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OVERVIEW

Tadalafil: 15 years' journey in male erectile dysfunction and beyond

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Enabling Technologies		Strategy, Management & Health Policy	
Hit, Lead & Candidate Discovery	Preclinical Research & Development	Clinical Research	Post-Market Research

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Abstract

Tadalafil, Cialis, Eli Lilly & Co./ICOS, (6*R*,12a*R*)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6] pyrido[3,4-*b*]indole-1,4-dione, was first discovered in 2003. It was reported to have high diastereospecificity for phosphodiesterase 5 (PDE5) inhibitions. The *cis*-(6*R*, 12a*R*) enantiomer is the most active enantiomer. Tadalafil showed PDE5 inhibition with IC_{50} = 5 nM. It possesses high selectivity for PDE5 versus PDE1-4 and PDE6. Tadalafil is more selective to PDE5 against PDE6 whereas sildenafil, another commercially available PDE5 inhibitor shows similar potencies to inhibit PDE5 and PDE6. Tadalafil is used for the treatment of male erectile dysfunction (MED), prostatic benign hyperplasia (PBH) signs and symptoms, and pulmonary arterial hypertension (PAH). Adcirca, another name for tadalafil, is used to treat PAH and improve exercise capacity. Recent clinical studies suggest the use of tadalafil for nonurological applications, including circulatory disorders (ischemia injury, myocardial infarction, cardiac hypertrophy, cardiomyopathy, heart failure, and stroke), neurodegenerative disorders, and cognitive impairment conditions. This review discusses tadalafil and its analogues reported in the past 15 years. It discusses synthetic pathways, structural activity relationships, existing and future pharmacological indications of tadalafil and its analogues. This work can help medicinal chemists developing novel PDE5 inhibitors with wider therapeutic indications.

KEYWORDS

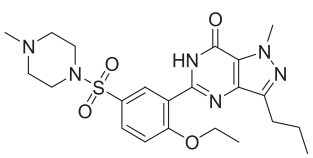
Alzheimer, autoimmune disease, cAMP, cGMP, chemotherapy, male erectile dysfunction, multiple drug resistance, neurogenesis, PDE5, Pictet–Spengler, pulmonary arterial hypertension, stereospecific, tadalafil

1 | INTRODUCTION

The nitric oxide/cGMP pathway is an essential pathway in many normal physiological functions; disruption of this pathway plays a role in the pathophysiology of several diseases. Nitric oxide (NO) binds to soluble guanylyl cyclase (sGC) an action that triggers (sGC)-cGMP signaling pathway. NO is synthesized by the oxidation of *L*-arginine, nitric oxide synthase (NOS) catalyzes the oxidation process in the presence of NADPH and O_2 as substrates. NO activates sGC, sGC converts GTP to cGMP. The formed cGMP activates cGMP-dependent protein kinase (PKG, cGK); such kinases activate a cascade of proteins resulting in numerous physiological effects. Therefore, NO-sGC-cGMP signaling pathway plays essential role in physiological processes like growth, cell viability, smooth muscle relaxation, neurotransmission, inflammation, and gene transcription. cGMP are hydrolyzed to GMP (inactive form) via cGMP specific PDE enzymes (PDE5, PDE6, and PDE9), which break its phosphodiester bond. PDE inhibitors block the action of PDE and thus elevate the levels of cGMP (Denninger & Marletta, 1999; Moncada, Palmer, & Higgs, 1991; Murad, 2006).

The synthesis of sildenafil (**1**), the first commercially available PDE5 inhibitor originally studied as antianginal agent, was a breakthrough in the treatment of erectile dysfunction (ED). Sildenafil discovery encouraged researchers to investigate novel clinical applications of PDE5 inhibitors. Although many PDE5 inhibitors were synthesized, sildenafil (**1**), tadalafil (**2**), and vardenafil (**3**) were the focus of scientific studies. Since sildenafil (**1**) was discovered, PDE5 inhibitors are perceived as the first line of therapy for ED. New PDE5 inhibitors were introduced to the market with clinical applications beyond male erectile dysfunction (MED). Sildenafil (**1**), tadalafil (**2**), vardenafil (**3**), lodenafil (**4**), and mirodenafil (**5**) are applied in the treatment of asthma, chronic obstructive pulmonary disease (COPD), pulmonary arterial hypertension (PAH), cardiac failure, autoimmune diseases, and ED (Maurice et al., 2014).

Tadalafil inhibits both PDE5 and PDE11 enzymes; PDE11 enzyme is abundant in skeletal muscle. Inhibition of PDE11 with tadalafil leads to the common side effects, namely, back and muscle pain (myalgia) (Makhlouf, Kshirsagar, & Niederberger, 2006). It was found that the catalytic site of PDE11 resembles that of PDE5, however, there is no available crystal structure for PDE11 and no adequate knowledge about its physiological role in human body. This lack of data restricts our understanding and limits our conception to how this PDE isoform works.



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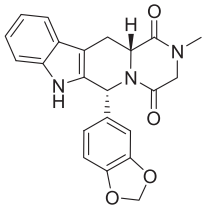

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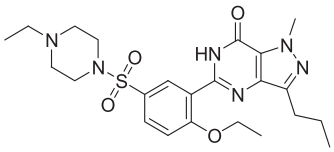

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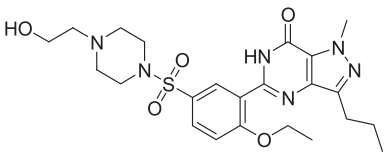

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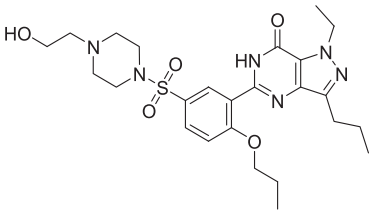

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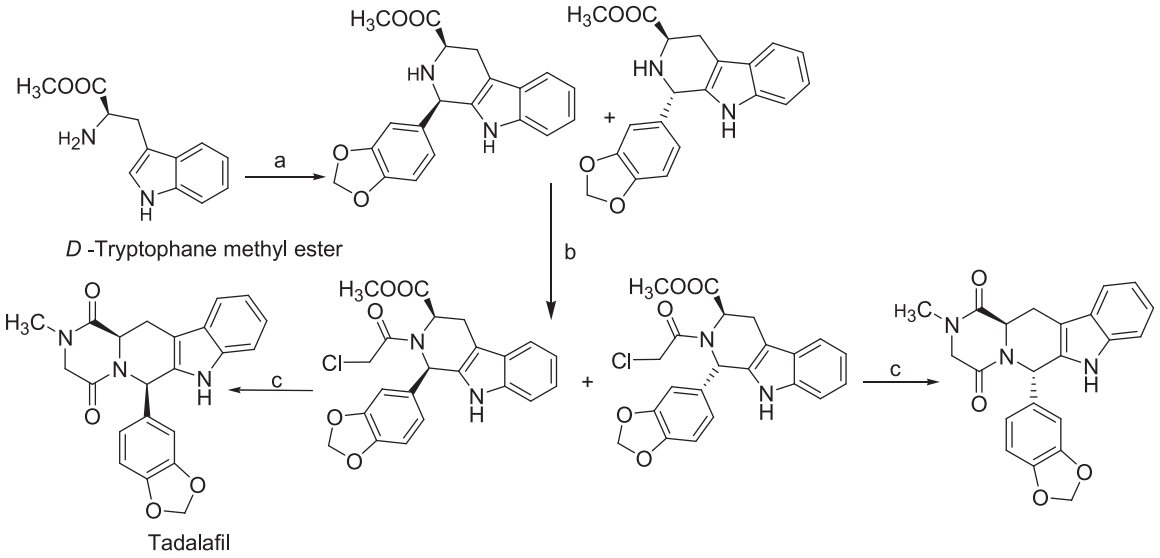
2 | ROUTES OF SYNTHESIS ADOPTED IN PREPARATION OF TADALAFIL AND ITS ANALOGUES

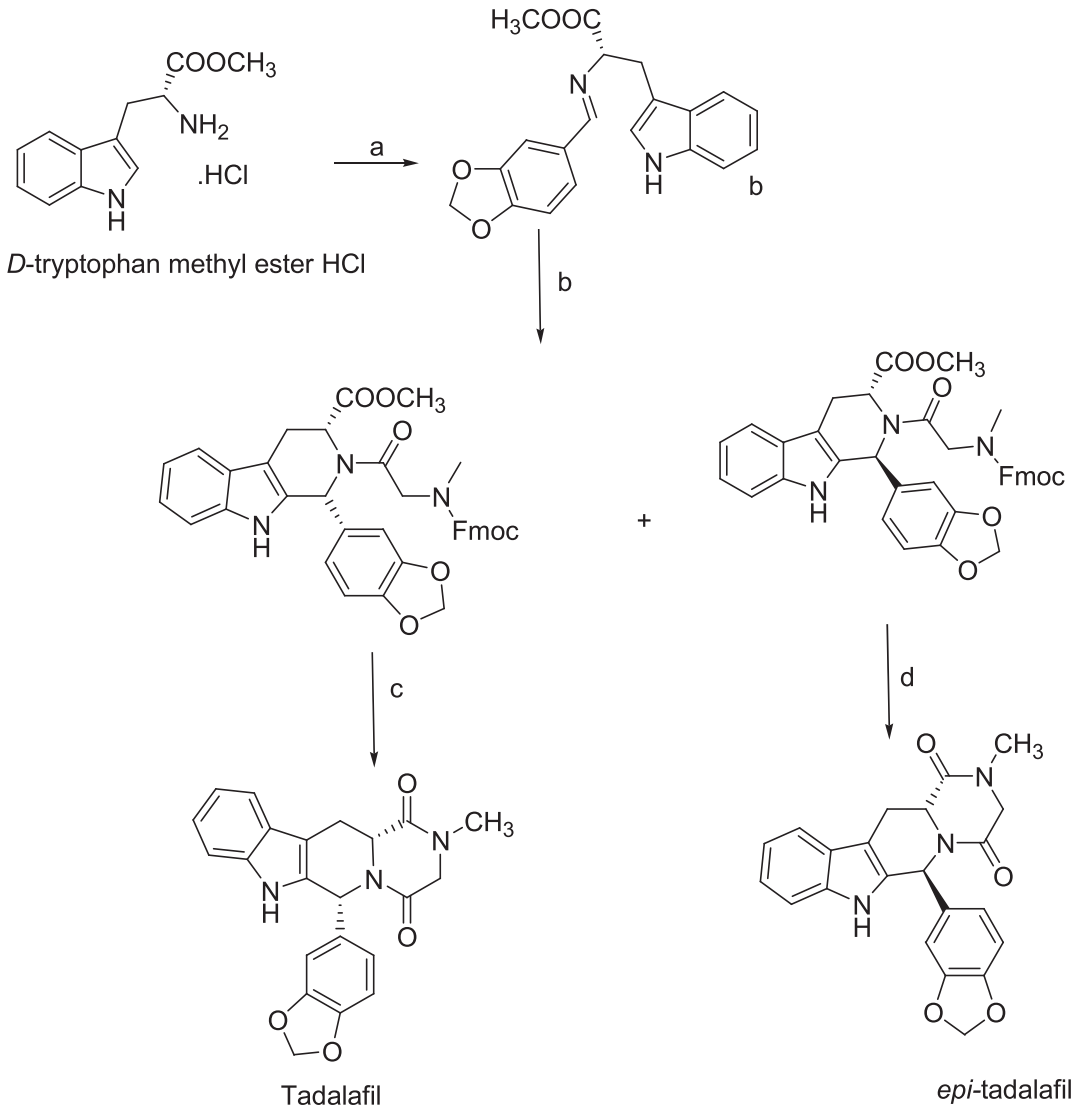
The huge success of tadalafil and its analogues have encouraged tremendous research that focuses on developing synthetic routes to these tetrahydro-β-carboline derivatives. A straightforward synthetic scheme was initially adopted for the preparation of Tadalafil (**2**). This scheme is based on the work of Saxena et al. using four main starting blocks, namely, *D*-tryptophan methyl ester, commercially available piperonal, chloroacetyl chloride, and methylamine (Saxena, Jain, & Anand, 1973).

