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Review

Phosphodiesterase 5 (PDE5): Structure-function regulation and therapeutic applications of inhibitors

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ABSTRACT

Phosphodiesterase 5 (PDE5) is one of the most well-studied phosphodiesterases (PDEs) that specifically targets cGMP typically generated by nitric oxide (NO)-mediated activation of the soluble guanylyl cyclase. Given the crucial role of cGMP generated through the activation of this cellular signaling pathway in a variety of physiologically processes, pharmacological inhibition of PDE5 has been demonstrated to have several therapeutic applications including erectile dysfunction and pulmonary arterial hypertension. While they are designed to inhibit PDE5, the inhibitors show different affinities and specificities against all PDE subtypes. Additionally, they have been shown to induce allosteric structural changes in the protein. These are mostly attributed to their chemical structure and, therefore, binding interactions with PDE catalytic domains. Therefore, understanding how these inhibitors interact with PDE5 and the structural basis of their selectivity is critically important for the design of novel, highly selective PDE5 inhibitors. Here, we review the structure of PDE5, how its function is regulated, and discuss the clinically available inhibitors that target phosphodiesterase 5, aiming to better understand the structural bases of their affinity and specificity. We also discuss the therapeutic indications of these inhibitors and the potential of repurposing for a wider range of clinical applications.

1. Introduction

The cGMP-binding, cGMP-specific phosphodiesterase 5 (PDE5) is a key regulator of cGMP signaling in the cardiovascular and other tissues. Typically, cGMP signal transduction pathway in a cell is initiated by ligand-mediated activation of a guanylyl cyclase (GC) enzyme, either soluble or receptor, resulting in an increased production of cGMP, which exerts its effect through activating effectors such as cGMP-dependent protein kinase G (PKG) and cGMP-regulated ion channels (Fig. 1). In this way, cGMP signaling pathway regulates a number of physiological processes including vascular tone, visual signal transduction, energy metabolism, renal function, intestinal fluid secretion, gut peristalsis, lipolysis, oocyte maturation, cerebellar motor control, transcription, cell growth, cell motility, anti-inflammatory activity, and apoptosis [1–9]. Several of these processes are regulated by the action of PDE5, which is allosterically activated by the increased level of cGMP in these cells via cGMP binding to its regulatory domain, resulting in an enhanced activity of the PDE5 catalytic domain, and subsequently, bringing the intracellular cGMP concentration to the basal levels. As a prerequisite for its function, PDE5 is expressed in a variety of tissues including the lung, brain, kidney, cardiac myocytes, gastrointestinal tissue, vascular smooth muscle cells, platelets, and penile corpus cavernosum [7,10–19]. In addition to the cGMP-mediated allosteric activation, PDE5 function is regulated at the genetic level through expression of various isoforms as well as by post-translational modifications such as phosphorylation, which also results in its activation [12,20] and nitrosylation, which results in its degradation through the ubiquitin pathway [21].

Specifically, PDE5 targets cGMP generated by nitric oxide (NO)-activated soluble GC [22] by catalyzing the hydrolysis of phosphodiester bond in cGMP through its catalytic domain, thereby converting cGMP to the inactive 5'-GMP form [23] (Fig. 1). In addition to the catalytic domain, which is located at the C-terminal of the protein, PDE5 contains regulatory GAF (mammalian c**G**MP-dependent phosphodiesterase, ***A**nabaena* adenylyl cyclase and *E. coli* **F**hlA) domains (GAFa-GAFb) in tandem in the N-terminal side. While structurally similar, the GAF domains in PDE5 play distinct roles. The N-terminal GAFa domain binds cGMP and allosterically modulates the catalytic domain activity [24], while the C-terminal GAFb domain plays a role in the dimerization of the enzyme [25].

Given its critical role in cGMP signaling, PDE5 has been

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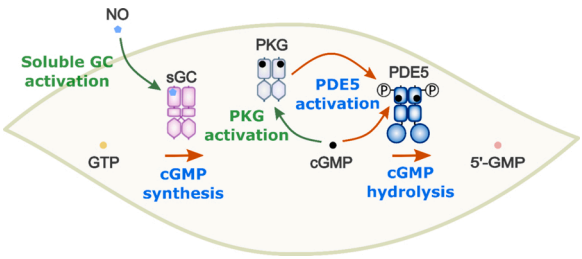

Fig. 1. Role of PDE5 in cGMP signaling pathway. cGMP is synthesized from GTP by the action of the NO-activated sGC. cGMP activates PDE5 directly by binding its GAFa domain, and indirectly by activating PDK which in turn phosphorylates PDE5 and enhances cGMP binding to the catalytic domain and its subsequent hydrolysis. Showing in the figure are the protein dimers.

