunity [33]. Treatment modalities of AD present with limiting problems of adverse events; therefore, developing topical and systemic PDE4is may be the solution [34]. A pilot study conducted on 16 adults demonstrated that apremilast, a PDE4i, reduced symptom severity in patients suffering from AD. In another study, apremilast showed a reduction in AD lesions at higher doses, but was associated with frequent adverse effects [21]. PDE4 inhibition is limited by its side effect, therefore novel topical agents such as crisaborole have been developed. In a study examining the latter therapy in AD patients aged 2 years and above, disease severity was reduced and adverse events were less frequent. In another study on the same medication, QoL was improved when compared to placebo [35]. Improvement from baseline eczema area and severity index score have been observed as early as week 1 with OPA15406, an agent that is highly selective for PDE4B. The results highlight that topical agents have favorable safety and efficacy profiles [36].

CNS application

Depression

Depression is a condition that affects 150 million people and is characterized by sadness and loss of interest. The condition is often underdiagnosed and undertreated [37]. There are many etiologies associated with the condition [38]. Current therapeutic agents include selective serotonin reuptake inhibitors, tricyclic antidepressants, and monoamine oxidase inhibitors [39]. These medications act on various signaling neurotransmitters. The effects of PDE5is on depression are not fully understood yet, but newer trials have shown their efficacy in alleviating the depressive symptoms; this sheds light on potential benefits for patients who suffer from ED and depression,

or patients who exhausted all means of traditional depression therapies. Results of a study that was performed on 40 male patients undergoing dialysis and suffering from ED have demonstrated that treatment with sildenafil or vardenafil have led to a statistically significant improvement in depression symptoms compared to pretreatment levels [40]. Moreover, a study that was conducted on 202 men suffering from ED and untreated depression, sildenafil citrate has demonstrated significant improvement in depression symptoms from baseline [41]. In another study conducted on 152 males, results demonstrated that patients who received sildenafil had a mean decrease of 10.6 in Hamilton depression scale scores vs. 2.3 mean score decrease in those receiving placebo [42]. Results from these trials reflect the potential benefits of PDE5is in treating depression.

Central nervous system (CNS) tumors

CNS tumors originate from exponential proliferation of mass tissue in the brain. CNS tumors are classified into primary and metastatic tumors. The latter affects around 150,000 people annually. Other conditions such as lung cancer contribute to the disease, as such, lung cancer leads to brain metastasis in ~40% of patients [43]. Various treatment modalities exist for CNS tumors including surgery, radiation, and others. Investigational therapies such as gene therapy, targeted toxins, and PDE5is offer a new hope as alternative treatments for brain tumors [44]. The latter have shown potential in improving blood brain barrier (BBB) permeability. PDE5Is can increase tumor sensitivity to standard treatment by preventing tumorigenesis and blocking drug efflux. They act as drug vehicles for cancer therapies to penetrate the BBB [45]. Vardenafil has been shown to increase drug uptake and transport of Herceptin in-vitro and in-vivo mice, and the combination has resulted in significantly longer survival time compared to untreated and Herceptin-treated mice suffering from intracranial lung cancer [44]. The combination has also resulted in significant

survival increase in mice suffering from intracranial breast tumor. The combination was beneficial to mice with high HER2+ expression only [44]. Administration of PDE5Is and an inhibitor of cGMP-specific PDE5 in rat brain tumor model has been shown to increase capillary permeability in gliosarcoma-bearing rats with no significant increase in normal brain capillaries [45]. Compared to Adriamycin only treated mice, those treated with the former and vardenafil had significantly longer survival. Another study that was conducted on a pediatric population established that adjunct chemotherapy with PDE5is performed greater when added with vincristine/etoposide/cisplatin combination to cause cell death in medulloblastoma cells [46]. The combination produced autophagy at the 12-hour mark post-treatment and enhanced the induction of chemotherapy-induced DNA damage. A patent that was published in 2009 claimed that sildenafil enhanced the permeability of the mammal BBB for a short period of time following administration, therefore allowing increased penetration of chemotherapeutic agents [40]. Current developments and investigational studies in PDE5is have shown enhanced lethality of chemotherapy on CNS tumor cells, thus highlighting their potential applications.