Pictet–Spengler (P–S) reaction is used to construct chiral tetrahydro-β-carbolines moieties. The P–S reaction of *D*-tryptophan methyl ester with piperonal in acidic medium is the fundamental step in the synthesis of tadalafil (**2**). Daugan et al. describe a process for the synthesis of tadalafil (**2**), *D*-tryptophan methyl ester reacts with a piperonal in methylene chloride in the presence of trifluoroacetic acid as a catalyst, and reaction is stirred for 5 days at 4 °C. The reaction gives a mixture of *cis*- and *trans*-tetrahydro-β-carboline derivatives (*cis*-/*trans*- 60:40). Reaction of the pure *cis*-isomer with chloroacetyl chloride in trichloromethane in basic medium (sodium bicarbonate or triethylamine in dichloromethane) form the *N*-chloroacetyl tetrahydro-β-carboline derivatives (62%), which then reacts with methylamine in methanol at 50 °C for 16 hr to furnish tadalafil (**2**) (70%) (Scheme 1) (Daugan et al., 2003b).

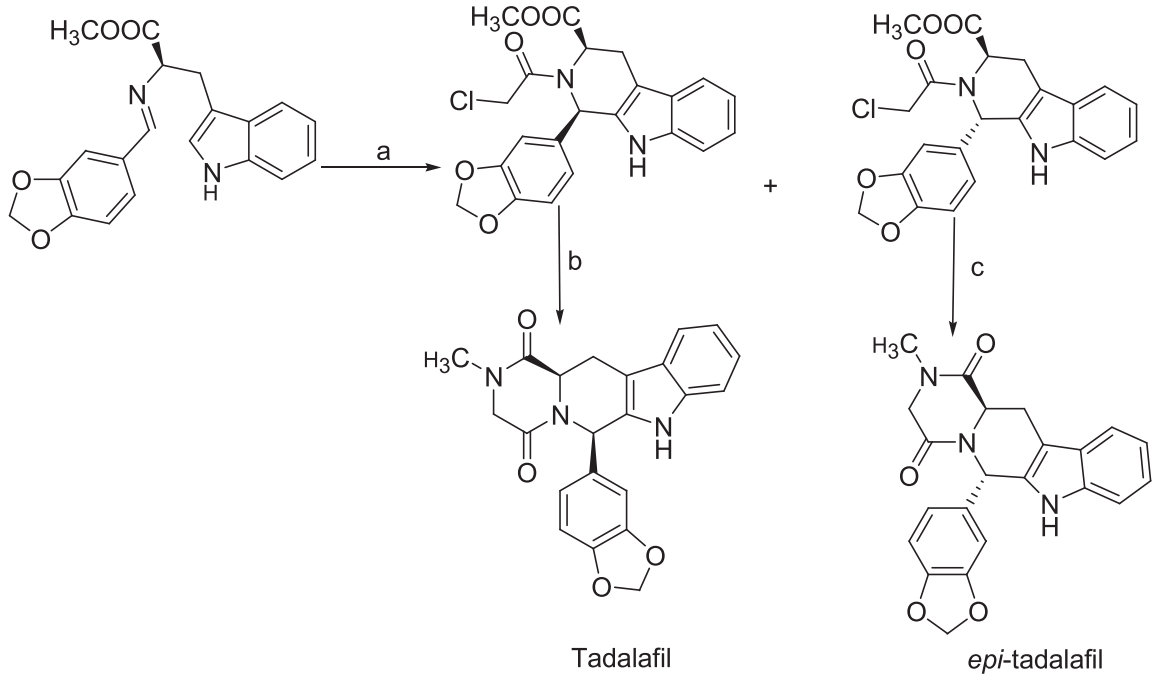
In 2004, two concise methods of synthesis were developed. A 2-day synthesis procedure was adopted instead of the 5-days synthesis adopted by Icos. In this route, piperonal and *D*-tryptophan methyl ester HCl react to produce an imine intermediate. The intermediate reacts with Fmoc–sarcosyl chloride to yield an acyl chloride derivative. Upon using Fmoc-sarcoyl chloride the *cis*-diastereomer undergoes smooth and rapid cyclization to tadalafil in the appropriate basic medium (Scheme 2) (Revell, Srinivasan, & Ganesan, 2004).



SCHEME 1 The Icos route to tadalafil (**2**). Reagents and conditions: (a) Piperonal, CF_3COOH, CH_2Cl_2 (70%, 60:40 *cis*:*trans*), (b) chloroacetylchloride, Et_3N, CH_2Cl_2 or $NaHCO_3$, $CHCl_3$ (62%), and (c) CH_3NH_2, MeOH, reflux (70%)



SCHEME 2 First generation *N*-acyliminium Pictet-Spengler route to tadalafil. Reagents and conditions: (a) 1.1 Equiv piperonal, 1 equiv Et_3N, 5 equiv $MgSO_4$, 24 hr (95%); (b) 3 equiv Fmoc-Sar-cl, 0.1 equiv DMAP, 3 equiv basic alumina, CH_2Cl_2, −25 °C to r.t., 2 hr (62%, 1.1:1 *cis*-:*trans*-); (c) 20% piperidine-DMF, 1 hr; and (d) 20% piperidine-DMF, 50 °C, 24 hr (86%)



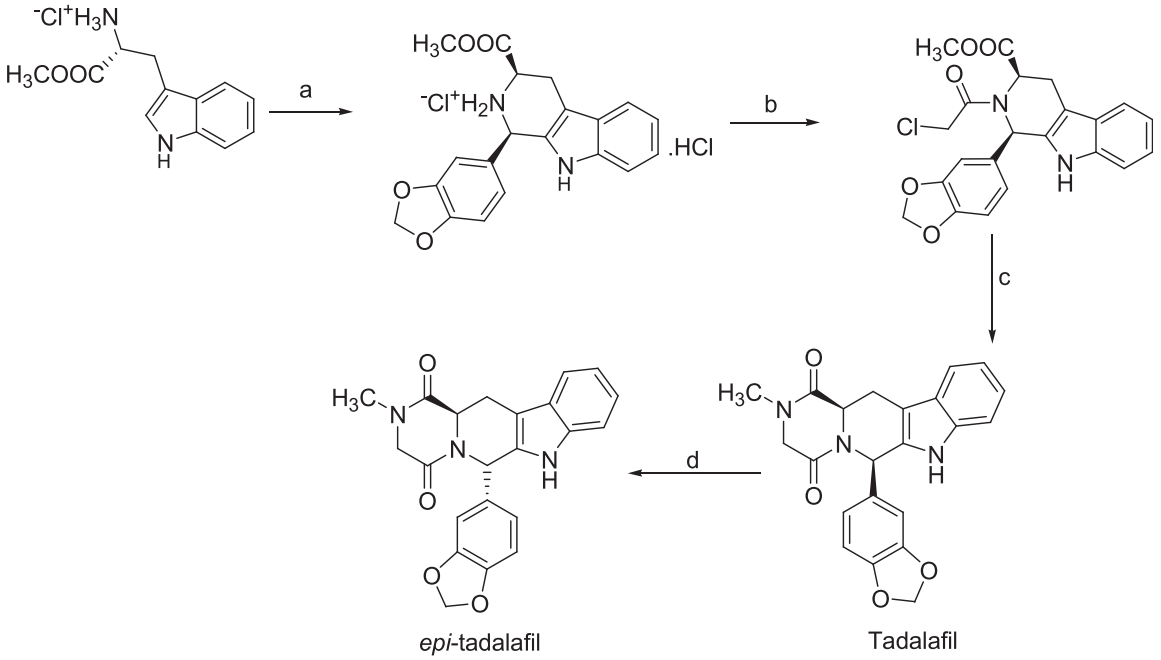
SCHEME 3 Second generation *N*-acyliminuim Pictet–Spengler route to tadalafil. Reagents and conditions: (a) 3 equiv $ClCOCH_2Cl$, 3 equiv DMAP, CH_2Cl_2, 2 hr (78%, 1.3:1 *cis*:*trans*), (b) $MeNH_2$, MeOH, 50 °C, 16 hr (92%), and (c) $MeNH_2$, MeOH, 50 °C, 24 hr (89%)

On an attempt to lower the cost of the reaction, chloroacetyl chloride was used as the acylating agent instead of the expensive Fmoc–sarcosyl chloride. The reaction of the imine intermediate with chloroacetyl chloride yielded an acyl chloride derivative (78%), a higher yield when compared to reaction with sarcosyl chloride (62%). Cyclization of the chloroacyl derivative using methylamine in methanol for 16 hr yielded tadalafil in 92% (Scheme 3) (Revell et al., 2004).

On attempt to improve stereoselectivity, Xiao et al. studied the stereoselectivity of P–S reaction under various conditions. They conducted the reaction using ester HCl to avoid the use of trifloroacetic acid (TFA); reactions were conducted in various solvents. This study concluded that in the absence of any catalyst, the reaction was slower and of a poor yield with no stereoselectivity. Furthermore, the use of an acid catalyst improved the yield, the reaction rate, and the stereoselectivity. Using benzoic acid gave the best results with high selectivity (*cis*-; *trans*- 92:8). Results showed that isopropanol, butanol, pentanol, nitromethane, acetonitrile, 1,2-dichloroethane and 1,1-dimethoxyethane were suitable solvents, those solvents improved both yields and stereoselectivities. Methanol, DMSO (dimethyl sulfoxide), and DMF (dimethyl formamide) provided only moderate yields and lower stereoselectivities. The best stereoselectivity was noticed with solvents that can precipitate the *cis*-isomer while the *trans*-isomer remains in the supernatant this stereoselectivity suggests that in certain solvents (e.g., acetonitrile or nitromethane) equilibrium develops between *cis*- and *trans*-tadalafil–(6*S*,12a*R*)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6] pyrido[3,4-*b*]indole-1,4- dione HCl epimers. The major driving force of this transformation was the large difference is solubility between the *cis*- and *trans*-isomers. It is noteworthy that this stereoselectivity was observed only when the *D*-tryptophane methyl ester HCl was reacted with piperonal. However, it could not be achieved using other ester salts or other aromatic aldehydes. To further extend the THBC HCl salt to the tetracyclic skeleton of tadalafil, the product of the P–S reaction was reacted with 1.5 equiv. of chloroacetyl chloride in dichloromethane at 0 °C, in a basic medium to form chloroacyl derivative (92%). This was followed by an overnight reaction with 5 equiv. methylamine in DMF at 25 °C to furnish tadalafil (95%). Epimerization of tadalafil during cyclization is noticed if the reaction was carried out in DMSO/*i* –PrOH under basic conditions (DBU: 1,8-Diazabicyclo [5.4.0]undec-7-ene) and refluxed at high temperature for 5 hr. 6a *epi*-tadalafil –(6*S*,12a*R*)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6] pyrido[3,4-*b*]indole-1,4- dione was obtained from tadalafil (98%) (Scheme 4) (Shi, Liu, Xu, & Xu, 2008).