pharmacologically targeted for the treatment of a number of pathological conditions including erectile dysfunction and pulmonary arterial hypertension [8,26,27]. The development and successful clinical application of a number of PDE5 inhibitors, such as sildenafil, to regulate cGMP levels in penile corpus cavernosum for the treatment of erectile dysfunction is a prime example of pharmacological targeting of proteins in the cGMP signal transduction pathway [28–31]. Importantly, these inhibitors of PDE5 were later approved for other clinical applications, such as pulmonary arterial hypertension (PAH) and lower urinary tract symptoms (LUTS) [32–34], while efforts to expand their clinical use to other diseases are still on going. In this review article, we outline the role of PDE5 in the cGMP signal transduction pathway and describe its inhibitors that are in clinical use. Specifically, we will describe the structural features of the protein, how its activity is regulated, and the pharmacological inhibitors that are used in its targeting. We envisage that this overview will highlight the mechanistic approach by which PDE5 inhibitors selectively bind to the enzyme and will guide future endeavors towards development of selective and potent inhibitors of this key cGMP regulator.

2. Structural and regulatory features in PDE5

PDE5 is a multidomain protein and can be divided into a regulatory part and a catalytic domain. The regulatory part is located at the N-terminal side and consists of two GAF (GAFa & GAFb) domains in tandem which are involved in controlling the catalytic activity as well as dimerization of the protein [35]. While multiple genes could encode a specific PDE family member, *PDE5A* is the only gene that encodes PDE5 protein and is expressed as three isoforms; PDE5A1, PDE5A2, and PDE5A3 [36,37]. All of these PDE5 isoforms share the exact same catalytic domain [38] but differ in the length of the N-terminal regulatory part [39]. Additionally, while PDE5A1 and PDE5A2 are widely expressed in a number of tissues, PDE5A3 is explicitly expressed in the vascular smooth muscle cells [12], thus providing additional layers of regulation.

The regulatory GAF domains in PDE5 are a member of a conserved family of regulatory domains that are found in a wide variety of proteins including several PDEs, adenylyl cyclases, and transcription factors [40]. Only one GAF domain of each PDE monomer binds cyclic nucleotide and in PDE5, GAFa is the cGMP-binding domain that mediates allosteric regulation of PDE5 activity [24,30,41]. In this respect, PDE5 is similar to PDE2, PDE6, PDE10 & PDE11 [24]. The unliganded GAFa domain of PDE5 exhibits high flexibility, indicating its capacity for large conformational change upon cGMP binding [53] . In fact, cGMP interacts by two hydrogen bonds - through its guanine group - with an aspartic acid residue present in the GAFa domain. This residue has been suggested to play a key role in the nucleotide selectivity of the GAFa domain, as mutating it disrupts the cGMP binding and increases the affinity for cAMP [53] . In further support of this, the aspartic acid is conserved in the GAFa of PDE11A and GAFb of PDE2A, both of which show a preference towards cGMP binding [42]. Mutation of other GAFa domain residues that are involved in cGMP binding render the isolated GAFa domain unable to change conformation in response to cGMP but does not affect the catalytic activity of the full length PDE5 [29].

A six-stranded anti-parallel β-sheet core (strand order – 3−2-1−6-5−4) and four α-helices form the GAF domains. The core β-sheet divides the plane into two faces with helices α2 and α5 on one face and helices α3 and α4 on other face. The helices α3-α4 face forms the cyclic nucleotide-binding site. The GAFa domain of PDE5A has a short α-helical turn, α2/3 that lies between β2 and β3. Some biochemical and X-ray crystallographic studies have suggested the role of strand β3 and helix α4 as lids that gate the cGMP binding pocket of GAFa domain [42]. Ueda et al. (2019) reported a PDE5A regulatory domain polymorphism (E90 K) in healthy dogs that they claimed to affect basal circulating cGMP concentration [43]. Such polymorphic variation in the regulatory domain makes it an attractive target for precision medicine. The GAFa domain of PDE5 was also reported to sequester and serve as a sink for cGMP [30,44]. In addition, the GAFa domain affinity for cGMP was described to be increased by substrate binding to the catalytic domain, with evidence that this increase in affinity and the decrease in cGMP off-rate from the GAFa domain is brought about by substrate-induced conformational change upon binding to the catalytic site, resulting in more efficient sequestration of cGMP by the GAFa domain [45]. Pandit et al. in 2009 had proposed that cGMP binding to the N-terminal regulatory domain of PDE regulates its catalytic activity by means of controlling access to the substrate binding pocket in the catalytic domain [46]. Their proposed model was based on analysis of the X-ray crystal structure of a PDE dimer that includes both catalytic and cGMP-binding regulatory domains of PDE2A. Similar to PDE5, cGMP binding to the N-terminal regulatory domain of PDE2 activates its catalytic domain. However, the allosteric regulation in PDE2 occurs through cGMP binding to the GAFb domain rather than to the N-terminal GAFa domain seen in PDE5. Structural analysis of the PDE2A dimer and site-specific mutations revealed that the catalytic domain H-loop of the dimer partner occludes substrate access to the active site when the enzyme is in the closed configuration, and cGMP binding to the GAF domain causes the two catalytic domains to swing out from each other (open configuration) and, therefore, relieving the catalytic domain inhibition, and facilitating access of the substrate to the substrate-binding pocket. It is strongly believed that such allosteric regulation also applies to the dimeric PDE5 through cGMP binding to the GAFa domain since the H-loop is a common feature to all PDEs. H-loop, a critical modulator of allosteric regulation of PDE5 activity [47] and substrate binding [48,49], is previously reported to be present in the crystal structure of PDE5 catalytic domain. Gulati et al. (2019) inferred a similar allosteric regulation model from analysis of the cryo-electron microscopy of the full length PDE6αβ2γ in which cGMP binding to the GAFa domain enhances PDE6γ subunit binding to the catalytic domain through its C-terminal residues, thereby stabilizing the open state conformation of PDE6αβ2γ, leading to the occlusion of the substrate-binding pocket and inhibition of the catalytic activity [50]. Understanding the structural regulations of catalytic site activity of PDE6 by the regulatory domain is of vital importance in the design of selective PDE5 inhibitors as many of the side effects exhibited by these inhibitors are mediated by binding to the closely related PDE6 enzyme [51,52].

