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Discovery of pyridoindole derivatives as potential inhibitors for phosphodiesterase 5A: *in silico* and *in vivo* studies

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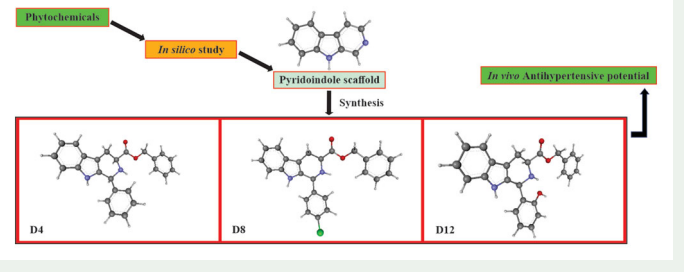
ABSTRACT

The aim of this work was to synthesise derivatives from identified plant based pyridoindole lead scaffold, and to assess phosphodiesterase 5A inhibitory potential by *in silico* and *in vivo*. Pyridoindole derivatives were synthesised by using six-stage reactor. *In silico* screening was carried out by grip-based docking methodology. In step-I, tryptophan as a starting material was reacted with different aldehydes and ketones to obtain 11 molecules. In step-II, obtained molecules were reacted with ethanol and benzyl alcohols to obtain D1 to D22 derivatives. *In silico* investigation resulted in best three molecules D12, D4 and D8 with promising BE score. Oral acute toxicity study of selected molecules resulted in LD50 value 500 mg/kg in rats. The result of *in vivo* antihypertensive study shown that molecule D12 was found to be the best antihypertensive lead molecule. This study could be a best platform to tailor novel biomolecules for inhibiting phosphodiesterase 5A enzyme in hypertension management.



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1. Introduction

Natural products are one of the best sources for obtaining a variety of therapeutic agents in drug discovery (Sircar 1982). Natural products contribute as a foundation of therapeutic agents and have shown valuable uses. Natural products are a rich source of biologically active compounds. Isolation, characterisation and pharmacological screening of natural products are the major challenging task in drug discovery as far as designing new leads from natural bioactives are concerned (Tariq and Siddiqi 1985).

Hypertension, a cardiovascular disease (CVD), is a global public health issue and the leading cause of death in the entire world (WHO 2017). Longstanding high blood pressure entertains major threat like coronary artery disease, heart failure, atrial fibrillation, dementia peripheral vascular disease, vision loss and kidney disorders (Lackland and Weber 2015).

Phosphodiesterase 5A (PDE5A) is an enzyme found in lung, cerebellum, heart, brain, platelets, cardiac myocytes, vascular myocytes, gastrointestinal tissues and penis. PDE5A inhibitors have mild systemic vasodilatory effects and could, therefore, represent a treatment for hypertension (Konstantinos and Petros 2009). The PDE5A causes vasoconstriction by preferentially hydrolysing the cyclic guanosine monophosphate (cGMP) to inactive GMP. PDE5A inhibition fosters intracellular accumulation of cGMP which inhibits calcium entry into the cell (Schellack and Agoro 2014; Abusnina and Lugnier 2017). This causes a decrease in intracellular calcium level and consequently results in relaxation of pulmonary and vascular smooth muscles (Yu et al. 1995; Chen 2010).

Recent research reported that nitrogen-containing 1H-Pyrazolo[4,3-d]pyrimidine and pyrazolopyrimidinones scaffolds could be used in PDE5 inhibition (Tollefson et al. 2010). A study reported that pyrrolo[2,3-d]pyrimidine scaffolds had antihypertensive application (Katouah and Gaffer 2019). Another nitrogen and oxygen containing scaffold, methoxyl-phenyl-pyrrol-thiophen have also been reported as a PDE5 inhibitor (Shang et al. 2014). Oxygen-containing benzofuran, naphthofuran, xanthene and chromene chemical backbones as well as nitrogen and oxygen containing furo quinoline, pyrano quinolizine and dioxalo isoquinolone backbones were also identified as lead scaffolds inhibiting PDE5A (Mali and Bhatia 2021).

Applying modern tools like computational technology offers an exclusive opportunity to screen a variety of natural products in discovering new drugs (Sircar 1982). The target-specific complex model was successfully applied for phytochemicals by using Grip-based docking methodology (Gaikwad and Jadhav 2019). In analysing receptor–-protein or drug–ligand interactions, molecular docking assists by getting the most excellent geometry of ligand/receptor composite, identifying the appropriate active sites in protein and calculating the binding energy for various molecules to design and optimise more useful ligands (Guedes et al. 2014). Similarity features for drug likeliness properties have been successfully described by using the pharmacophore modelling approach (Gaikwad and Jadhav 2021).