Alzheimer's Disease (AD)

AD is the most common form of dementia affecting millions of patients worldwide [47]. AD is characterized by β -amyloid plaques and hyperphosphorylation of Tau. The former is protein fragment that is eliminated in healthy brains and the latter is a component of mature neurons [48]. AD is a progressive irreversible disease that affects memory and thinking skills [47]. There is no cure for AD; failure to find a cure has resulted in a shift towards non-amyloid-based approaches, one of such with promising results is the use of PDEIs [49,50]. PDE5 enzymes are present in the brain and are critical in neural control [47,50,51]. A study conducted by Teich *et al* [52]. to confirm the presence of PDE5 in human neurons detected

its presence using various techniques [52]. PDE5is have shown efficacy in different neurological conditions due to its ability to produce anti-inflammatory and neuroprotective effects [47]. PDE5is increase NO synthases, accumulate cGMP, and activate protein kinase G. These pathways play important roles in diseases such as AD, Parkinson's disease, and multiple sclerosis. The predicted effect of PDE5is based on the mechanism of action is to reduce the degeneration process of the neuron [47]. FDA reports and studies have demonstrated that sildenafil and vardenafil can cross the BBB making them candidates for AD [53-54]. One mechanism through which PDE5is improve AD symptoms is the upregulation of cAMP and/or cGMP. Regulation of the latter promotes increased cerebral blood flow [55]. A study that examined the effects of sildenafil on astrocytes found that sildenafil reduced Ca^{2+} response. Astrocytes regulate the formation and functioning of the BBB and play an important role in regulating cerebral blood flow which affects cognition and memory [56]. Another novel mechanism through which PDE5is improve AD symptoms is by promoting gene transcription through activating cAMP response element-binding (CREB). Activation of the latter signaling pathway by PDEIs may ameliorate AD symptoms through restoring synaptic function [57]. CREB phosphorylation leads to transcription of memory-associated genes and its disruption leads to memory impairment [58].

PDE5 effect on cognition

Cognitive decline is one of the most common signs and symptoms of AD [59]. There is no treatment for AD and the goal of pharmacotherapy is to slow down the decline of cognitive function [60]. The results of a study conducted on 27 patients with ED to assess the effect of repeated dosing of udenafil on cognition revealed that the scores of International Index of Erectile Dysfunction, frontal assessment battery, Seoul verbal learning test, and physical health questionnaires have significantly improved from baseline after

2 months of treatment highlighting that repeated dosing of PDE5is increases cognition [61]. Cognition improvement has been also confirmed in another study. A study that was performed on 10 healthy young males who received an oral dose of 100 mg revealed that the participants showed enhanced ability to focus attention and select relevant target stimuli in auditory tasks [62]. Another study conducted on male Swiss mice who received sildenafil found that the 3mg/kg dose demonstrated a significant improvement in cognition function and memory retention [63]. Another study found that PDE5is were able to reverse age-induced retention deficits of mice and helped improve cognition. These studies highlight the potential of PDE5is in slowing down cognitive decline making it a possible pharmacological option for decreasing AD symptoms.

PDE5 effect on memory loss

Memory loss is one of the first symptoms of AD [64]. Many studies conducted on mice demonstrated how the use of PDE5is can slow it down. One study showed that sildenafil has anti-AD effects when given at advanced stages of the disease in mouse models. The results of the latter study highlighted that sildenafil reduced Tau hyperphosphorylation, decreased glycogen synthase kinase 3 β activity and reduced cyclin-dependent kinase 5. Moreover, sildenafil also increased levels of brain-derived neurotrophic factor and the activity-regulated cytoskeletal-associated protein [65]. Another study conducted on mice found that sildenafil enhances phosphorylation of CREB through cGMP elevations leading to beneficial outcomes in amyloid disposition mouse model [66]. In another study, tadalafil was found to reverse memory deficits in mouse models. PDE5is main activity was towards Tau pathology [67]. Another study conducted on mice found that sildenafil influenced retention by modulating time-dependent mechanisms involving memory storage [67,68]. Therefore, the administration of sildenafil 30 minutes prior to training enhanced retention performance. PDE5is have demonstrated through studies on

humans, mice, and primates promising results on memory and cognition impairment that are associated with AD highlighting their importance and potential use for AD management.