In addition to structural studies of the apo- and holo ([53])-GAFa domain of PDE5, the cGMP-induced conformational change in the PDE5 GAFa domain has also been demonstrated using an intramolecular Bioluminescence Resonance Energy Transfer (BRET) [29,30,54,55] and Fluorescence Resonance Energy Transfer (FRET) [56] constructs. However, allosteric inhibitors of the regulatory site have not been described yet, although Zhang et al. (2020) have described allosteric inhibitors that bind the catalytic domain of PDE5 [57]. The GAFa domain allosteric inhibitors would have the advantage of inhibiting the GAFa-mediated stimulation of the catalytic domain rather than inhibiting the basal PDE5 catalytic activity as seen with conventional catalytic-site binding

PDE5 inhibitors [29,30].

On the other hand, the GAFb domain contributes to dimerization of PDE5 [25]. Another region of about 20 amino acids to the N-terminal side of GAFa was also found to be involved in the dimerization [53]. The GAFb domain was also reported to increase the binding affinity of the catalytic domain to the PDE5 inhibitor, vardenafil and vardenafil-based analogues, although it had no effect on the binding affinity of other inhibitors such as sildenafil and tadalafil [25].

In addition to the GAF domains, the N-terminal regulatory region of PDE5 has a cGMP-dependent protein kinase (PKG) phosphorylation site which is conserved across all PDE5 isoforms (PDE5A1, PDE5A2 and PDE5A3). The phosphorylation was reported to enhance the catalytic activity of the enzyme as well as cGMP binding to the GAFa domain [58–60]. It was also reported that cGMP binding to the GAFa domain induces a conformational change in the enzyme that exposes the phosphorylation site [31]. Although it does not bind cGMP, GAFb domain still deemed necessary for this cGMP-activated serine phosphorylation of PDE5 [61].

The catalytic domain of PDE5 is located at the C-terminal of the protein and shares homology with all the other 10 PDEs encoded in the mammalian genome [62]. Crystallographic studies have illustrated the presence of three helical sub-domains in the PDE5 catalytic domain: a N-terminal cyclin-fold region, a linker domain, and a C-terminal helical bundle. The C-terminal helical bundle adopts the substrate pocket at its center, which is composed of four subsites: M site (metal-binding site), Q pocket (core pocket), H pocket (hydrophobic pocket) and L region (lid region) [63]. The M site at the bottom of the pocket contains zinc and magnesium ions that coordinate with the invariant metal-binding residues of PDE5, thus stabilizing the structure and activating the active-site hydroxide to facilitate cGMP hydrolysis. This site has a zinc coordination sphere with three His residues, an Asp residue, and two water molecules, and a magnesium coordination sphere which consists of the same aspartate and five water molecules, out of which, one is shared with zinc moiety. In this way, the M site creates an octahedral metal ion coordination geometry. Additionally, the M site interacts directly with the cGMP substrate, and indirectly with the inhibitors [63].