The potential of natural compounds in providing lead molecules have been accepted by researchers but it has certain limitations like appropriate screening methodology for lead constituents, optimisation of leads and its target suitability.

Moreover, identification of lead scaffolds and their optimisation have less tried till date. This demands the need for exploration of phyto-lead scaffolds for drug discovery.

Considering these facts; the present study was attempted to focus on exploration of nitrogen-containing pyridoindole lead scaffold which was identified by *in silico* methodology from natural database (Mali and Bhatia 2019). The objective of the present study was to synthesise pyridoindole derivatives from amino acid tryptophan as a starting material, *in silico* screening of derivatives for PDE5A inhibition, assessment of oral acute toxicity and *in vivo* antihypertensive potential of selected molecules.

2. Results and discussion

2.1. Lead scaffold identification

Docking result exhibited varying patterns in H-bonding, ionic, π-stacking and van der Waals interactions of ligands with protein (Hansen et al. 1996; Yun et al. 2014). Fourteen nitrogen-containing tricyclic molecules having three different basic chemical backbones as lead scaffolds namely pyridoindole, tetrahydro-pyridonaphthyridine and dihydropyridoquinazoline were identified (Mali and Bhatia 2019).

2.2. Synthesis of derivatives of pyridoindole

Amongst identified tricyclic leads, nitrogen-containing lead scaffold have preferred for lead scaffold optimisation because standard PDE5A inhibitor (sildenafil) (Wilkens et al. 2001) contains nitrogen heteroatom in its basic skeleton. Critical interpretation of the docking interaction progressed in the identification of vital structural functionalities whose optimisation may lead to improved potency of the selected scaffold. The importance of aromatic hetero ring, substitution at R1 and R2 is evident from the docking studies of molecules bearing it. Considering the results of virtual screening, substitutions were proposed on the pyridoindole ring system at R1, R2 and –RCOOH positions using the protocol described in Section 3. This led to the synthesis of 22 pyridoindole derivatives (D1 to D22).

For synthesis, tryptophan was used as a starting material having indole ring in it, which makes the structure electron-rich in nature. Initially, various aldehyde and ketones were used for ring-closing reaction. The optimum yield was obtained as the reaction was refluxed to 80 °C in acetic acid. Moderate reaction temperature helps in achieving pure products with minimum by-products and could be separated with suction filtration and washing. After drying, the product was used for the second step namely esterification. The carboxyl functionality on the third position of the intermediate compound was esterified with the use of either ethanol or benzyl alcohol in $SOCl_2$ to obtain designed novel ethyl/benzyl ester.

A number of signals (neglecting splitting) in ^{1}H NMR were observed for confirmation of the presence of different hydrogen environments. The number of signals in the ^{13}C NMR spectrum along with intensity were observed for deciding the carbon number in the proposed structure. The physical, chromatographic and spectral characterisation information of D12, D4 and D8 as given in Table S1.

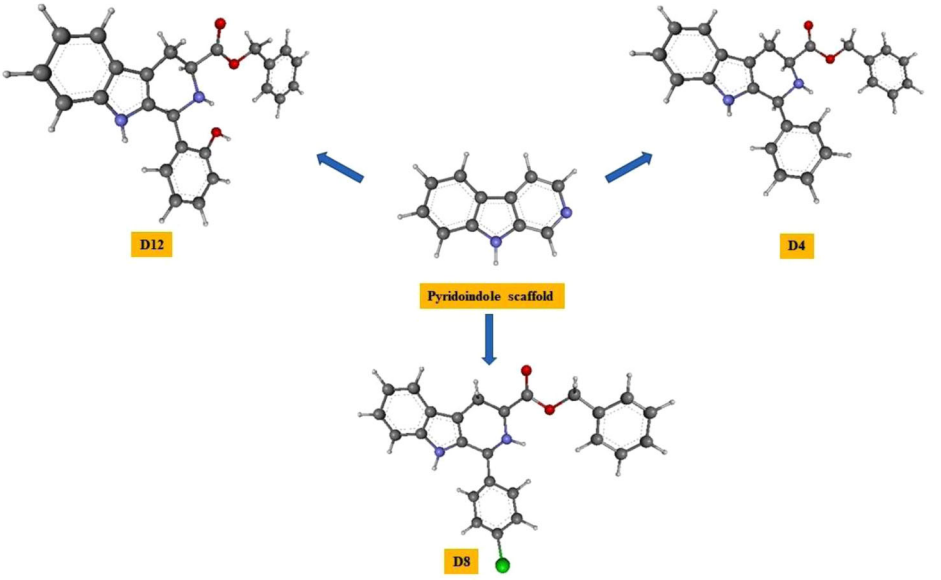


Figure 1. Structures of pyridoindole scaffold, D12, D4 and D8.

