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Inhibition of Phosphodiesterase 5 Enzyme by Pterine- 6 Carboxylic Acid from *Baphia nitida* – Related to Erectile Dysfunction: Computational Kinetic

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Authors' contributions

This work was carried out in collaboration among all authors. Author WI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SKM and EAP managed the analyses of the study. Authors AAU and MOW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Treatment of erectile dysfunction is associated with inhibition of Phosphodiesterase 5 enzyme. This study deals with the evaluation of Pterin-6-carboxylic acid inhibitory activity on phosphodiesterase 5 (PDB ID: 4OEW) using *in silico* docking studies. Pterin-6-carboxylic acid from *Baphia nitida* was isolated using GC-MS and docked into PDE5 active site. The docking result showed that pterin-6-carboxylic acid bind to the active site of phosphodiesterase 5 with the binding energy value of -7.1

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and 2.05A° - 2.23A° when compared with other compound found in the plant. Moreso, docking analysis with the ligand identified specific residues such as: Ile 778, Phe 820, Gln 817, Ser 815 and Gln 775 within the binding pocket which played an important role in the ligand binding affinity to the protein. Result from our *In silico* studies hypothesized that pterin-6-carboxylic acid can be an inhibitory agent for PDE5 protein which could be a potential drug candidate for the treatment of erectile dysfunction.

Keywords: Pterin-6-carboxylic acid; Baphia nitida; molecular docking and erectile dysfunction.

1. INTRODUCTION

Male sexual arousal is a complex process that involves the brain, hormones, emotions, nerves, muscles and blood vessels. Erectile dysfunction (ED) can result from a problem with any of these. Occasional ED is common among men who indulge in strenuous activities. Furthermore, other male sexual dysfunction like premature ejaculation, delayed or absent ejaculation, lack of sexual interest may likely contribute to reproductive abnormalities [1,2,3].

This condition is not limited to elderly men alone since its etiology often involves a combination of vascular, neurological, endocrine and psychological factors. Other risk factors such as cardiovascular disease, hypertension, diabetes, hypercholesterolemia, and smoking have been strongly associated with an increased prevalence of ED [4].

Historically, a limited understanding of the physiological mechanism of erections restricted the treatment of ED to vacuum-constriction devices, prosthetic implants, intra-cavernosal injections, and intra-urethral suppositories, [5]. Since its advent, the class of agents known as type-5 phosphodiesterase (PDE_5) inhibitors has revolutionized the management of ED [5]. PDE_5 inhibitors have become the first-line therapy for ED, as recommended by the American Urological Association (AUA) and the European Association of Urology (EAU) [5,6].

Numerous plant agents are going into the drug discovery process at the initial stage but few molecules make it to the final stage and become the potential new therapy. The failure of a candidate molecule may occur due to different factors such as adverse effects, poor pharmacokinetics and lack of efficacy and commercial reasons [7].

The use of medicinal plants in folkloric medicine is still prevalent in developing countries [8]. *Baphia nitida* is used as garden tree, wood-dye, ornament and also medical purpose [9]. Recent studies have shown the presence of numerous phytochemical components with therapeutic effects in the leaves including saponins, tannins, flavonoid and glycosides, the plant is also used to enhance sexual performance [10].

The AutoDock offers different types of search algorithms to search the conformational space. Among these, the Genetic Algorithm is the most modern and sophisticated. Genetic Algorithms are a family of powerful mathematical functions derived from the concepts of language of molecular genetics. Other types of search algorithms in AutoDock include Simulated Annealing and Local Search [11,12].

2. MATERIALS AND METHODS

The materials and methods used are bioinformatics which involves the knowledge of Biology, Computer and Online Resources. The tools included; a linux-like OS (Ubuntu 14.04 LTS), docking software (pymol), babel, Protein Data Bank (PDB) repository,NCBI Pubchem compound, Swiss model server, Data warrior, Chembl database.