Binding of cGMP to the active site occurs through direct coordination bonds between two oxygen atoms of the cyclic phosphate group of cGMP on one side and the Zn^{2+} and Mg^{2+} ions present in the M-subsite on the other side. The Zn^{2+} is also critical for catalysis as it acts as a Lewis acid and activates a hydroxyl group - derived from a water molecule that bridges the metal ions – that is inserted at the cyclic phosphate group of cGMP hydrolyzing the phosphodiester bond. The Q pocket, on the other hand, accommodates the guanidine group of cGMP and the guanidine-mimicking group of PDE5 inhibitors. It is a highly conserved region in the PDE5 catalytic domain and includes the invariant Gln817, Phe820, Val782 and Tyr612 amino acid residues [63]. The γ-amide group of the conserved Gln817 forms bidentate hydrogen bond with the guanine group of cGMP, stabilizing cGMP structure in the active site [63]. Other residues in this pocket form hydrophobic interaction with cGMP. Importantly, the invariant Gln817 residue is considered as key specificity determinant in PDEs, which could hydrolyze either cGMP or cAMP, because of its ability in recognizing the purine moiety in cAMP and/or cGMP through a “glutamine switch” mechanism controlled by surrounding residues that fix the glutamine residue in a specific orientation, depending on the biochemical nature of the PDEs, for binding either cGMP and/or cAMP binding [38], thus accounting for the substrate selectivity of different PDEs. In PDE5, this is achieved by immobilization of the side chain amide group of Gln817 through a sophisticated network of hydrogen bonds that include Gln817 to Gln775 and Gln775 to Ala767 as well as to Trp853 [38,63,64]. The H-pocket consists of variable hydrophobic residues that contributes to inhibitor selectivity in PDEs. Last but not the least, the L-region in the catalytic domains is known to play a role in inhibitor binding by changing its conformation from a closed to an open form [65].

One of the key features of the PDE5 catalytic domain is the binding of metal ions that are critical for PDE activity. Incubating PDE5 with metal ions such as Mg^{+2} or Mn^{+2} was described to induce stimulatory effect in the PDE5. Using metal ion chelators such as EDTA blocks the catalytic activity of the enzyme and also blocks the PDE5 inhibitor, sildenafil, from binding to the catalytic domain [29,66], thereby converting PDE5 to an inactive conformation. This indicates the role of divalent metal ions in the activity of the enzyme and their ability to induce a confirmation change, a property shared with other mammalian PDEs [12,62].

3. PDE5 inhibitors

Competitive PDE5 inhibitors reported so far exclusively bind to the catalytic domain preventing cGMP (substrate) binding and its subsequent catalysis. [67] This inhibition leads to the accumulation of cGMP in cells of various tissue with several therapeutic benefits. Food and Drug Administration (FDA)-approved PDE5 inhibitors include sildenafil (approved in 1998), tadalafil (approved in 2003), vardenafil (approved in 2003), and avanafil (approved in 2012) (71), also considered as a second generation PDE5 inhibitor [69]. These inhibitors differ in their selectivity, potency, indication, onset and duration of action, cost, administration considerations, precautions, and adverse effects profiles. Other clinically available non-FDA approved PDE5 inhibitors include lodenafil, udenafil [70] and mirodenafil [39] (Fig. 2). Novel PDE5 inhibitors with dual targets have also been described in the literature [71,72]. In addition to PDE5, some of these inhibitors can selectively inhibit other enzymes including acetylcholine esterase [73], histone deacetylase [74], cyclooxygenase [72], or act as nitric oxide donors [71].

The relative potency (affinity) of vardenafil for PDE5 was reported to be the highest, followed by tadalafil, and then sildenafil [67]. The relatively higher potency of vardenafil compared to the other two inhibitors was attributed to its heterocyclic double ring [67]. The binding affinity of the inhibitors is increased in presence of cGMP, which is mediated by cGMP binding to the GAFa domain, indicating that each inhibitor indirectly potentiates its own binding to the catalytic site by