In 2008, Anumula et al. developed an alternative pathway for the synthesis of tadalafil avoiding the use of toxic chloroacetyl chloride and expensive solvents. The protocol also circumvented the need for column chromatography meeting the standards of International Conference on Harmonization (ICH). This method adopts P–S reaction to produce the tetrayhydro-β-carboline skeleton, the tetrayhydro-β-carboline HCl salt is subjected to amidation conditions with sarcosine ethyl ester hydrochloride in presence of DCC (*N*,*N*′-dicyclohexyl carbodiimide)/HOBt (*N*-hydroxybenzotriazole). Pure tadalafil is obtained (55%) (Scheme 5) (Anumula et al., 2008).

Tadalafil was also prepared from N-Boc-*D*-tryptophan. The N-protected tryptophan was treated with ethyl chloroformate to generate its mixed anhydride which reacted in situ with sarcosine ester to yield an intermediate (a) in 50% yield. The reaction of the anhydride intermediate with piperonal using TFA as a catalyst and toluene as a solvent at high temperature (110 °C) gave a *trans*-(*S*,*R*)-tadalafil product with 70% yield, while at a lower temperature (45 °C) *cis*-(*R*,*R*)-tadalafil in 50% yield was observed via an intermediate formation

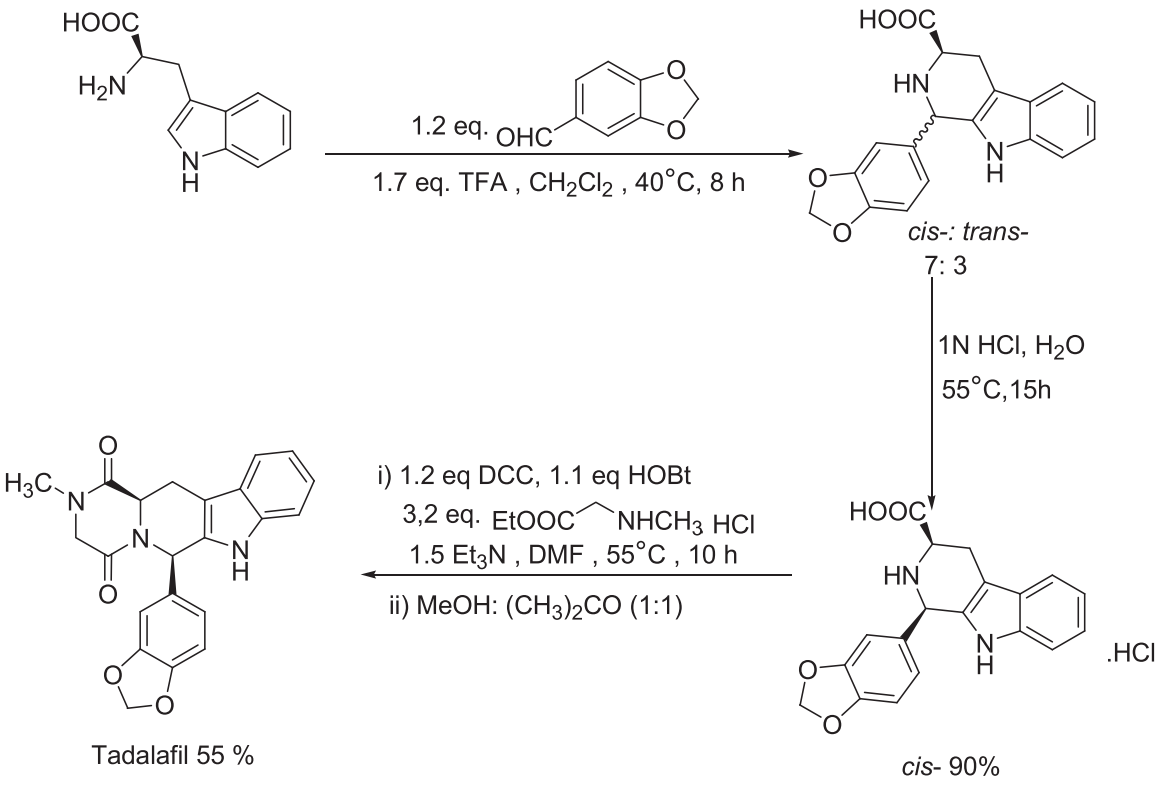


SCHEME 4 Stereoselective synthesis of tadalafil and *epi*-tadlafilreagents and conditions: (a) 1 equiv. of piperonal, refluxing in nitromethane for 4 hr (yield 94%); (b) 1.5 equiv. of chloroacetyl chloride and 3 equiv. of triethylamine, 0–5 °C for 2 hr in CH_2Cl_2 (yield 92%); (c) 5 equiv. methylamine overnight in DMF (yield 95%); and (d) 2 equiv. of DBU, 83 °C for 5 hr in a mixed solvent of DMSO and *i* –PrOH (1:5) (yield 98%)

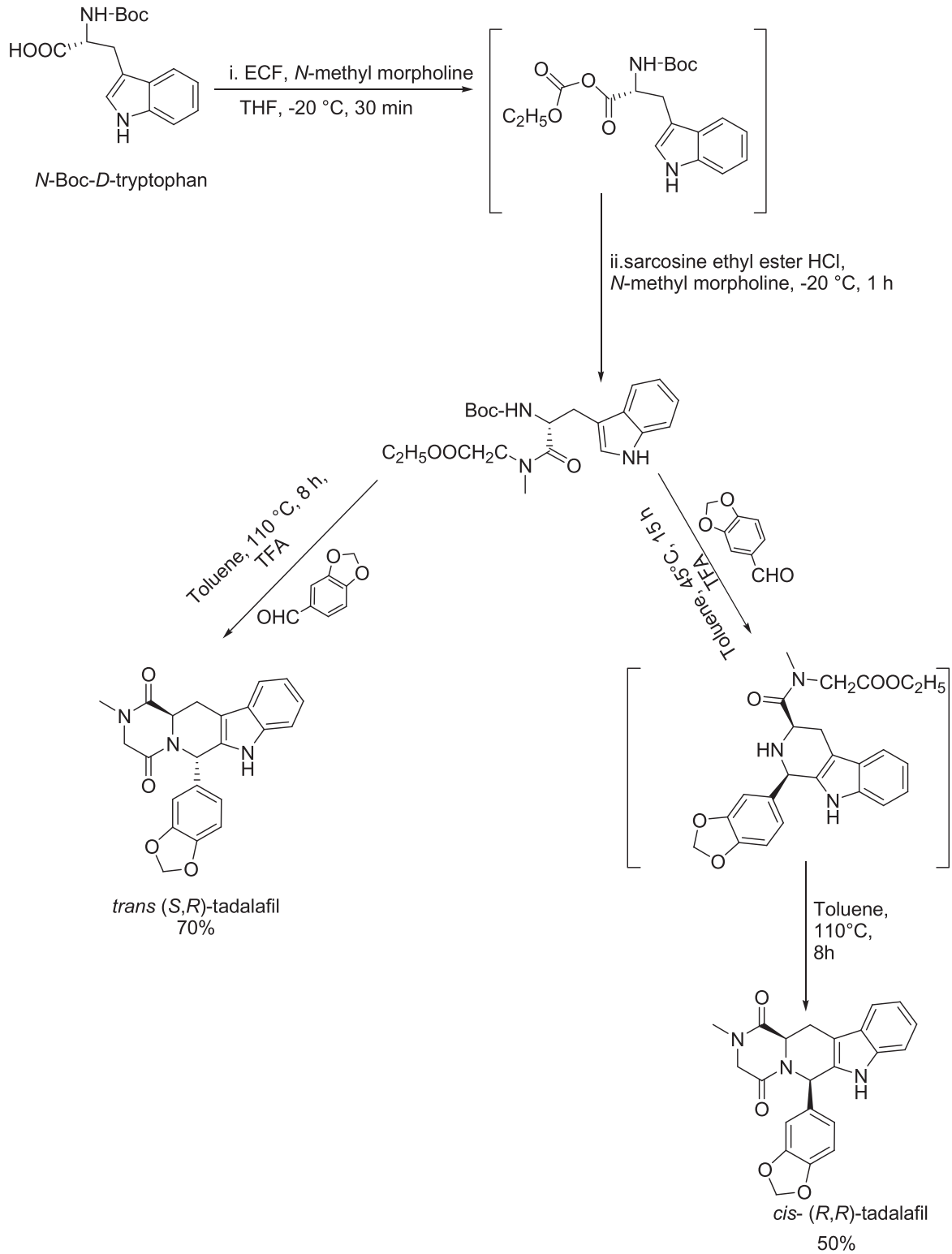
(Scheme 6) (Vedantham, Shanmugam, Vetukuri, Khagga, & Bandichhor, 2012).

Crystallization Induced Asymmetric Transformation (CIAT) was adopted to produce the *cis*-6*R*,12a*R* isomer as the major product (Xiao et al., 2009).

P–S reaction of *D*-tryptophan ester HCl with piperonal using nitromethane and toluene mixture as a solvent showed that a strict control over of the ratio of both solvents is crucial for high stereoselectivities and high yields. *cis*-tadalafil was obtained as pure enantiomer by recrystallization or flash chromatography of the neutralized