2.3. In silico study

The developed three feature pharmacophore model disclosed that the molecular assembly of sildenafil fundamentally encompasses one aromatic centre, one aliphatic centre and one centre of hydrogen bond acceptor at specific distance (Figure S1). The novel derivatives of pyridoindole were designed considering pharmacophoric features required to be present in them and were synthesised. Those derivatives which have all three features, and showing good interactions and binding energy (BE) score, are ranked at top whereas those with less than three features are ranked at bottom in Table S2. *In silico* docking studies revealed that, within 22 synthesised pyridoindole derivatives, few show satisfactory BE score as compared to standard. Considering this post-synthesis docking, all molecules were ranked according to their BE score and selected top three molecules D12, D4 and D8 with promising BE score −75.5608, −74.4264 and −70.6706, respectively (Table S2). Although the remaining molecules were showing marginally poor BE score, we have restricted our pharmacological study to these three molecules only and these molecules were shortlisted for screening of their acute oral toxicity and antihypertensive potential.

Amongst all synthesised compounds, the chemical structures of D12, D4 and D8 which have shown promising docking interactions with PDE5A are depicted in Figure 1.

2.4. Acute oral toxicity studies

After giving 300 mg/kg of D12, one animal of the group was dead, whereas after giving 300 mg/kg of D4 and D8, all animals of group were alive. In the next step, dosing

of three additional animals at 2000 mg/kg dose level was carried out where all animals for all three compounds were dead. For all three synthesised drugs (D4, D8 and D12) the LD50 value was found to be 500 mg/kg.

2.5. Ex vivo vasodilatory study pertaining to PDE5A inhibition

Results of % of relaxation of aortic endothelium showed that amongst D4, D8 and D12, the D12 was having maximum activity 60.26 ± 3.15% and 81.34 ± 2.10% as compared with standard sildenafil 63.45 ± 4.95% and 83.22 ± 3.83% with lower (0.25 μg/mL) and higher (0.5 μg/mL) concentration, respectively (Table S3).

2.6. In vivo antihypertensive activity

The antihypertensive potential of D4, D8 and D12 were estimated on rats, in which the hypertension was induced with the help of methyl prednisolone acetate, and was compared with the antihypertensive effect of standard sildenafil drug (Table S4).

The systolic blood pressure of group of untreated rats was checked initially and was found to be in the range of 113 to 119 mm Hg. Also, the rats in which hypertension was induced the systolic blood pressure was found to be in the range of 176 to 183 mm Hg. The oral administration of D4, D8 and D12 suspension (50 mg/kg) minimised the hypertension up to 120, 122 and 119 mm Hg, respectively, within 6 h, and then reached 171, 178 and 153 mm Hg, respectively, at 12 h (Figure S2). Compared with the antihypertensive effect of standard sildenafil (50 mg/kg), oral suspension of D4 and D8 have shown a gradual decrease in blood pressure up to 6 h nonetheless its action reversed and blood pressure raised to above the regular value at 12 h. The effect shown by D12 was found to be best because it controlled the blood pressure to normal (119 mm Hg) at 6 h and its antihypertensive effect remains moderately active at 12 h (153 mm Hg) as compared to the effect shown by sildenafil.

The above result suggested that the antihypertensive potential of optimised lead compound D12 was better than the sildenafil; whereas other optimised leads D4 and D8 have moderate potential. Structurally, it was observed that the benzyl ($O–CH_2–C_6H_6$) ester derivatives are more active than ethyl ($O–C_2H_5$) esters. Results suggest that if the benzene ring was attached on pyridine then the activity increases than that if alkyl substituents were attached. It could also be concluded that if the electron donating group was present on the benzene ring which was attached to pyridine then antihypertensive potential increases (inhibit PDE5A). The presence of two oxygen atoms at the ester group acts as hydrogen bond acceptor whereas two heterocyclic nitrogen moieties acts as hydrogen bond donor are important parts for the therapeutic activity. Also, the aromatic ring in the pyridoindole scaffold is essential for the activity of these derivatives. The results of *in vivo* pharmacological studies validate the results obtained in *in silico* studies. It was also observed that the synthesised lead compound D12 hold promises for the managing of hypertension that requires to be further authenticated by the clinical trial. The screening of remaining derivatives for their antihypertensive potential will be the future scope of this study.

3. Experimental

3.1. Lead scaffolds identification

The pyridoindole lead scaffold was identified from selected antihypertensive phytochemicals as per our previous *in silico* work (Mali and Bhatia 2019).