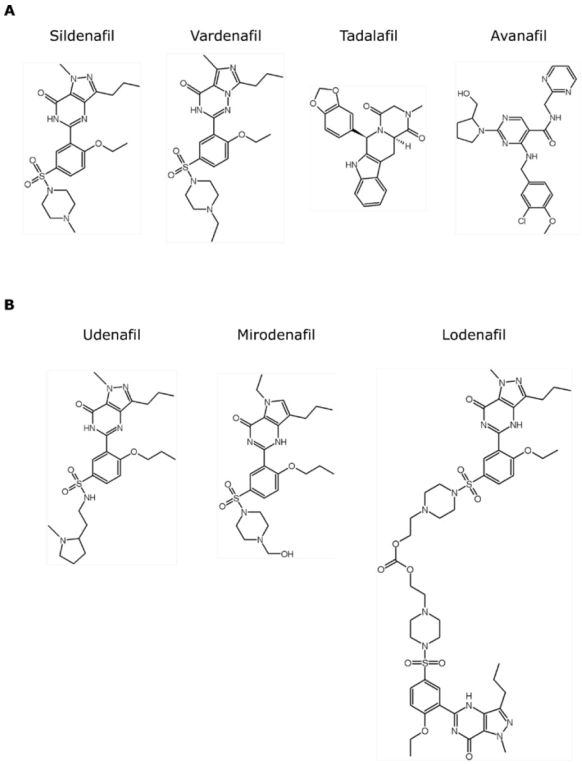

Fig. 2. Chemical structure of PDE5 inhibitors. (A) Chemical structure of FDA-approved PDE5 inhibitors. (B) Chemical structure of other clinically approved PDE5 inhibitors.

directly inhibiting cGMP degradation, whereas in the absence of an inhibitor, cGMP accumulation would potentiate its own degradation by binding to the GAF regulatory domain and enhancing the catalytic domain-mediated hydrolysis of cGMP [67,75]. Moreover, some PDE5 inhibitors induce structural changes in the structure of PDE5 upon binding, which has been proven by crystallography as well as *in vitro* studies [29,48]. These structural changes were reported to enhance cGMP binding to the GAFa domain as well as the phosphorylation of the enzyme, both of which increase binding affinity of the inhibitors. Utilizing a full-length PDE5A2-containing intramolecular BRET sensor that allows detection of the structural change induced by cGMP binding to the GAFa domain, we confirmed that sildenafil was able to induce a structural change in the enzyme whereas IBMX was not [29]. Importantly, the sildenafil-induced structural change was independent of cGMP binding to the GAFa domain [29]. Crystal structure analysis revealed that IBMX bind to a sub-pocket of the catalytic domain of PDE5 that is common for binding non-selective inhibitors of PDEs [76] while sildenafil, vardenafil, and tadalafil occupy the same pocket [67] as indicated by their ability to induce a conformational change in the enzyme [77]. Therefore, it seems that the exact position where the inhibitor bind in the catalytic domain may affect their ability to induce a conformational change in the enzyme.

A change in the conformation of the inhibitors for a better fit in the active site that will increase their binding affinity has also been reported [63]. Crystal structure studies have revealed that PDE5 and PDE4 share similar topological fold in the catalytic domain except for three regions in the M-loop, H-loop, and an additional region. Since the M-loop and H-loop are involved in the selective binding of the inhibitors, their different conformations in the active site of different PDEs most likely contributes to the inhibitors selectivity [76].

3.1. Sildenafil

Sildenafil interacts with the catalytic domain through the Q pocket, the H-pocket, and the L region. It does not form direct interaction with the M subsite. Sildenafil does not interact directly with the metal ions Zn^{2+} and Mg^{2+} of the M-subsite, rather, its pyrazole N_2 atom forms a hydrogen bond with a water molecule that, in turn, forms two hydrogen bonds, one with Tyr612 in the Q-pocket and the other with a water molecule that coordinates to the Zn^{2+} ion [63]. The amide group of the pyrazolopyrimidinone moiety of sildenafil interacts through bidentate hydrogen bond with the side chain amide group of the conserved Gln817. This interaction resembles the bidendate hydrogen bond formed between Gln817 and the purine ring of cGMP. The pyrazolopyrimidinone also interacts through π-stacking against the Phe820 in the Q-pocket [49,63,79]. Both interactions are commonly shared by the majority of the PDE5 inhibitors [47]. Sildenafil also forms several other hydrophobic interactions with hydrophobic residues in the Q-pocket through its pyrazole ring [63,79]. While these hydrophobic interactions provide a substantial source of binding energy, the orientation of the invariant Gln817 is important for subtype selectivity of the inhibitors [64]. On the other hand, the hydrophobic H-pocket accommodates the ethoxyphenyl moiety of sildenafil. Crystal structure studies revealed that this moiety will be sterically hindered to fit in the H-pocket of other PDEs such as PDE4, indicating the role it plays in the selectivity of sildenafil for PDE5 [63]. The methylpiperazine moiety of sildenfafil interacts with the L region of the active site and is exposed at the protein surface. It forms hydrophobic interactions with non-conserved, non-polar residues in this region which is an additional reason for the relative selectivity of sildenafil on PDE5. The sulphonyl group of sildenafil is not involved in any hydrogen bonding formation [63], indicating that it is dispensable.