SCHEME 5 Alternative synthesis of tadalafil by Anumula et al

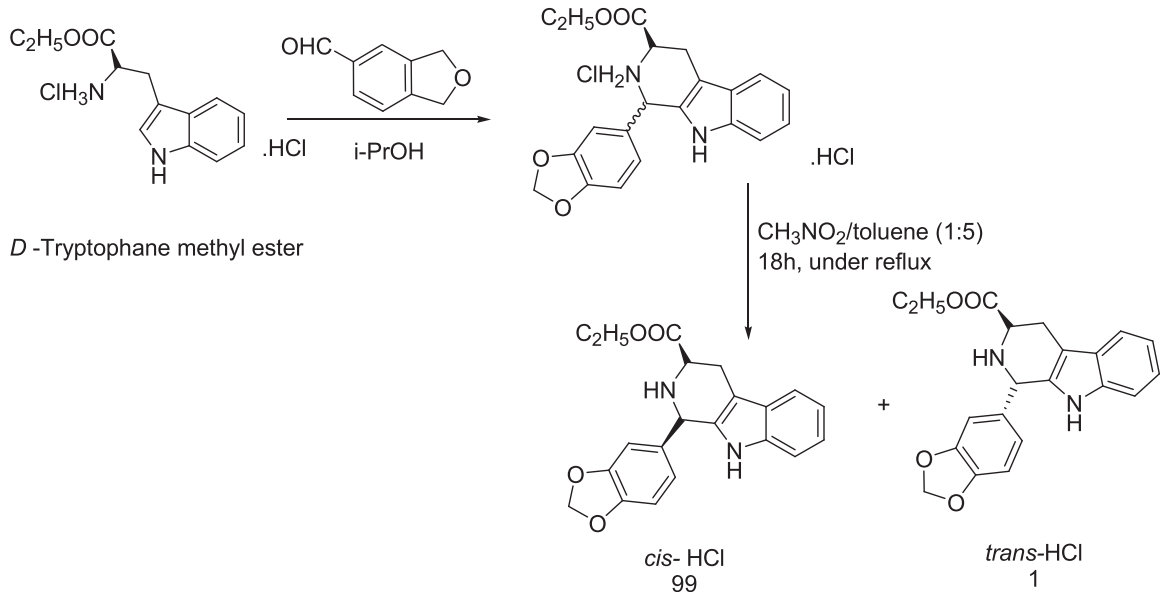


SCHEME 6 Synthesis of Tadalafil 2 from N-Boc-*D*-tryptophan

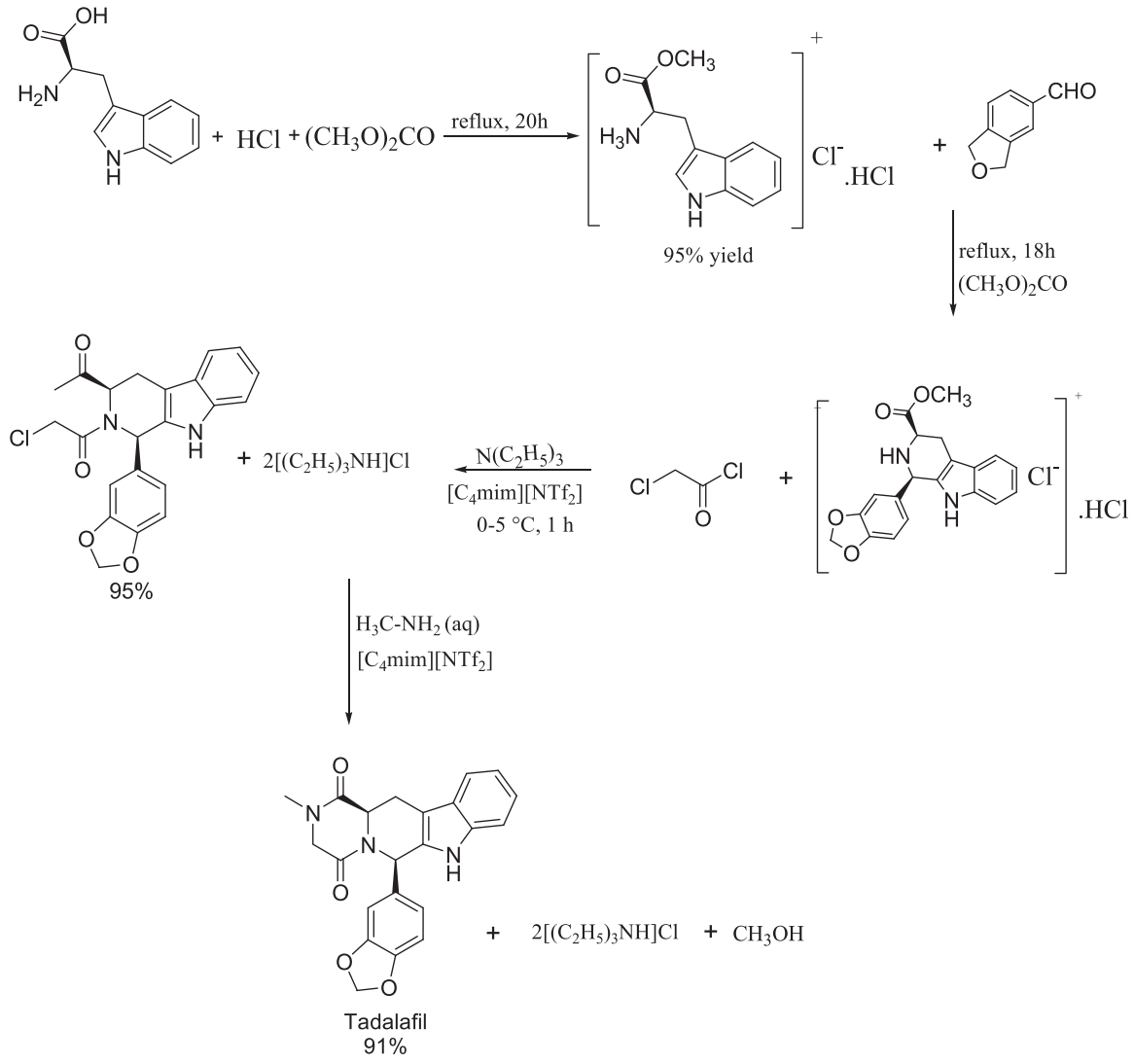
HCl salt. The pure *cis*-form is used as precursor for tadalafil synthesis Scheme 7.

In 2014, Earle et al. suggested overall improved processes for the synthesis of 6*R*, 12a*R* tadalafil using cheaper solvents, less isolation, and purification steps. The major differences between the suggested and traditional tadalafil synthesis methods included esterification of tryptophan using dimethyl carbonate instead of methanol; it serves as solvent as well. Dimethyl carbonate proved to be a good alternative to commonly adopted solvents (nitromethane or acetonitrile) for the diastereoselective P–S reaction. The acetylation and amination of the ester HCl salt were carried out stepwise in a single reactor using an ionic liquid, bis{(trifluoromethyl)sulfonyl}amide) $[C_4dmim][NTf_2]$, as the solvent (Scheme 8) (Earle, Noè, Perosa, & Seddon, 2014).

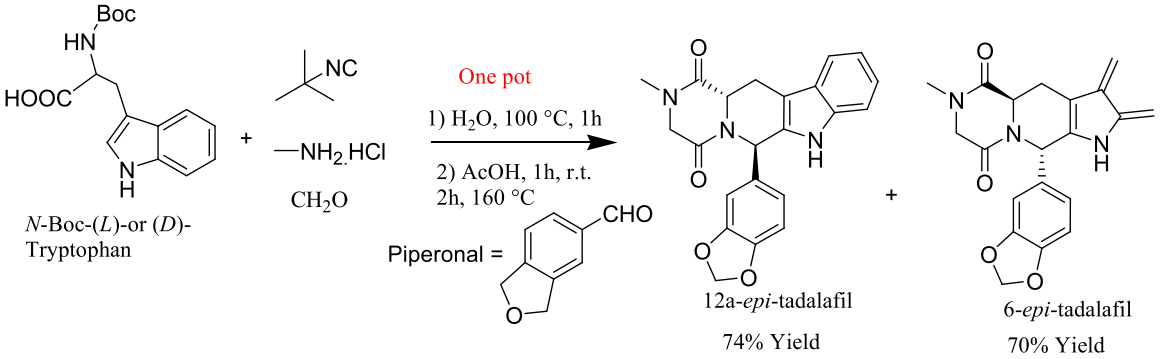
A simple, effective, and environmentally friendly synthetic scheme was adopted to prepare the two pure enantiomers of tadalafil: 12a-*epi*-tadalafil and 6-*epi*-tadalfil. N-Boc-(*L*)- or (*D*)-tryptophan were



SCHEME 7 Two-step synthesis of *cis*-2-HCl and *trans-2*-HCl of tetrahydro-β-carboline via CIAT process



SCHEME 8 Improved synthesis of tadalafil using dimethyl carbonate and ionic liquids



SCHEME 9 One-pot synthesis of tadalafil epimers

used as starting materials, a three-step one-pot reaction was adopted, using the Ugi reaction in water. The Ugi reaction involves the condensation of a carbonyl component, an amine, a carboxylic acid and an isocyanide, to produce α-acylaminoamides, which was followed by a Boc-deprotection, a P–S reaction (with piperonal) and lactamization in acetic acid (Scheme 9) (Jida & Ballet, 2018).

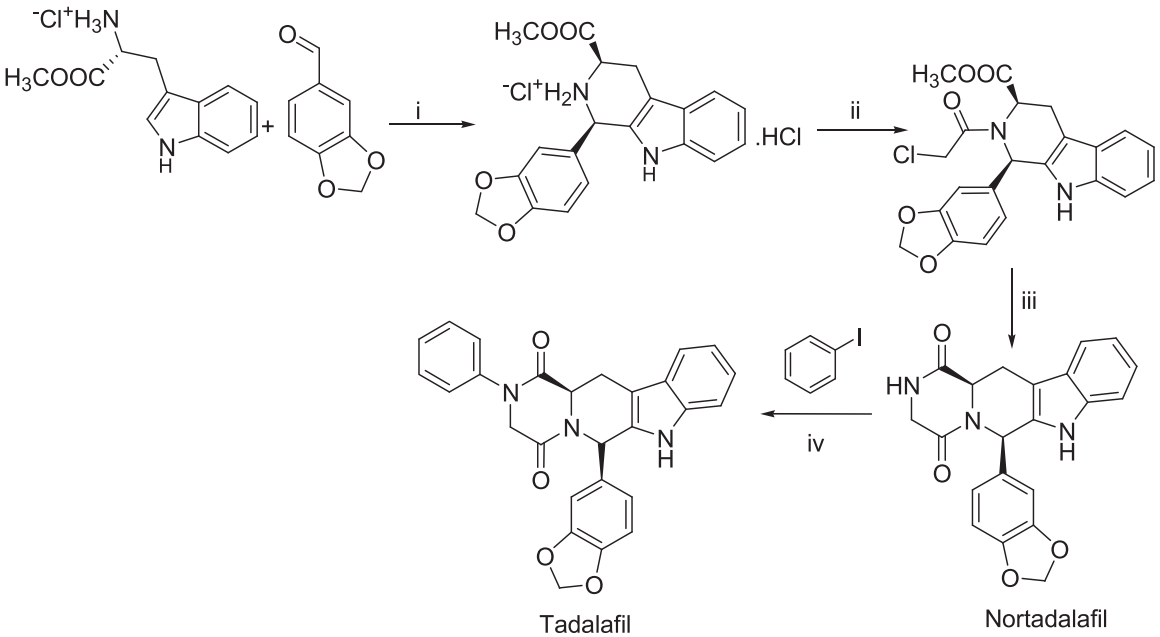
Attempting to replace the methyl group of tadalafil by various aryl groups, a method to access totally new analogues of tadalafil was explored. The Buchwald reaction was adapted. Introduction of aryl substituent proceeded via the use of 0.2–10 mol% of cuprous iodide, 5–20 mol% of a diamine ligand, and a mineral base such as K_3PO_4 in toluene or DMF. To avoid isomerization to the less active *trans*-isomer, lower temperature and lower amount of base was used and a higher stoichiometry of catalyst was involved (Scheme 10). (Beghyn, Hounsou, & Deprez, 2007)

In summary, many successful attempts were carried out to improve the yield and upscale the synthesis of tadalafil to the industrial scale, as well as to adopt a greener chemistry approach. Attempts were also successful to improve diastereoselectivity toward 6*R*, 12a*R* tadalafil up to 95%. Optimization of the reaction was possible by using tryptophan ester HCl, fine tuning of the solvent to allow preferential precipitation of one of the diastereomers, manipulating the acid catalyst needed in P–S reaction. Also, variation of the solvent and time of amination and cyclization steps helped in inducing or avoiding epimerization.

3 | STRUCTURAL AND STEREOCHEMICAL ASPECTS OF TADALAFIL AND ITS ANALOGUES

Phosphodiesterases (PDEs) are hydrolyzing enzymes, the PDEs comprises 11 families that hydrolyze cyclic nucleotides. They regulate cAMP and cGMP intracellular levels, the levels of those second messengers influence all cell functions. PDEs inhibitors block the degradative action of this class of enzymes.