2.1 Gas Chromatography- Mass Spectroscopy (GC-MS) Analysis

1 ml of prepared Sample solution was transferred into a 2 ml vial ready for GS-MS analysis. The GC-MS was carried out using Agilent 7890A-5975C GC-MS system employing the following condition;

1. HP5-column (30 m×0.25 mm×0.25 µm) operating in electron impact mode at 70eV
2. Carrier gas flow (a constant)= 1 ml/min
3. Injection volume= 0.5 µl
4. Split ratio= 10:1
5. Injector temperature= 250°C
6. Ion source temperature =280°C
7. Oven temperature: initial =70°C(hold 2 mins); 70°C to 280°C at 15°C/min(hold 5 min)

8. Mass spectra were taken at 70eV
9. Interpretation of the mass spectrum GC-MS was conducted using the NIST database.

The name, molecular weight and structure of the components in the test materials were ascertained [13,14].

2.2 Protein Preparation for Docking

The crystallized 3D structure of the human Phosphodiesterase 5 (PDE5) receptor was downloaded from the protein data bank (www.rcsb.org) with the PDB ID 4OEW, titled; crystal structure of the PDE5 catalytic domain in complex with novel inhibitors. The protein was viewed on pymol to show the amino acid sequence and the co-crystallized ligand (the ligand crystallized together with the protein, so it is downloaded in complex with the protein). The crystallized ligand was extracted to show the active site or the grid around the binding site of the protein. This grid is called the 'config.txt'. The config.txt defines the region around the active site of the target. The receptor was also generated in the pdbqt format on the pymol software. The grid center was placed in the active site pocket center. The grid boxes included the entire binding site of the enzyme and provided enough space for the ligand translational and rotational movement.

Table 1. Grid coordinates

	Grid center	Grid size
X	-29.37	22.50
Y	14.75	22.50
Z	-19.69	22.50

2.3 Ligand Preparation for Docking

Following the GC-MS analysis, a library of compound from the GC-MS result was generated by downloading the various plant constituents from the NCBI pubchem database in the 2D sdf format. The ligand (Pterin-6-carboxylic acid) in sdf was converted to pdb using the babel command. The ligand (Pterin-6-carboxylic acid) pdb was further converted to the pdbqt format using the Autodock MGLTool for ligand preparation.

2.4 Molecular Docking

Molecular docking methods are commonly used for predicting binding modes to proteins and energies of ligands [15]. Using the Autodock vina program compiled under Ubuntu 14.04 LTS, Pterin-6-carboxylic acid was docked into the target protein to get the respective binding affinity. The binding affinity predicts the strength of the molecular interaction of the ligand-protein complex. The binding results were validated using the chembl Database. The fasta sequence of the protein was gotten from Pubmed and blast on www.ebi.ac.uk/chembl/, and the search result was downloaded in the text format, using the IC_{50} chembl activity type. The smile format of the compounds were converted to sdf using Data warrior software and saved as 2D. These 2D structures were converted to pdb and pdbqt using Babel and lig prep command lines respectively to generate the 3D structure of the compounds. The 3D-generated compounds were docked into the PDE5 target using the vina command line and the corresponding docking score was plotted against their pubchem values to get the correlation value [15].

The results were analyzed using binding energy. For each ligand, a docking experiment consisting of 100 stimulations was performed and the analysis was based on binding free energies and root mean square deviation (RMSD) values, and the ligand molecules were then ranked in the order of increasing docking energies. The binding energy of each cluster is the mean binding energy of all the conformations present within the cluster, the cluster with the lowest binding energy and higher number of conformations within it was selected as the docked pose of that particular ligand. The clusters were ranked by the lowest-energy representative of each binding mode. The rest of the parameters were set as default values. Atthe end of a docking experiment with multiple runs, a cluster analysis was performed. Substrate docking with natural Plant phytochemical was performed on to PDE5 model with same parameters and PMV 1.4.5 viewer was then used to observe the interactions of the docked compound to the PDE5 model.

3. RESULTS

3.1 The Name, Molecular Weight and Percentage Composition of *Baphia nitida* Hexane Extract Using GC-MS

The result shown in Table 2, revealed that l-Guanidinosuccinimide, Benzeneethanamine, 2,5-difluoro-ß,3,4-trihydroxy-N-methyl-, Pterin-6-carboxylic acid, Actinobolin, Benzeneethana-

mine, 2,5-difluoro-ß, 3,4-trihydroxy-N-methyl-, 1-Pentanol, 4-amino, Benzeneethanamine, 2,5-difluoro-ß,3,4-trihydroxy-N-methyl-, 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester, Imidazole, 2-amino-5-[(2-carboxy) vinyl]-, Benzeneethanamine, 2,5-difluoro-ß,3,4-trihydroxy-N-methyl-, Phemethylamine, p,α-dimethyl-, Benzeneethanamine, 2- fluoro-ß,5-dihydroxy-N-methyl-, N-dl-Alanylglycine and Benzeneethanamine, 2-fluoro-ß,5-dihydroxy-N-methyl- were present and based on their percentage total, Pterin-6-carboxylic acid had higher percentage (23.44%) when compared with other compound present in the extract.