3.2. Vardenafil

Vardenafil is closely related in structure to sildenafil except the difference in the substitution on the piperazine ring (methyl in sildenafil vs ethyl in vardenafil) as well as in the orientation of the piperazine ring in the active site [64]. They also differ in the heterocyclic ring system that simulates the purine ring of cGMP. Both inhibitors were reported to share similar binding interactions [38,48,63,76]. Despite this structural and binding similarities, vardenafil is a more potent and more selective PDE5 inhibitor than sildenafil [79]. It has at least 20 times higher affinity to the enzyme than sildenafil [80]. Interestingly, however, the crystal structure of the fully active, non-mutated PDE5 in complex with vardenafil revealed three differences from that of the PDE5-sildenafil complex: *i*) the molecular configuration of bound vardenafil was different; *ii*) the conformation of the H-loop of PDE5 was different; and *iii*) there was a loss of divalent metal ions upon binding of vardenafil [47]. The involvement of different amino acids in the binding interaction of vardenafil from that of sildenafil was also noted and attributed to the different positions of the M-loop and the H-loop [47]. In addition, due to the difference in the heterocyclic ring system, one can assume that vardenafil interacts with more and/or stronger binding interactions (i.e. hydrogen bonding and pi-stacking) through its heterocyclic ring with residues in the Q pocket. The latter seems more likely as Gln817 was described to be a major determinant for the potency of vardenafil and mutating this site resulted in more than 600 folds decrease in the potency of vardenafil, more than other inhibitors such as sildenafil (48 folds) and IBMX binding (60 folds) [81]. In fact, it has been suggested that the part of an inhibitor that interacts with the Q pocket could act as a scaffold for the design of selective and potent inhibitors [64].

3.3. Tadalafil

Tadalafil has about 200–600 folds more affinity for PDE5 than PDE6, making it more selective than sildenafil or vardenafil [63]. Tadalafil was reported to bind PDE5 in a different manner than sildenafil in that it forms different binding modes with the Q pocket and does not interact with the L region. The γ-amide group of Gln817 forms a single, rather than bidendate, hydrogen bond with the NH of the indole ring in tadalafil [63]. Pires de Oliveira et al. (2018) confirmed the role of Gln817 in the affinity of tadalafil for the catalytic site of PDE5. They studied the effect of Gln817 mutation on the binding affinity of tadalafil using molecular dynamics simulation approach coupled to Adaptive Biasing Force method. The results confirmed the role of Gln817 in forming a well-oriented single hydrogen bond with the indole ring of tadalafil stabilizing its structure in the active site of PDE5 [78]. The methylenedioxphenyl group of tadalafil accommodates the H-pocket and forms extensive hydrophobic interaction with its residues which accounts for the high affinity of tadalafil despite not forming any interactions with the L region [63]. Moreover, the rigid chemical structure of tadalafil with only one rotatable bond suggests a role in its high affinity as the loss of entropy is much less upon protein binding compared to sildenafil or vardenafil [64]. It is important to note that the phosphorylation of PDE5 increases the binding affinity of tadalafil independent of and more than cGMP allosteric binding. An additive effect on the binding affinity of tadalafil was observed when the enzyme was treated with both cGMP and phosphorylation [82].

3.4. Avanafil

Avanafil is the only second generation PDE5 inhibitor that is FDA-approved. Avanafil has superior PDEs isoform selectivity and side effects profile than the first generation PDE5 inhibitors. Hsieh et al. (2020) has described the structural basis of avanafil isoform selectivity, specifically the off-target effect toward PDE1, PDE6, and PDE11. They reported the crystal structure of PDE5 complexed with avanafil and characterized its molecular interaction with the enzyme. Their results showed that avanafil binds as a competitive inhibitor to PDE5 through interaction with residues known to be non-conserved among PDE isoforms which account for its selectivity towards PDE5. More importantly,

they highlighted the involvement of a halogen bond between the chloride atom of avanafil and a backbone oxygen of an α-helix located in the active site. This was confirmed by site specific mutations of residues involved in the halogen bonding, resulting in a marked decrease in the inhibitory potency of avanafil [69]. In fact, the role of halogen bonding in the design of PDE5 inhibitors has been previously verified by X-ray crystal structure of a PDE5 complexed with a series of halogenated compounds designed based on lead-optimization [83].

4. Clinical applications of PDE5 inhibitors

Currently, the clinically approved indications of PDE5 inhibitors include erectile dysfunction (ED), lower urinary tract symptoms (LUTS), and pulmonary artery hypertension (PAH) (Fig. 3) [39,68,84]. During normal penile erection, parasympathetic stimulation, among other factors, enhances NO release from penile endothelial cells leading to increased production of cGMP by action of soluble GC [84]. cGMP signal propagation through downstream effectors leads to a decrease in the intracellular Ca^{2+} concentration in the corpus cavernosum smooth muscles causing smooth muscle relaxation and a reduction in arterial blood drainage and erection sustainability [16,39]. PDE5 inhibitors competitively and reversibly inhibit PDE5 found in the corpus cavernosum responsible for hydrolysis of cGMP, and thus lead to accumulation of cGMP produced in response to NO release and, subsequently, sustainability of penile erection [39]. Oral PDE5 inhibitors that are FDA-approved for ED include Sildenafil [85], tadalafil, vardenafil, and the recently approved avanafil [68]. Other clinically available non-FDA approved PDE5 inhibitors for ED include lodenafil, udenafil [70], and mirodenafil [39].