Upon superimposing of all reported inhibitors bound to PDE4D, PDE4B, and PDE5A, it was clear that all PDE inhibitors bind to the



SCHEME 10 Synthesis of novel arylated analogues of tadalafilreagents and conditions: (i) isopropanol reflux, (yield 92%); (ii) chloroacetylchloride, chloroform, triethylamine, −10 °C, 85%; (iii) NH_3, methanol, 45 °C, (yield 70%); (iv) CuI 10 mol%, (±)-trans-1,2-diaminocyclohexane (2 equiv), 20 mol%, iodobenzene (1 equiv), K3PO4 (3 equiv), dioxane,14 °C, 15 days, (yield 95%)

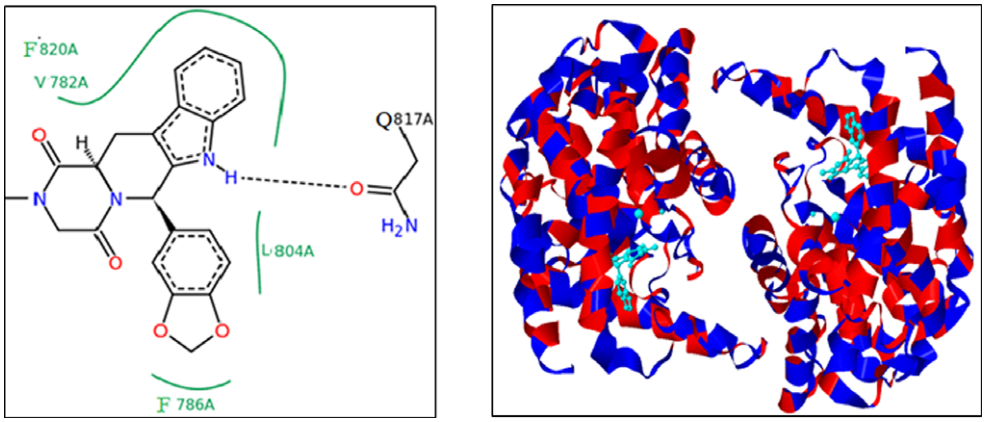


FIGURE 1 2D and 3D view of tadalafil bound to human PDE5 (PDB 1UDU) (Sung et al., 2003)

binding cavity of PDEs in conservative manner, despite their major differences in their chemotypes. All these inhibitors possess a planar ring fitted by a hydrophobic clamp (one jaw is a conserved phenylalanine (F) in all PDEs whereas the other jaw is always a hydrophobic residue). These inhibitors form monodentate or bidentate H-bond with an invariant glutamine (Q), a residue that is preserved among all PDE enzymes. The selectivity of PDE enzymes toward cAMP or cGMP relies on the ability of essential amino acids lining the active site to anchor the invariant Q in different orientations; a glutamine switch takes place to allow binding to either cAMP or cGMP. The active site of PDEs is the common binding site for all reported PDEs inhibitors; primarily they bind to the Q (core pocket) and may extend to the relatively large M pocket (metal-binding site) depending on the size of their substituents. There is no direct binding between PDEs inhibitors and metal ions of the M pocket; they rather bind indirectly via a network of water molecules (Zhang et al., 2004). Tadalafil binds to PDE5A in a unique manner; tadalafil forms a single hydrogen bond between carbonyl of Q817 and heterocyclic nitrogen in its indole ring. Q817 amide group rotates nearly 90° compared to its orientation with sildenafil and vardenafil, such flip allows accommodation of a single H-bond donor from tadalafil. The C-6 pendant aryl ring of tadalafil appears fitting in the Q_2 hydrophobic pocket, this pocket is occupied by ethoxy group of sildenafil and vardenafil. Tadalafil does not show any interaction with the metal ions in the M pocket, not even water mediated interactions. Moreover, the relatively rigid chemical structure of tadalafil has increased its binding affinity, this may be attributed to the fact that it has only one nonterminal rotatable bond, upon binding to PDE5 enzyme tadalafil can form more stable complex than sildenafil and vardenafil due to loss of little entropy (Figures 1 and 2) (Sung et al., 2003).

Tadalafil was co-crystallized with human PDE5 (PDB: 1UDU) with 2.8 Å resolution, while sildenafil was co-crystallized with human PDE5 (PDB: 2H42) with 2.3 Å resolution. The analysis of this PDB data showed that some essential residues near the binding site are not resolved in 1UDU. In 2011, Mohamed et al. superimposed the two crystal structures of PDE5 and highlighted the differences between the two crystal structures; they deduced that the I665 residue appears in 2H42 but is missing 1UDU. In their work, they assumed substantial hydrophobic interactions between Ile665 and the N-alkyl substituent of tadalafil and its analogues. They concluded that the inversion of the 6*R* chiral center of tadalafil to 6*S* induces a 180° flip of the central scaffold. This flip leads to the loss of essential H-bond between carbonyl of Q817 and the indole NH- of tadalafil. Additionally, the 6*S* configuration shows a steric clash between one carbonyl group of piperazinedione and the carbonyl of Q817, this clash has a significant deteriorating effect on binding affinity. The stereoinversion had no effect on the π-π stacking interaction with P820. Their work highlighted an important finding; the chiral center at C-6 rather than at C-12a is the determinant factor of activity. More analogues were thus prepared to focus on producing only the 6*R* analogues starting with the less expensive *L*-tryptophan (Mohamed et al., 2011).

3.1 | Tadalafil analogues with modification on C-6 pendant aryl

All active tadalafil analogues bear an aryl or heteroaryl ring at C-6, this ring is reported to fit into the Q pocket of the PDE5 enzyme via hydrophobic interaction. The size, lipophilicity, and position of the

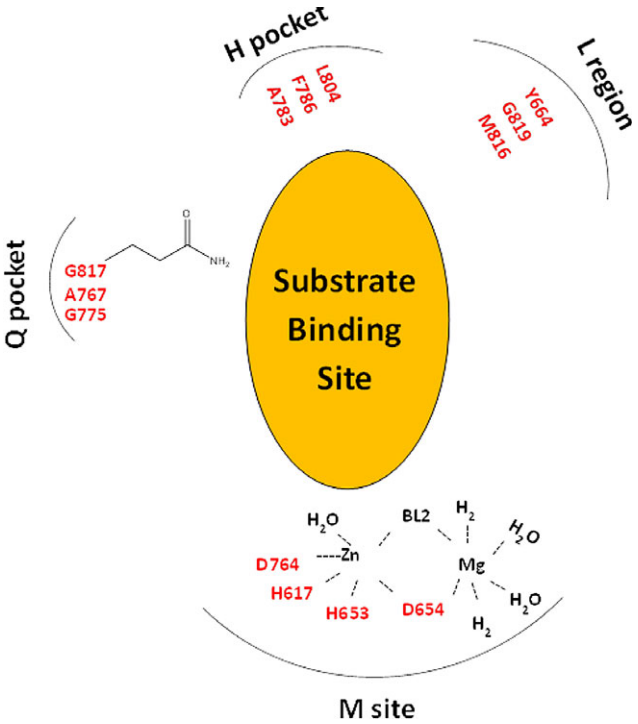
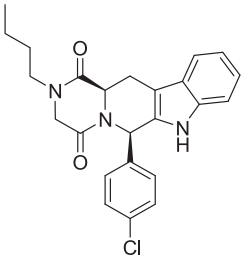


FIGURE 2 A schematic representation of the PDE5 active site

substituents on the aryl ring play a role on both potency and selectivity. The pendant aryl moiety occupies the narrow Q2 pocket, where the pocket governs the selectivity of the ligands toward the enzyme. Substitution on the aromatic ring with mild electron withdrawing groups (**6**) (IC_{50} = 15 nM) improved the PDE5 inhibition compared with compound (**7**) (IC_{50} = 90 nM) bearing, whereas the introduction of 4-cyano group (**8**) (IC_{50} = 765 nM) resulted in a marked decrease in PDE5 inhibition, it seems that substitution with strong electron withdrawing groups lowers the electron density on the C-6 aromatic ring, this can potentially influence the essential π-π interaction. (Daugan et al., 2003b).



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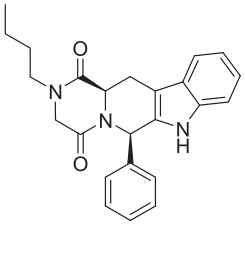

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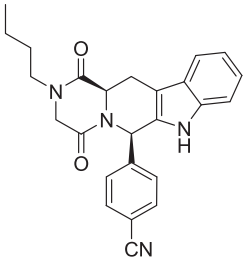

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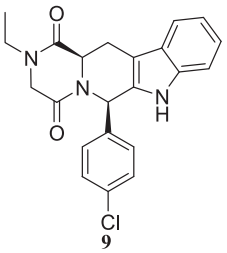
A *para* chloro substitution on the pendant aryl yielded an equipotent tadalafil analog (**9**) (IC_{50} = 3 nM) with remarkable selectivity toward PDE5 versus PDE11(Ahmed et al., 2011). The modification of benzodioxol of tadalafil to dichlorophenyl derivatives was adopted, 2,4-Dichlorophenyl (**10**) showed equipotent PDE5 inhibitory activity compared to tadalafil (IC_{50} = 2 nM) whereas 2,6-Dichlorophenyl analogues (**11**) lacked PDE5 inhibitory activity. Docking studies proved that structural isomers (**11**) and (**12**) may be sterically locked in a conformation that prevents binding to PDE5 (Mohamed et al., 2011). It is worth mentioning that compound (**10**) showed remarkable activity against PDE 11A (IC_{50} = 11 nM), the exact mechanism of this inhibitory activity is still unknown. More recent studies adopted in silico approaches including molecular modeling and virtual screening protocols to design tadalafil analogues with better PDE5/PDE11 selectivity profile (Kayık, Tüzün, & Durdagi, 2017). Findings of those studies went along with Abadi et al. results indicating PDE5/PDE11 selectivity is governed by the tetracycle terminal ring interactions rather than the substitution on the pendant aryl (Mohamed et al., 2011).



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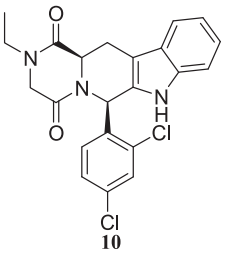

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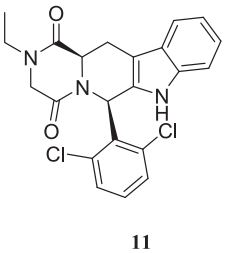

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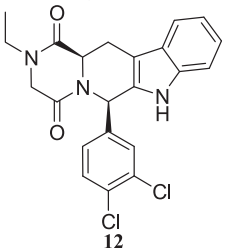

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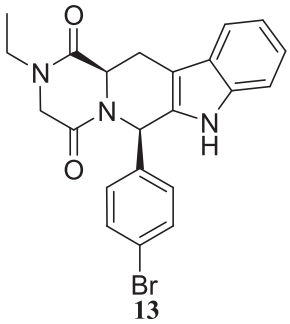
Changing the *para* chloro substituent of the pendant aryl to *para* bromo yielded an equipotent analog (**13**) (IC_{50} = 3 nM) (Ahmed et al., 2012). Conversely, changing the bromine position from *para* to *ortho* led to analog (**14**) which is 100 times less potent than (**13**) (IC_{50} = 320 nM) (Abadi, Gary, Tinsley, Piazza, & Abdel-Halim, 2010). 4-methoxyphenyl analog (**15**) showed potent PDE5 inhibition (IC_{50} = 5 nM) (Daugan et al., 2003b). 3,4 dimethoxyphenyl analog (**16**) showed no PDE5 inhibitory activity. The 3,4 dimethoxy group is sterically compact when compared with 3,4-methylenedioxy of tadalafil. The constrained conformation of 3,4-methylenedioxy group seems to be an essential determinant for activity. The presence of 3,4 dimethoxy free rotating groups was assumed to prevent the optimal orientation of the C-6 aromatic ring (Ahmed et al., 2010).