Novel delivery systems for PDE5is

Optimal uterine endometrial thickness is essential for successful pregnancy. Endometrial thickness depends on blood flow among other factors. Refai *et al* [69], investigated the use of sildenafil-loaded liposomes in improving uterine blood flow through vaginal delivery of the drug in rats. The histopathological examination of the rats that received chitosan-coated sildenafil liposomes demonstrated that this group had greater dilatation and congestion of the endometrial blood vessels, as well as increased endometrial layer thickness compared to the control group. The results of this study highlighted the potential benefits obtained from using chitosan-coated liposomal formulation [69]. Pulmonary arterial hypertension (PAH) is a condition that is characterized by increased pulmonary vascular resistance and vascular remodeling. The progressive thickening of the pulmonary arteries (PAs) is mainly caused by proliferation of the smooth muscle cells. Li *et al* [70], hypothesized that targeting overexpressed glucose transport-1 (GLUT-1) on PAs smooth muscle cells would result in higher delivery of apoptosis-inducing drugs; thus, inhibit the proliferation of these muscle cells and reverse monocrotaline-induced PAH in rats. To achieve the objective, the investigators associated sildenafil-loaded liposomes with glucuronic acid (GlcA) resulting in glucuronic acid modified liposome (Glc-Lip). Results of the study demonstrated that Glc-Lips loaded with sildenafil significantly inhibited PA remodeling and reduced PA pressure by 32.4%, medial thickening by 41.3%, and improved right ventricular hypertrophy by 44%. This study highlighted new opportunities for using targeted therapies in treating PAH [70]. A study conducted by Shahin *et al* [71], evaluated the effectiveness of using sildenafil-loaded

hydrogel microparticles inhaler in treating PAH. The investigators hypothesized that the latter formulation would provide improved lung disposition, controlled release, and superior local and systemic kinetic profile compared to oral formulation. In-vivo experiments were conducted on male albino rats. The results of the study demonstrated that compared to the oral formulation, the inhaled preparation showed fast initial drug release in the lungs, higher drug concentration, significantly higher (by 4-fold) relative drug bioavailability, and significantly higher C_{max}. Additionally, the inhaled formulation displayed higher half-life and almost double mean residence time (MRT) value. Mean PA pressure was reduced by 21-42% with the inhaled formulation. In summary, the results show that the formulated inhalable spray-dried hydrogel microparticles can potentially be considered as an alternative to oral route in treating PAH [71]. Wound healing is a process that spans 3 phases including inflammation, proliferation, and remodeling. Kulshrestha *et al* [72]. evaluated the effectiveness of topical sildenafil formulations in promoting wound healing, as well as investigated their dermal toxicity. The study was conducted on female Sprague Dawley rats using various sildenafil concentrations. The results demonstrated that none of the concentrations caused adverse events and skin primary irritation index (PII) decreased with increasing sildenafil concentration with the 3% formula exhibiting similar PII to that of placebo. Additionally, the 5% and 10% concentrations demonstrated equivalent and higher healing rates than the 3% concentration. The re-epithelization rate was found to be highest in the 5% and 10% concentration groups compared to the control group. The effects of formulations on NO levels were significantly high in the 5% and 10% concentration groups. Similarly, the 5 and 10% concentrations were found to exhibit high and significant effects on increasing the content of collagen, hydroxyproline, and total protein. In conclusion, the results demonstrate that the hydrogel formulations of sildenafil were safe and effective in promoting skin healing in