3.2. Docking parameters

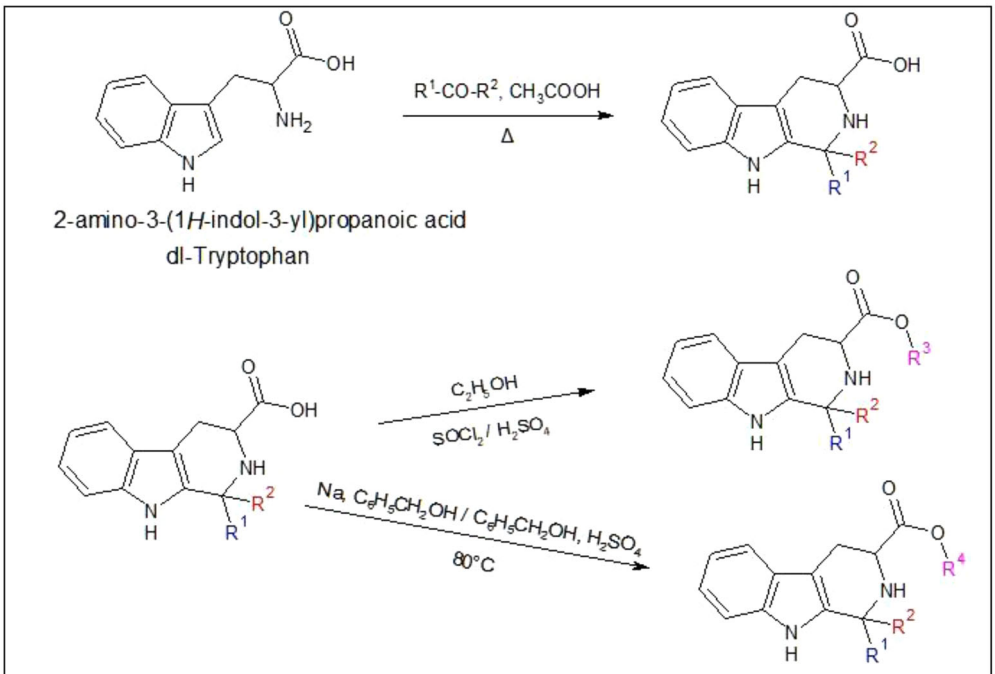
GRIP type of docking was performed in which the ligand was kept nonflexible, rotation angle was set at 10°, a number of placements were 30 and ligand wise 10 results (poses) were selected. PDB ID: 1TBF, was selected as a docking template, which was in complex with sildenafil, a reference PDE5A inhibitor was used. Docking was carried out using vLife MDS 4.4 software (vLife Sciences Technologies Pvt. Ltd., Pune, India). Docking analysis of the enzyme/ligand complex, was based on the parameters such as hydrogen bond interactions, π–π interactions, BE and orientation of the docked compound within the active site.

3.3. Synthesis of derivatives of pyridoindole

To develop novel derivatives of pyridoindole scaffold synthesis scheme was designed (Figure 2). Tryptophan as a starting material having an indole ring was used. Bischler–Napieralski or Pictet–Spengler reactions method with some modifications was designed with step I and II (Milen et al. 2005). In step I, DL-Tryptophan was reacted with required aldehyde or ketone in glacial acetic acid with stirring and heated till reflux. Different aldehydes and ketones were used and it includes acetaldehyde, benzaldehyde, *p*-nitrobenzaldehyde, *p*-cholorobenzaldehyde, *p*-methoxybenzaldehyde, 2-hydroxybenzaldehyde (salicylaldehyde), ethyl methyl ketone, acetone, diethyl ketone, isopropyl methyl ketone (3-methyl-2-butanone) and propionaldehyde. The reaction mixture was then cooled, was filtered by suction filtration. Residual acetic acid was removed by using water and then the product of the first step was dried to give 11 products in step 1. At step 2, ethanol and benzyl alcohols were reacted with step 1 products in the ice salt bath with the reaction mixture containing $SOCl_2$ for 20 min with stirring. After that the ice bath was taken aside. The mixture was then slowly heated to reflux for 4.0 h. The mixture was cooled and stirred into 500 mL of ice water. NaOH solution was used to adjust the pH to neutral and the white precipitate was formed. Water was used to wash the precipitate, then filtered, and dried using the vacuum to obtain respective novel ethyl/benzyl ester. To check the reaction development, the TLC technique was used. The proposed structures of new synthesised analogues were confirmed by taking their melting point, IR and NMR spectral information.