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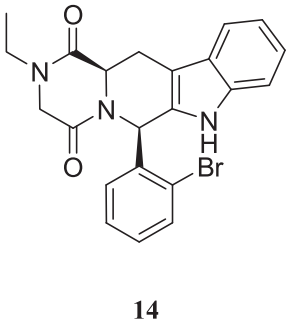

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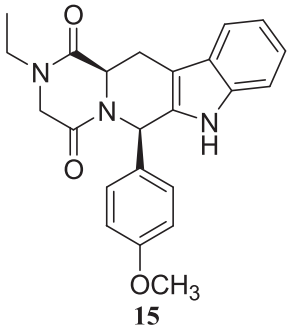

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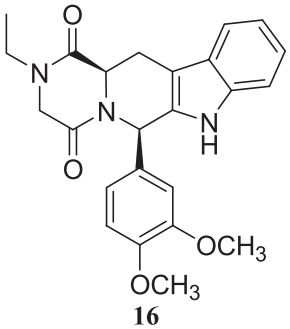

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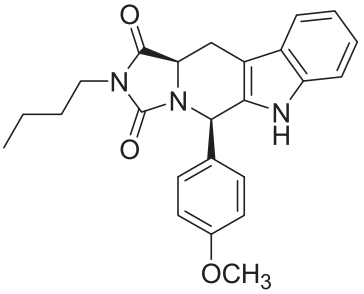
3.2 | Tadalafil analogues with modifications at C-5

Several tadalafil analogues were prepared to bear a hydantoin (Imidazolidine-1,3-dione)/thiohydantoin (2-Thioxo-imidazolidin-4-one) ring instead of the piperazinedione ring. C-5 of the analogues was designed to bear different aryl/heteroaryl rings including 2,5-dimethoxyphenyl (Abadi et al., 2009), 2,4-dimethoxyphenyl (Abadi, Lehmann, Piazza, Abdel-Halim, & Ali, 2011), 2-bromopheny (Abadi et al., 2010), 3-pyridinyl (Ahmed et al., 2010), 5-bromothienyl (El-Gamil et al., 2013), 5-thienyl furanyl rings (Daugan et al., 2003a). Analogues with either a *para* methoxy or *para* chloro group at the phenyl ring showed improved potency, whereas the cyano derivatives lacked activity. Substitution at position 2 with either chloro or methoxy groups prevented the optimal orientation of the phenyl ring. It is obvious that same substituents on pendant C-5 or C-6 enhance PDE5 inhibition activity in piperazinedione and hydantoin analogues. A remarkable difference between piperazinedione derivatives and their hydantoin congeners is that both *cis*- and *trans*-isomers are nearly equipotent. PDE5 inhibition showed no diastereoslectivity preference, hydantoin analogues (**17**) (IC_{50} = 5 nM) and its diastereomers (**18**) (IC_{50} = 8 nM) are of nearly equal activity**.** For hydantoin derivatives, deletion of one carbonyl group (**19**) (IC_{50} = 60 nM) caused a marginal decrease in inhibitory activity, whereas the deletion of both of carbonyls caused a remarkable decrease in activity (**20**) (IC_{50} > 10,000 nM; Daugan et al., 2003a).



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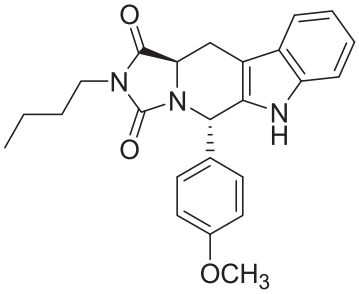

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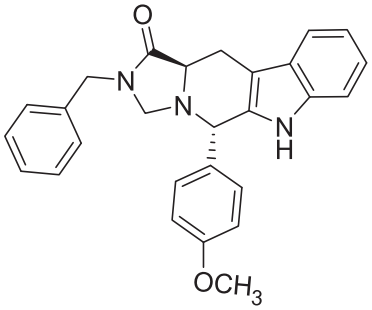

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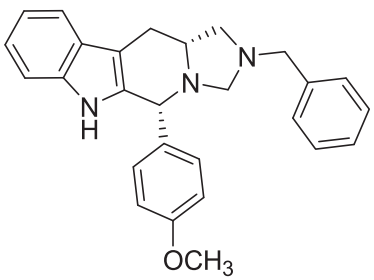

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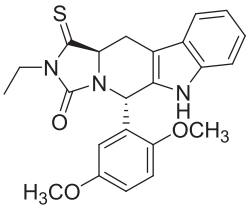
The fusion of tetrahydro-β-carboline skeleton to thiohydantoin ring was also investigated; this modification abolished PDE5 inhibitory activity (**21–23**). Loss in activity can be attributed to the lower electronegativity of the sulfur atom compared with the oxygen atom (Abadi et al., 2009, 2010, 2011).



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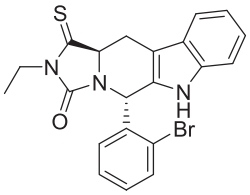

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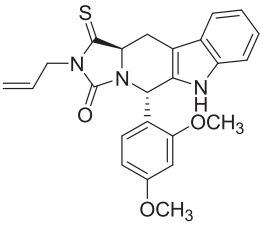

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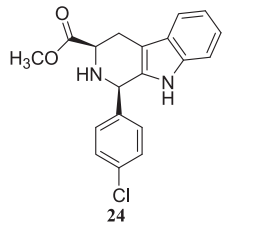
The presence of two carbonyl groups is essential for activity, while the size of the fused ring is highly tolerated.

1,3 disubstituted tetrahydro-β-carboline derivative (**24**) demonstrated no PDE5 inhibitory activity. Compounds bearing two carbonyl groups at position C-1 and N-2 (Ahmed et al., 2010), or analogues with two carbonyl groups at position C-1 and N-2 with an additional terminal N-alkyl substituents showed no inhibitory activities as well (**26–29**) (Ahmed, 2010). This indicates the need for a fused tetracyclic ring to ensure proper binding to PDE5 active site. Even Introduction of one carbonyl group (**24**) or two carbonyl groups (**25–29**) was not sufficient to yield compounds with inhibitory activity even in the presence of terminal N-alkyl substituent suggesting that a rigidification of the carbonyls in either a hydantoin or piperazinedione ring is a requirement of activity. The rigidity offers an essential interaction because the carbonyl groups can interact via water-bridging with H613/N662 as well as with backbone of M816 (Ahmed et al., 2012; Zoraghi, Francis, & Corbin, 2007).



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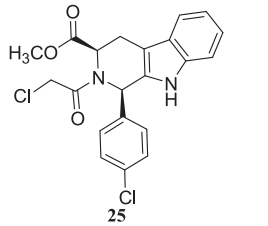

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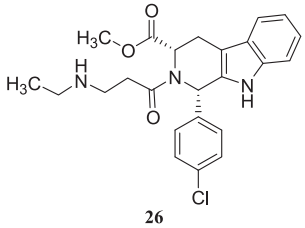

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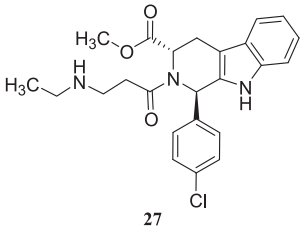

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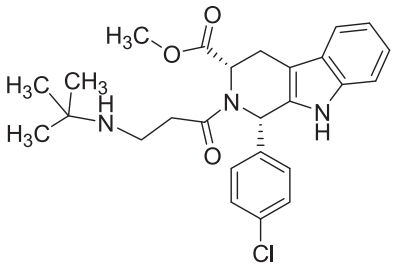
The fusion of THBC to a diazepane-dione (**30**) was reported only once, where only the *trans*-isomer was successfully synthesized to yield a 7-membered ring hybrid, which retained PDE5 inhibitory activity in the nanomolar range (Jiang et al., 2004). All those findings indicated that the catalytic domain in PDE5 can tolerate tetracyclic-β-carboline moieties with 5, 6, or even 7 membered fused rings. The presence of the carbonyl groups is more essential than the size of the rings and the rigidity is a determinant factor for proper PDE5 inhibition.



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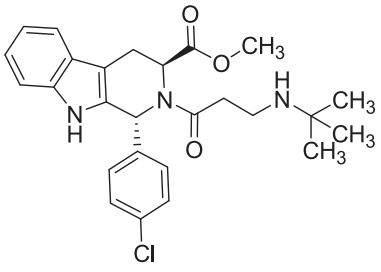

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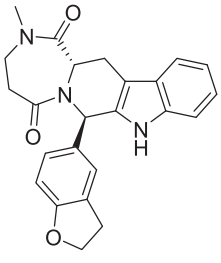

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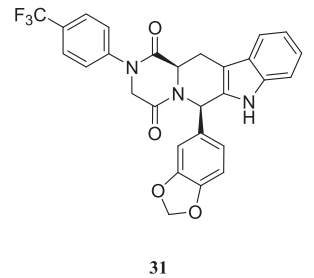
3.3 | Tadalafil analogues with modification on N-substituent on the terminal ring

Daugan et al. suggested that piperazinedione ring of tadalafil can tolerate a wide range of N-alkyl and aryl substituents (Daugan et al., 2003b). In 2007, new analogues of tadalafil bearing various aryl groups were explored (Beghyn et al., 2007). The obtained results showed that the aryl substituents are generally well-accepted. Both electron-donating and electron withdrawing groups at the *meta* or *para* positions of the N-phenyl ring yielded active analogues. Lipophilic substituents showed diminished

activity (**31**) IC_{50} = 377 nM, whereas heterocycles like thiophene (**32**), pyridine (**33**), and pyrimidine (**34**) showed an improved IC_{50} = 18, 28, 53 nM, respectively. Despite the fact that all analogues were weaker than tadalafil yet this work offered a ground for the design and synthesis of more selective PDE5 inhibitors with better solubility (Beghyn et al., 2007).

Abadi et al. proved via ensemble docking studies that both the size and steric properties of the N-substituents of hydantoin or piperazinedione rings must be optimized to improve activity and selectivity parameters. The results of the ensemble docking showed that some essential residues that are close to the binding site are not resolved in PDB 1UDU mainly I665 and T664.

The results showed that N-substituents on both hydantoin and piperazinedione of tadalafil and its analogues occupied a hydrophobic pocket formed by Y612, L765, A767, I768, Q775, I778, and V782 and therefore the stability of the PDE5 inhibitor complex was strongly dependent on the size and bulkiness of the terminal N-substituent (Ahmed et al., 2012). Furthermore, the ensemble docking revealed that the N-alkyl substituents are extending nearby to the H-loop residues N661, S663, and I665 for the 5/6 *R*-isomers as well as to Y12 and Q775 for the 5/6 *S*-isomers. Such findings suggested that substituting the commonly adopted N-alkyl group with N-polar groups having acceptor atoms or halogen atoms might lead to more potent and more selective tadalafil analogues. Another study based on the previous findings prepared tadalafil analogues with N-alkylhydroxy or alkylamino substituents on the piperazinedione nitrogen, the polar substituent are designed to interact with some of the hydrophilic residues in the binding pocket. Moderately



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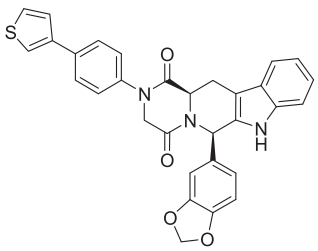

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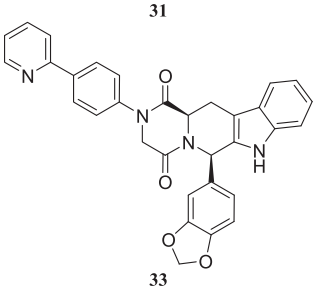

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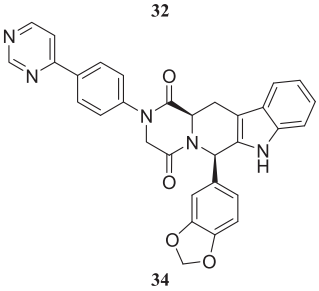

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active analogues (**35–37**) (IC_{50} = 0.6–0.9 μM) were obtained, the analogues bear 6S configuration. The results of this study suggested a new chemical pool from which novel PDE5 can be designed, compounds bearing 5*S* or 6*S* configuration and polar terminal N-substituent can serve as basis for active tadalafil analogues (Elhady et al., 2015).