Several clinical studies showed improvements in LUTS with the use of PDE5 inhibitors, specifically LUTS secondary to benign prostatic hyperplasia (BPH) [86–90]. However, tadalafil is the only currently approved drug for treating LUTS regardless of erectile function [32]. PDE5 inhibitors are believed to reduce moderate to severe LUTS by improving lower urinary tract oxygenation, relaxation of smooth muscles, downregulation of proliferation of prostate stromal cells, modulation of afferent nerve activity, and reduction of inflammatory response in the prostate [32,39]. Furthermore, PDE5 inhibitors are clinically effective in treating PAH [33,91]. They act as vasorelaxants by relaxing and dilating smooth muscles cells in the pulmonary vasculature where high levels of PDE5 exist [39]. Sildenafil and tadalafil are FDA-approved for managing PAH [33,34].

Several meta-analyses studies discussed the potential of PDE5 inhibitors in treating other conditions such as premature ejaculation either alone [92,93] or in combination with selective serotonin reuptake inhibitors (SSRIs) [92], for improving hemodynamic parameters in patients with heart failure [94], and for improving sperm motility and morphology in infertile men with oligospermia [95]. In fact, some experimental studies have indicated that the administration of PDE5 inhibitors enhances Leydig cell secretory function [96] and increases testicular androgen-binding protein secretion [97] suggesting their potential benefits as adjuncts for the treatment of semen disorder and male infertility [98–100]. On the other hand, several meta-analyses concluded a risk for developing melanoma with the use of PDE5 inhibitors [101–103], while others suggested that the increased risk seen is not causal and may be associated with potential bias and limitations of previous studies [104].

Numerous animal models investigated the potential role of PDE5 inhibition in stroke. In these studies, PDE5 inhibitors demonstrated neuroprotective effects with increased neurogenesis [105], angiogenesis, and cerebral blood flow to the ischemic region in animal models [105–107]. Eventhough preclinical studies using animal models provide valuable data, interspecies variations in clinical pharmacokinetics of PDE5 inhibitors pose a limitation for some animal models as indicated by Tian *et al.* (2020) [108]. Therefore, the scarce evidence for using these drugs in human stroke patients warrants human clinical studies [106,107].

Dunkerly-Eyring et al. (2020) and Samidurai et al. (2020) have noted the role of PDE5 inhibitors in attenuating microRNA upregulation in cardiomyocytes following chronic alcohol ingestion [109] and hypertrophic cardia remodeling [110] indicating their cardioprotective effect. Korkmaz-Icoz et al. (2020) described similar cardioprotective evidence of these inhibitors in their careful repurposing for the treatment of heart failure and myocardial ischemia [111]. In addition, PDE5 inhibitors