3.2 Docking Result of Pterin-6-Carboxylic Acid on Phosphodiesterase 5 (PDE5)

As shown in Table 3, the Co-crystallized ligand (the inhibitor that came with the enzyme) had a binding score of -8.7 which showed a very high binding affinity for the enzyme active site. Pterin-6-carboxylic acid one of the bioactive compound from the plant (*Baphia nitida*) under study showed a higher docking score of -7.1 when compared with other compounds. However, 9-Octadecanoic acid showed high docking score of -6.9 but not as compared to Pterin-6-carboxylic acid.

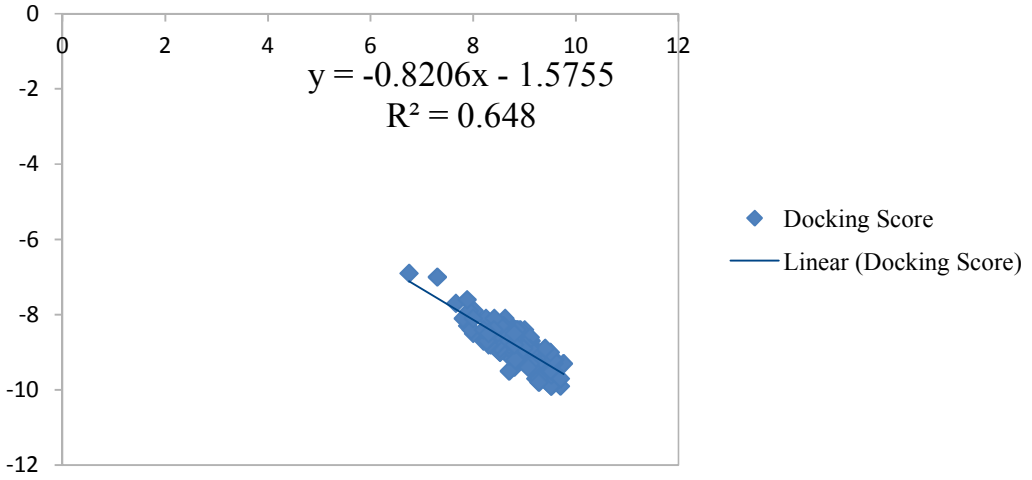

Fig. 1. Correlation between the PDE5E docking result with the investigated extracts and IC_{50} for fasta sequence of PDE5E

Table 2. The name, molecular weight and percentage composition of *Baphia nitida* hexane extract using GC-MS

S/N	Name of compound	Molecular formula	MW	% of Total
1	l-Guanidinosuccinimide	$C_5H_7N_3O_2$	141	3.11
2	Benzeneethanamine, 2,5-difluoro-ß,3,4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	3.01
3	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207	23.44
4	Actinobolin	$C_{13}H_{20}N_2O_3$	300	8.12
5	Benzeneethanamine, 2,5-difluoro-ß,3,4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	13.33
6	1-Pentanol, 4-amino	$C_5H_{13}NO$	103	4.24
7	Benzeneethanamine, 2,5-difluoro-ß,3,4-trihydroxy-N-methyl-	$C_9H_{12}FNO_2$	185	3.52
8	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	18.74
9	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	$C_6H_7N_3O_2$	153	6.76
10	Benzeneethanamine, 2,5-difluoro-ß,3,4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	3.95
11	Phemethylamine, p,α-dimethyl-	$C_{10}H_{15}N$	149	2.65
12	Benzeneethanamine, 2- fluoro-ß,5-dihydroxy-N-methyl-	$C_9H_{12}FNO_2$	185	2.83
13	N-dl-Alanylglycine	$C_5H_{10}N_2O_3$	146	3.36
14	Benzeneethanamine, 2-fluoro-ß,5-dihydroxy-N-methyl-	$C_9H_{12}FNO_2$	185	2.94

3.3 2D Chemical Interaction of Pterin-6-Carboxylic Acid and PDE5 Active Site

The binding interaction of pterin-6-carboxylic acid as shown in plates 1 and 2 revealed a better interaction of the bioactive compound to phosphodiesterase 5 enzyme active site by interacting favorably with the amino acid residues around the catalytic site of the enzyme.