3.4. Acute oral toxicity studies

Institutional animal ethics committee permission was obtained as per CPCSEA guidelines (Approval No: BVCPK/CPCSEA/IAEC/01/23) for carrying out the study on animals (OECD 2001).



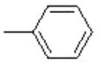
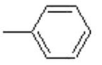
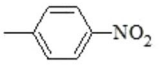
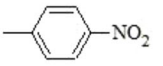
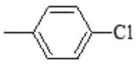
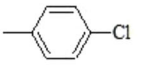
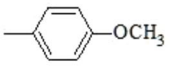
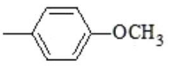
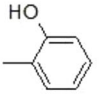
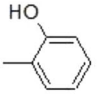
	$R^3 = -C_2H_5$			$R^4 =$	
	R^1	R^2		R^1	R^2
D1	-H	$-CH_3$	**D2**	-H	$-CH_3$
D3	-H		**D4**	-H	
D5	-H		**D6**	-H	
D7	-H		**D8**	-H	
D9	-H		**D10**	-H	
D11	-H		**D12**	-H	
D13	$-CH_3$	$-C_2H_5$	**D14**	$-CH_3$	$-C_2H_5$
D15	$-CH_3$	$-CH_3$	**D16**	$-CH_3$	$-CH_3$
D17	$-C_2H_5$	$-C_2H_5$	**D18**	$-C_2H_5$	$-C_2H_5$
D19	$-CH_3$	$-CH(CH_3)_2$	**D20**	$-CH_3$	$-CH(CH_3)_2$
D21	-H	$-C_2H_5$	**D22**	-H	$-C_2H_5$

Figure 2. Scheme for synthesis.

3.5. Ex vivo vasodilatory study pertaining to PDE5A inhibition

The goat aortic strips were brought from slaughterhouse. Aortic rings were tied to stainless steel hooks placed in Krebs–Henseleit physiological solution maintained at 37 °C. About 2 g weight was given to all tissues for the creation of basal tension and changes in basal tension were recorded (Biopac Systems). The contraction of each prepared aorta was maximised by administration of KCl (120 mM) and recorded. The tissues were pre-incubated with D4, D8 and D12 (0.25 and 0.5 μg/mL). Sildenafil was used as standard (0.25 and 0.5 μg/mL). Relaxation was expressed as a percentage change from KCl contracted levels, i.e. by comparison between maximum vascular contraction before and after addition of samples and calculated using the following formula:

$$\% \text{ Relaxation} = T_c - T_t/T_c \text{ x } 100$$

where T_c stands for tension due to contracting agents and T_t stands for tension after adding compounds.

3.6. In vivo antihypertensive activity

A total of 15 rats, aged 3- to 5-month-old of both sex and weighing between 180 and 200 g were used in the course of this study. Five groups with three rats in each group were used for the testing of optimised leads after molecular modelling studies. The rats were housed in stainless steel cages with sterilised bedding which were changed every day. Slight movement and aggression by the animal create the difference in BP reading and to avoid this training to the animals was given for staying in the restrainer. Group I was kept as control. To the animals of other four groups (Groups II to V), hypertension was developed by subcutaneous injection of 20 mg/kg/week of methylprednisolone acetate for 2 weeks as per the reported method described by Aqil et al. (2006). Before the drug administration on the day of the actual experiment, hypertensive rats from Group II to V were observed and BP was recorded. Group II was treated with standard drug sildenafil. Group III, IV and V were given an oral suspension (50 mg/kg) of test drug D4, D8 and D12, respectively, with a feeding needle. Suspension of the synthesised compounds was made in 1% w/v sodium CMC and administered at a lower and higher dose per body weight. The rat was then placed in the restrainer where the movement of animals was restricted. The dirty matter on the tail was removed with moist cotton and surface of tail was made smooth by spraying the talcum powder. The pulse transducer and tail cuff were attached around the tail and the blood pressure from the tail was recorded by CODA non-invasive BP system (a tail-cuff method, Kent Scientific Corporation) at predetermined time intervals up to 12 h. The systolic, diastolic and mean blood pressure of the rats were monitored.

4. Conclusion

Nitrogen-containing pyridoindole lead scaffold was successfully explored to discover potent derivatives by *in silico* methodology from natural database. Furthermore, *in silico* investigation of 22 compounds resulted in best three molecules D12, D4 and D8

with promising BE score. Oral acute toxicity study of D12, D4 and D8 showed the LD50 value 500 mg/kg. *Ex vivo* and i*n vivo* study results show that molecule D12 was found to be the best antihypertensive lead molecule. This study would provide a novel platform for drug discovery of antihypertensive leads from the natural resource.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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