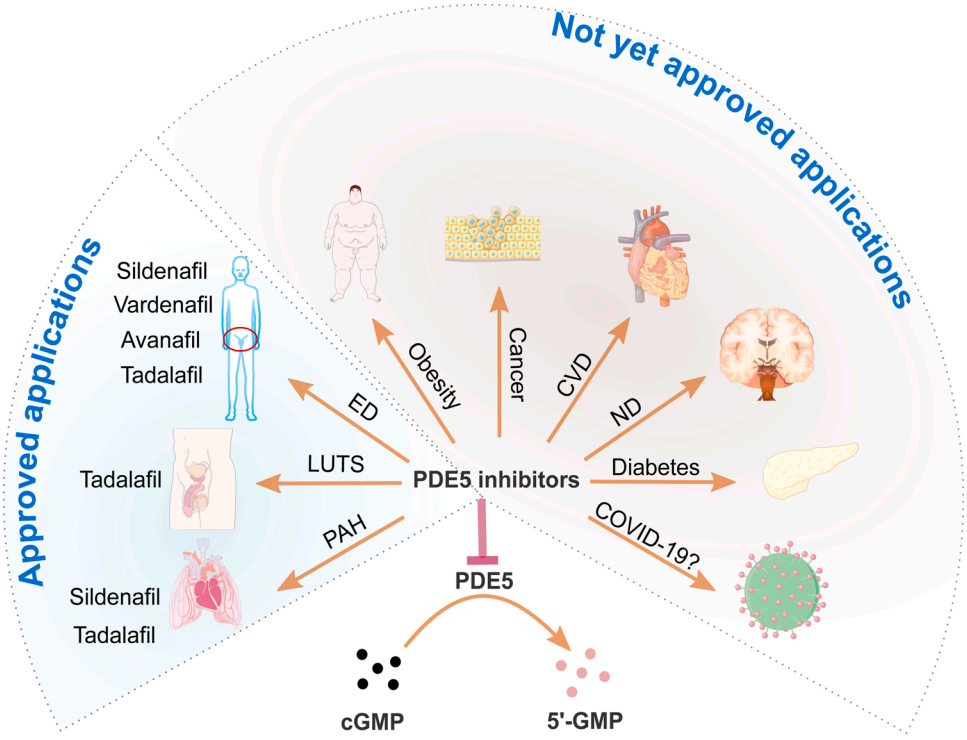

Fig. 3. Therapeutic applications of PDE5 inhibitors. On the left side (light blue shade) are FDA-approved therapeutic applications of PDE5 inhibitors. On the right side (light purple shade) are investigational therapeutic applications of PDE5 inhibitors. PAH: pulmonary artery hypertension, LUTS: lower urinary tract symptoms, ED: erectile dysfunction, CVD: cardiovascular diseases, ND: neurological disorders (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

were described to be generally safe when used in combination with other drugs used for the treatment of cardiovascular diseases [112]. For instance, Uschner et al. (2020) demonstrated the safety of these inhibitors in experimental animal model when used in combination with non-selective beta blockers, and their additional benefit in attenuating the aggravating effect of beta blockers on erectile dysfunction [113]. In fact, a review on the cardiovascular safety profile of PDE5 inhibitors by Kloner et al. (2018) based on published literature of the last two decades concluded the cardiovascular safety of these inhibitors [112]. In a large study by Xanthopoulos et al. (2020) that included more than 13,000 participants, the use of PDE5 inhibitors after left ventricular assist device implantation was found to be associated with lower rates of thrombotic events and improved survival [114], although Gulati et al. (2020) argued the clinical safety of these inhibitors in patients undergoing heart transplant or left ventricular assist device therapy and the need for careful prospective studies before repurposing [115]. Additionally, Ribaudo et al. (2020) described in detail the role of PDE5 and its inhibitors – specifically sildenafil and tadalafil which can cross the blood brain barrier – for the treatment of neurodegenerative diseases (Fig. 3) [116]. Huang et al. (2020) and Zuccarello et al. (2020) have recently synthesized novel inhibitors for this specific application [117, 118].

Several authors have discussed the utility of PDE5 inhibitors in the treatment of the 2019 novel corona virus disease (COVID-19) [119–121]. For instance, Isidori et al. (2020) discussed the role of the NO-cGMP-PDE5 axis in modulating COVID-19 complications considering the predominant expression of PDE5 in the lungs [121]. In fact, this axis has been the target of at least six clinical trials, including a pilot study (NCT04304313) that explores the effect of daily dose of sildenafil in the rate of disease remission. Other clinical trials have explored the potential of repurposing PDE5 inhibitors for other clinical indications including type II diabetes and its associated complications (NCT01566006, NCT01238224, NCT02601989, NCT00692237, NCT01803828), obesity (NCT02554045) and effect on insulin secretion and sensitivity in obese men (NCT02595684), cardiovascular conditions (NCT02819440, NCT02611258, NCT01803828, NCT01275339, NCT02450253), Solid (NCT02544880, NCT01697800, NCT00894413, NCT02466802) and non-solid (NCT01374217) tumors, and kidney stones (NCT03229889, NCT02519153) (Fig. 3).

5. Conclusion

PDE5 plays a key role in regulating a multitude of cGMP-mediated physiological processes involving multiple regulatory mechanisms including allosteric structural changes and posttranslational modifications such as phosphorylation. Unsurprisingly, it has been targeted with a variety of pharmacological inhibitors for the treatment of a number of diseases including erectile dysfunction and pulmonary hypertension. First, understanding the binding interaction of these inhibitors with the active site and the structural bases of their affinity and selectivity will provide great advantage in the design of novel potent and highly selective PDE5 inhibitors. Second, while PDE5 inhibitors have several therapeutic applications, only three of these therapeutic applications are FDA-approved. There is increasing evidence that PDE5 inhibitors could play a role in management of a number of additional diseases including cancer and COVID-19 complications by modulating the NO-cGMP-PDE5 axis, suggesting their use as adjuncts in the treatment of COVID-19 infection. However, clinical studies are warranted in order to extend the therapeutic applications of these inhibitors to a wider range of diseases. Further, much remains to be explored with respect to allosteric regulation, specifically through pharmacological agents, of PDE5 that can be employed for fine tuning its activity in a tissue and disease context-dependent manner.

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Declaration of Competing Interest

Authors declare no conflict of interest.

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