The N-substituents are crucial structural determinants for PDE5 inhibition, the versatile nature of the amino acids lining PDE5 H-loop offers a large chemical space from which novel tadalafil analogues bearing alkyl, aryl, hydrophobic, or hydrophilic substituents can be designed.

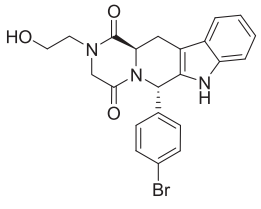
In summary, it is essential to have a *6R* configuration, *cis*-analogues are more potent than *trans*-analogues yet an *R* absolute configuration at position 6 is more crucial than an *R* absolute configuration at position 12a. The size of the terminal fused ring is well-tolerated as long as at least one carbonyl group is present. *N*-substituents on the terminal fused dione ring range from small alkyl to bulky aromatic rings with no deleterious effect on activity. Replacing the lipophilic groups with polar groups still needs further optimization. Substitution on the indole ring mostly lowered the potency. The pendant aryl ring at C-6 plays a role in both potency and selectivity. Lipophilic, electron donating, or mild electron withdrawing are highly favored at *para* position.

4 | THERAPEUTIC POTENTIAL OF TADALAFIL AND ITS ANALOGUES IN MED AND BEYOND

Many normal physiological functions are governed by NO/cGMP pathway. Disruption of this essential signaling pathway contributes to pathophysiology of several diseases. Sildenafil (**1**), the first reported PDE5 inhibitor introduced PDE5 inhibitors as the first line of therapy for MED. The success of PDE5 inhibitors of different chemical classes in the treatment of MED encouraged more research in the field of PDE5 inhibition. Many new clinical applications, such as the treatment of lower urinary tract symptoms (LUTS) associated with benign prostatic hyperplasia (BPH) and PAH were investigated. PDE5 inhibitors are clinically approved for the aforementioned indications. However, PDE5 inhibitors have been further investigated for novel indications beyond MED, CVS, and LUTS, some of these novel indications are in the clinical trials phase.

4.1 | Tadalafil in MED

More than 100 million men worldwide suffer from ED whereby they fail to reach and maintain an adequate erection (Ayta, McKinlay, & Krane, 1999). Sexual stimuli induce the release of NO in the corpus cavernosum, thus increasing the production of cGMP. cGMP is hydrolyzed via PDE5, an action that is terminated by Tadalafil (**2**). Tadalafil allows an increase in the concentration of cGMP to allow relaxation of erectile tissues and dilatation of the corpora cavernosa arteries in patients with MED. The higher blood flow causes the engorgement of the corpora cavernosa. This engorgement diminishes penile venous



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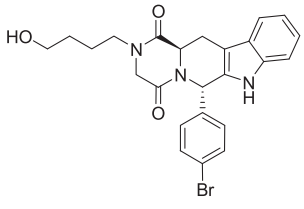

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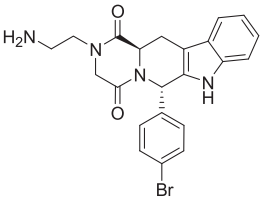

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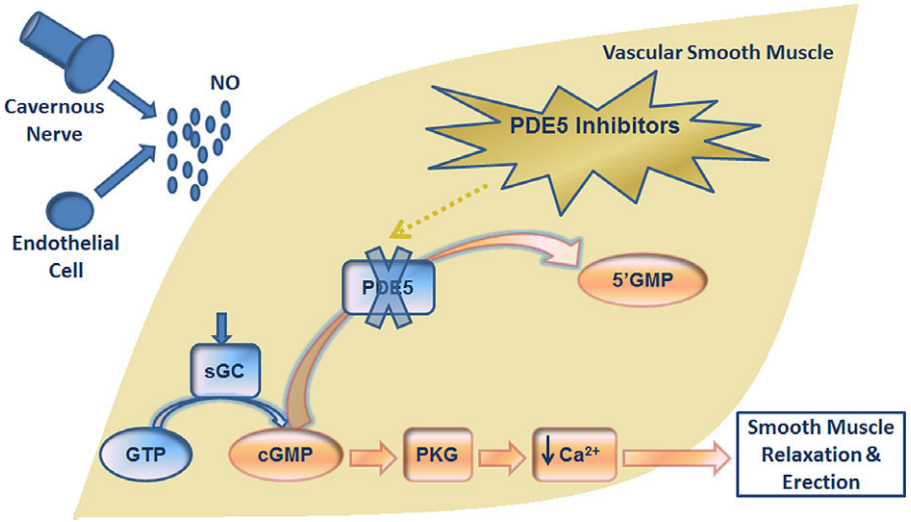


FIGURE 3 Cellular mechanisms involved in penile erection

outflow and causes higher penile blood pressure, resulting in a physiological erection (Figure 3) (Lincoln & Cornwell, 1991).

Randomized and nonrandomized controlled trials were carried out to compare sildenafil (**1**) and tadalafil (**2**), no difference were reported in terms of efficacy and side effect rates. However, tadalafil was reported to improve the psychological experience of the patients. Additionally, side effects like myalgia and back pain rates were more common with tadalafil yet the flushing rate and visual symptoms were lower. This is attributed to the higher PDE5 selectivity of tadalafil versus PDE6 and PDE11 (Gresser & Gleiter, 2002).

4.2 | Tadalafil in prostatic benign hyperplasia

In 2011, FDA approved the use of tadalafil for the treatment of lower urinary tract symptoms (LUTS) in prostatic benign hyperplasia (PBH). Male LUTS is increasing in particular with age. Symptoms include and are not limited to voiding (obstructive) symptoms; storage (irritative) symptoms and postmicturition symptoms (Abrams et al., 2003). The presence of LUTS is associated with prostate growth, urethral compression, and blockade, this leads to bladder irritability. Some research data strongly suggest that BPH is an immune inflammatory disease, this can further help design novel drugs for BPH if the nature of immune dysregulation is well-studied (De Nunzio et al., 2011; Kramer, Mitteregger, & Marberger, 2007).

The pathophysiology of LUTS is still not clear and further studies are needed to understand its complexity. As PDE5 are highly expressed in all parts of the genitourinary tract, it was logic to start using PDE5 inhibitors in LUTS. Tadalafil exerts its activity through improving the oxygenation of LUT, tadalafil contributes to smooth muscle relaxation decreasing compression and engorgement of urethera, tadalafil can interfere with prostate cells proliferation and differentiation. All these mechanisms can reduce prostate inflammation (Andersson, 2011; Giuliano et al., 2013).

4.3 | Tadalafil in cardiovascular protection

PDE5 inhibitors have a powerful protective effect against myocardial ischemia/reperfusion (I/R) injury, doxorubicin cardiotoxicity, ischemic and diabetic cardiomyopathy, and cardiac hypertrophy. PDE5 shows 2–6 upregulation in human heart disease (Shan & Margulies, 2011). PDE-5 inhibitors could potentially release endogenous cardioprotective mediators, including adenosine and bradykinin.

PDE5 inhibitors may act via NO-cGMP-PKG signaling pathway. PKG can open mitochondrial KATP channels resulting in the cardioprotective effects (Fisher, Salloum, Das, Hyder, & Kukreja, 2005; Koka & Kukreja, 2010; Kukreja et al., 2004).

4.4 | Tadalafil in PAH

Tadalafil is marketed as Adcirca by Eli Lilly for PAH treatment. PAH results from vasoconstriction of small pulmonary arteries, this leads to advanced increase in pulmonary vascular resistance and increased right ventricle afterload. PAH may in some cases lead to premature death. PAH has a complicated pathophysiology yet is mainly attributed to the compromised production of vasodilators such as NO, prostacyclin, and to overproduction of vasoconstrictors, such as endothelin-1 (Hampl & Herget, 2000). PDE5 is highly expressed in the lung; this was a base evidence for the use of PDE5 inhibitors in the treatment of PAH. PDE5 is up-regulated in conditions associated with PAH, thus PDE5 inhibition may contribute to vasodilatation and anti-proliferative effects on pulmonary vascular smooth muscle cells (Corbin, Beasley, Blount, & Francis, 2005). However, the efficacy of PDE5 inhibitors is limited by other factors, namely, presence of sufficient amounts of endogenous NO to activate sGC and generate cGMP. PDE5 inhibitors are the oral treatment option for children with PAH. A combination therapy of PDE5 inhibitors and endothelin receptor antagonists, prostacyclin (PGI2) analogues, calcium channel blockers, and sGC stimulators is a commonly adopted choice (Figure 4) (Desai & Desouza, 2017).

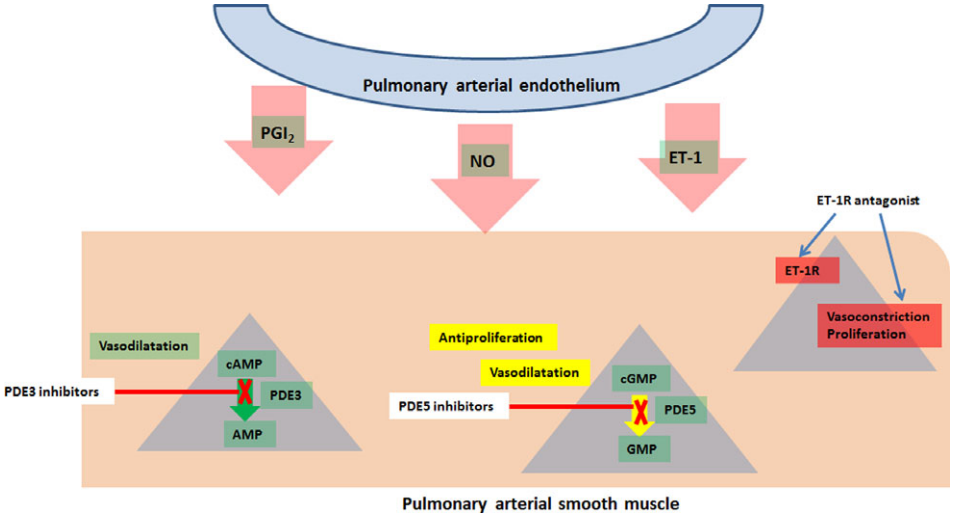


FIGURE 4 The principal targets for current therapies for PAH

4.5 | Tadalafil in central nervous system

Tadalafil enhances cGMP signaling, this secondary messenger affects neural communication through interference with presynaptic release and postsynaptic intracellular pathways of neurotransmitters. PDE5 is abundant in the hippocampus, cortex, and cerebellum of the brain (Heckman, Blokland, Ramaekers, & Prickaerts, 2015). PDE5 is involved in many central nervous system (CNS) functions such as neurotransmitter release, neuroplasticity, neuroprotection, and strengthening of cognitive abilities (Domek-Łopacińska & Strosznajder, 2010; Francis, Blount, & Corbin, 2011).

Mild Alzheimer's disease (AD) patients have significantly lower cGMP levels in the cerebrospinal fluid (CSF) when compared to healthy control subjects. This may be attributed to over expression of PDE5 in the cortex of AD patients (Ugarte et al., 2015). AD mouse models treated with tadalafil showed improvement in memory and recognition (García-Barroso et al., 2013). In 2017, Jian Li et al. prepared a series of tadalafil analogues with improved penetrability to Blood Brain Barrier. The novel analogues act as inhibitors to both AChE and PDE5 and are investigated as a novel therapeutic method for the treatment of AD. The lead compound (**42**) demonstrated significant memory-enhancing effects in AD mouse model (Mao et al., 2018). However, compound (**42**) was nearly insoluble in water and was then further optimized to produce a series of more water-soluble analogues (**43**) (Ni et al., 2018).