traumatic wounds.⁷² Thyroid tumors represent a rising global challenge. Despite the majority being well differentiated and can be treated with radiation and surgery, there is a minority that is less differentiated with poor response to radiation and surgery. De Rose *et al* [73]. demonstrated that blocking overexpressed PDE-5 with sildenafil or tadalafil inhibited the proliferation of thyroid cancer cells. In this study, the investigators, with regard to free drug, tested the antiproliferative properties of PEGylated nanoliposomal preparations of sildenafil and tadalafil on TPC-1 and BCPAP human thyroid cancer cell lines. Encapsulated drugs demonstrated significant antiproliferative activity even at 1 μ M in TPC-1 cell line. However, in the BCPAP cell line, much more significant inhibition of growth was obtained with tadalafil nanoliposome at a concentration of 0.1 μ M. In summary, the study demonstrated that nanoformulations of sildenafil and tadalafil enhanced the antiproliferative properties of these drugs [73]. Avanafil is a drug that has been approved by the FDA and the European Medicines Agency (EMA) for treating erectile dysfunction. Kurakula *et al.* investigated methods to improve the solubility and bioavailability profiles of avanafil through using solid-lipid nanoparticles (SLNs) technique. Ex-vivo permeation study was conducted on rats' skin. The results of the study demonstrated that after 24 hours, the proportion of the drug that permeated from the HPMC formula was higher compared to the chitosan formula. In addition, SLNs were efficiently delivered to the deeper layers of the skin as seen through the confocal laser scanning microscopy; this finding suggests that avanafil SLNs penetration into dermal tissue is expected to improve bioavailability of the drug. In conclusion, the study highlights the potential benefits that could be obtained from using this route as an alternative for oral administration [74]. Cancer is a global problem. Multidrug treatment represents a valuable strategy to improve efficacy. Combination therapy comes with various problems ranging from molecule-molecule interaction to severe adverse events. de Melo-Dogo *et al* [75]. evaluated the impact

of incorporating 2 chemotherapeutic agents (crizotinib and palbociclib) with sildenafil in a nanocarrier made of amphiphilic polymeric micelles on the cytotoxic efficacy of the 2 drugs. The formulation was intended to tackle the hallmarks of non-small cell lung cancer (NSCLC) and drug-resistance mechanisms. The cytotoxic efficacy of the formulation was tested on non-small human lung adenocarcinoma cell line (A549). Results demonstrated that the cytotoxic effects of the triple combination resulted in a significant decrease in tumor cell viability (1.78-fold decrease). Moreover, the triple combination produced a synergistic effect and exhibited extensive cytotoxic activity. In summary, the study demonstrated the cytotoxic efficacy of the nanocarrier encapsulated triple therapy in lung cancer cells highlighting its potential use in treating NSCLC [75]. In another study involving cancer that was conducted by Marques *et al* [76], the cytotoxic efficacy of micellar vehicles containing crizotinib and sildenafil on breast cancer cells was evaluated. The authors hypothesized that incorporating the 2 latter agents in a PEG-PLA micellar carrier would produce a synergistic cytotoxic effect on MCF-7 breast cancer adenocarcinoma cell line. Results revealed that after 48 hours, the viable breast cancer cells were reduced by 96% with the sildenafil-crizotinib combination micellar formula, which highlights the synergistic effects between the 2 drugs. Moreover, the results of the study revealed a significant improvement in intracellular bioavailability as well as hemo- and biocompatibility of the formulation. In summary, incorporating sildenafil and crizotinib in nanomicelles resulted in synergistic cytotoxic activity on breast cancer cells highlighting the potential benefits of the formulation in cancer therapy [76].

Conclusion

PDEs have been extensively studied as targets for treating of a wide range of disease states. Their potential application in condition such as AD, COPD, and CHF has been demonstrated in

various in-vitro and in-vivo studies. With the promising results of these studies, one can conclude that new potential applications of PDEIs are likely to arise.

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