In Addition to the potential use for AD treatment, tadalafil produces antidepressant-like effects (Liebenberg, Harvey, Brand, & Brink, 2010) and was studied for the regulation of addictive behaviors as well. Tadalafil potentiates behavioral cocaine sensitization, and reduces threshold to generate hippocampal long-term potentiation (LTP) by increasing the levels of cGMP (Gabach et al., 2013).

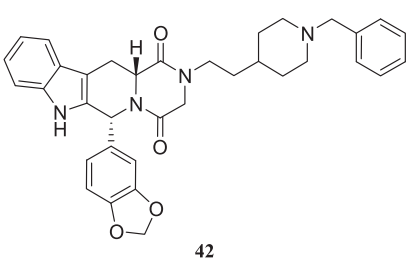
4.6 | Tadalafil in autoimmune disease

Tadalafil is the first PDE5 inhibitor with FDA approval for BPH, an autoimmune disease that involves symptomatic enlargement of the prostate gland. Tadalafil improved the overall quality of life of the patients. This effect is suggested to take place through relaxation of prostatic smooth muscle and possible improvement of pelvic blood flow and affects sensory nerve signaling from the bladder and prostate (Andersson et al., 2011).

Tadalafil has shown efficacy in the treatment of Raynaud's phenomenon associated with systemic sclerosis autoimmune disease as well. Studies showed that tadalafil significantly decreased symptoms including frequency, and duration of attacks (Roustit et al., 2013). In animal models, tadalafil proved efficacy in alleviating symptoms of osteoarthritis, a mechanism that involved reduction of cell influx and Tumor necrosis factor (TNF) release (Shenoy, Agarwal, Agarwal, Misra, & Naik, 2011).

4.7 | Tadalafil in cancer

Cancer is the final step in a series of morphological and structural changes that drive the normal cells into highly malignant derivative



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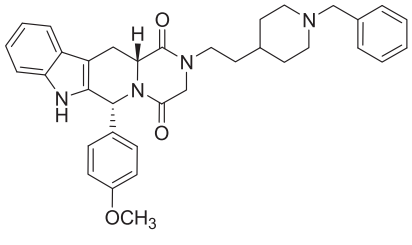

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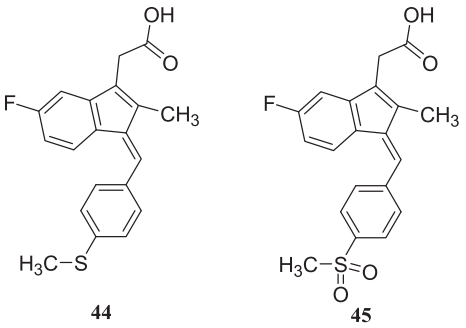
cells (Stanley, 1995). Both activation of protooncogenes, and inhibition in the tumor suppressor genes are likely to be early steps in the development of cancer (Liotta, Steeg, & Stetler-Stevenson, 1991). It is reported that PDEs are overexpressed in many tumor cells, such as breast and colon cancer cells, particularly PDE5 enzyme, inhibition of this enzyme will lead to an increase in the cGMP level inside the cell, resulting into possible apoptotic and anti-proliferative mechanisms leading to cell growth arrest and stimulation of apoptotic cascade (Hirsh et al., 2004). PKG is expressed in lower levels in the tumor cells compared to normal cells (Hou et al., 2006), which adds to the importance of PDE5 inhibitors as anticancer agents. The apoptotic effect of some agents has been attributed to their PDE5 inhibition. Agents like sulindac sulfide (**44**) and exiSulind (**45**), which is the sulfone metabolite of sulindac, both had shown remarkable activity in inducing apoptosis in colon and breast cancer cell lines, their action mainly resulted from PDE2/PDE5 inhibition (Tinsley & Piazza, 2012).



The inhibition of PDE5, prevents the degradation of cGMP increasing its levels, which in turn activates PKG, which mediates the phosphorylation and the proteolysis of B-catenin, an oncogenic protein involved in the transcription of growth regulatory genes associated with the promotion of tumor cell proliferation and survival (Li et al., 2002); (Zhu, Vemavarapu, Thompson, & Strada, 2005). Also, PKG phosphorylates MEKK1-SEK1-JNK1 signaling pathways that activate a number of caspases leading to cellular apoptosis (Figure 5) (Zhu et al., 2005).

It is also worth mentioning that the increase in the cGMP level induces mitotic arrest by increasing the mitotic inhibitor p21WAF1/CIP1 in carcinoma cells with enhanced PDE5 expression. The up-regulation of p21 is anti-proliferative as it arrests the cell cycle at the G2/M phase (Zhu et al., 2005).

PDE5 inhibition also increases the antitumor immunity reducing myeloid-derived suppressor cell function, this potentiates intratumoral T-cell infiltration and activation, PDE5 inhibitors can thus be used to improve adoptive T-cell therapy efficacy (Serafini et al., 2006).

MDR is another major challenge to chemotherapy, where by time cancer cells become resistant to chemotherapeutic drugs, leading to multidrug resistance (MDR). MDR is caused by the overexpression of ATP-binding cassette (ABC) transporters, which are membrane proteins responsible for the active transport of chemotherapeutic agents. The overexpression of ABC transporters results in efflux of chemotherapeutic agents out of cancer cells. This leads to lower intracellular concentration of chemotherapeutic agents which in turn results in therapeutic failure.

Researchers worked on development of ABC transporters modulators yet results were limited by toxicity or lack of efficacy of the new modulators. More researchers are supporting the use of PDE5 inhibitors as an alternative, based on the fact that cGMP binds to both PDE5 and ABCC5 with the same affinity therefore PDE5 inhibitors are ideal candidates to modulate ABCC5 activity. Therapeutic doses of PDE5 inhibitors effectively inhibit various ABC transporters, this allows the use of tadalafil therapeutic dose in combination with a variety of chemotherapeutics (Figure 6) (Ding et al., 2011). In a phase II clinical trial, tadalafil proved efficacy in patients with head and neck squamous cell carcinoma (HNSCC) (Califano et al., 2015). The success of tadalafil in reversing tumor-specific immune suppression encourages its use in combination with immune checkpoint inhibitors and targeted treatments to potentially increase efficacy (Hassel et al., 2017).

Cyclic nucleotide signaling pathways are crucial players in the development and progression of tumors. Overexpression of PDE isoforms in some malignancies impairs cAMP and cGMP levels in the body, PDE5 inhibitors can act to restore the balance. Thus, PDE inhibitors offer hope to future cancer treatment.

PDE5 inhibitors can contribute to the fight against cancer by targeting different aspects of the development of tumors. PDE5 inhibitors can induce apoptosis or autophagy; they may interfere with tumor invasion and metastasis; they can have a role in preventing tumor angiogenesis. PDE5 inhibitors can attenuate immunity system. Only too little is known on the antitumor effects of PDEs and their mechanism in fighting cancer.

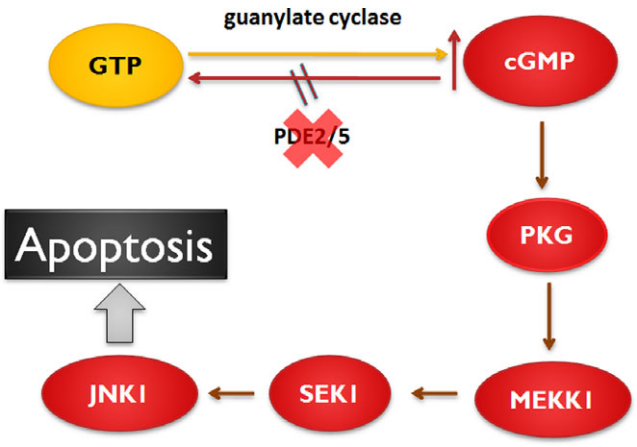


FIGURE 5 PDE5 inhibitors as pro-apoptotic agents

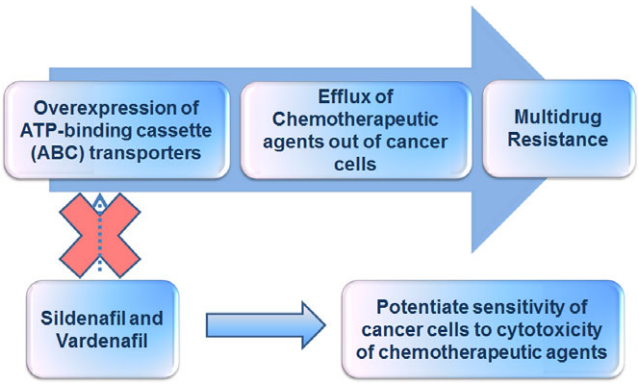


FIGURE 6 PDE5 role in MDR

Among other strategies for tumor treatment, PDE inhibitors in general and PDE5 inhibitors in particular seem very promising.

4.8 | Future therapeutic uses of tadalafil

The potential therapeutic uses of tadalafil include in addition to MED, Raynaud's syndrome, COPD, heart failure, cognition enhancement, PAH, and BPH. Adcirca was approved for the treatment of PAH in May 2009, whereas tadalafil was approved for treating the signs and symptoms of BPH in October 2011. Based on tadalafil pharmacodynamics; researcher have investigated several other possible clinical applications. Despite some of these indications have been supported by in vitro and in silico studies, yet further work needs to be done and clinical trials are still lacking in most of these researches.

Several new therapeutic indications are currently investigated. Screening a small organic compounds library to identify hits that can be used as inhibitors of Zika virus infection revealed the potential of tadalafil to inhibit Zika virus infection in nanomolar level. Tadalafil, therefore, provides an excellent template for the development of inexpensive and orally available anti-Zika drugs (Micewicz, Khachatoorian, French, & Ruchala, 2017).

Preclinical studies on early and advanced stage diabetic mice with peripheral neuropathy showed improvement in neurological functional outcome when treated with tadalafil. An 8 week consecutive administration of tadalafil enhanced the velocity of nerve conduction the sciatic nerve of a diabetic mouse.

Tadalafil should therefore be investigated in patients with diabetic peripheral neuropathy (Wang, Chopp, & Zhang, 2017).

A recent therapeutic indication for tadalafil is the treatment of female sexual disorders. Tadalafil was reported to improve some aspects of sexual function, such as desire, arousal, orgasm, and sexual satisfaction. A suggested mechanism involves elevating the levels of cGMP leading to relaxation of clitoral and vaginal smooth muscle, and thus, increasing local blood flow (Borghi & Dell'Atti, 2017).

5 | CONCLUSION

In conclusion, tadalafil is an important drug lead investigated by researchers worldwide for MED. Tadalafil is a unique member of PDE5 inhibitors family, it has a characteristic chemical scaffold (tetrahydro-β-carboline) and desired pharmacokinetics properties (essentially longer half-life). Tadalafil significantly improved psychological outcomes. Furthermore, the patients and their partners preferred tadalafil over sildenafil, and no significant difference was found in the adherence and persistence rates between tadalafil and sildenafil (Gong et al., 2017). Despite sildenafil was the first in the PDE5 inhibitors family to offer a new approach in the field of treating ED, tadalafil seems to introduce new perspectives for the treatment and cure of ED. Tadalafil discovery stimulated further research on the discovery of other significant analogues with novel biological activities. It is anticipated that in the near future, there will be more promising compounds in terms of efficacy, selectivity, and new targets.

In this review article, author worked on presenting the progressions made in the last 15 years in the tadalafil research domain. Author hopes it will assist researchers interested in PDE5 family, PDE5 inhibitors, their approved, investigated and future applications.

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