3.4 Validation of Docking Result

The docking result from the investigated plant correlated positively with the result from the fasta sequence of the enzyme PDE5E (R^2=0.648) as shown in Fig. 1.

4. DISCUSSION

It has been discovered that Phosphodiestrase 5 (PDE5) is an active signaling enzyme that greatly antagonize the activities of cyclic guanosine monophosphate (cGMP) in the penile erectile tissues via inhibition of guanylyl cyclase enzyme necessary for conversion of GTP to cyclic GMP [16]. Furthermore, it has been shown that inhibiting phosphodiesterase 5pathways yielded a prolonged erection and delayed ejaculation [16]. In this study, the binding interactions of phytochemical agents from *Baphia nitida* plant were evaluated on phosphodiesterase 5 active site.

Molecular docking methods are commonly used for predicting binding modes to proteins and energies of ligands [17]. Using the Autodock vina compiled under Ubuntu 14.04 LTS, the phyto-ligands were docked into the target protein to get the respective binding affinity. The binding affinity predicts the strength of the molecular interaction of the ligand-protein complex. The docking of the plant phytochemicals to the target protein showed that the plant constituents have an inhibitory effect on the receptor. Though the plant phytochemicals do not bind better than the co-crystallized ligand (-8.7), the lead compound Pterin 6-carboxylic acid showed a good binding affinity (-7.1) to the protein, showing its inhibitory effect on the protein. Also, the analog (4-[2-(2-amino-4-oxo-1H-pteridin-6-yl) ethyl]benzoic) of the lead compound generated from the zinc database showed a better binding interaction (-9.8) to the target. Furthermore, the result showed a positive correlation with the experimented value with a correlation value of 0.65 after the correlation graph was plotted. This confirmed the validation of the docking result. We can hypothesize that Pterin 6-carboxylic acid may likely be considered as an important inhibiting agent on phosphodiesterase 5 activity and found as the most active compound in the respective target site. This Pterin 6-carboxylic acid can be promising candidate for the development towards the design of one of the key targets for erectile dysfunction drug as therapeutic compound.

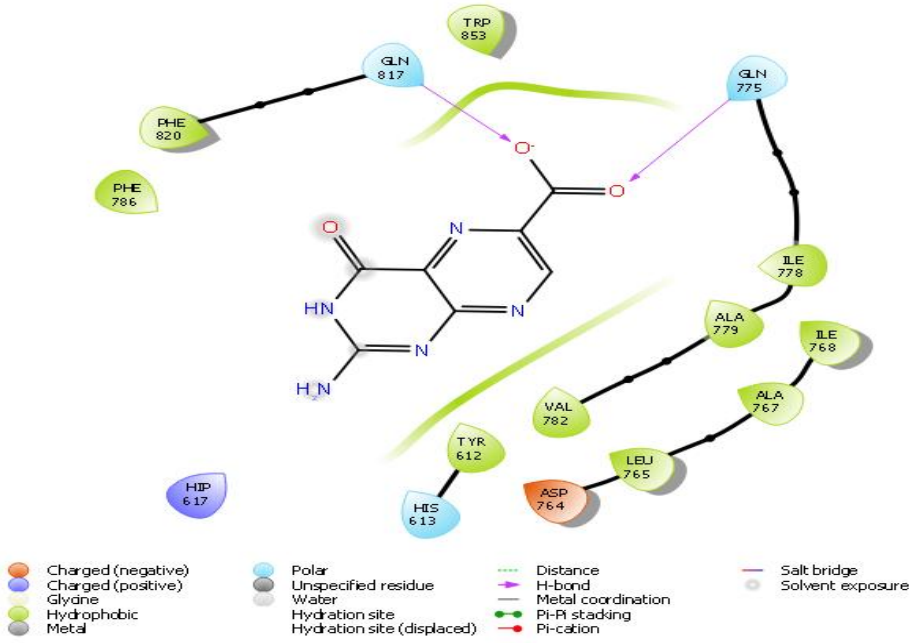

Plate 1. 2D chemical interaction of pterin 6 carboxylic acid and PDE5 active site

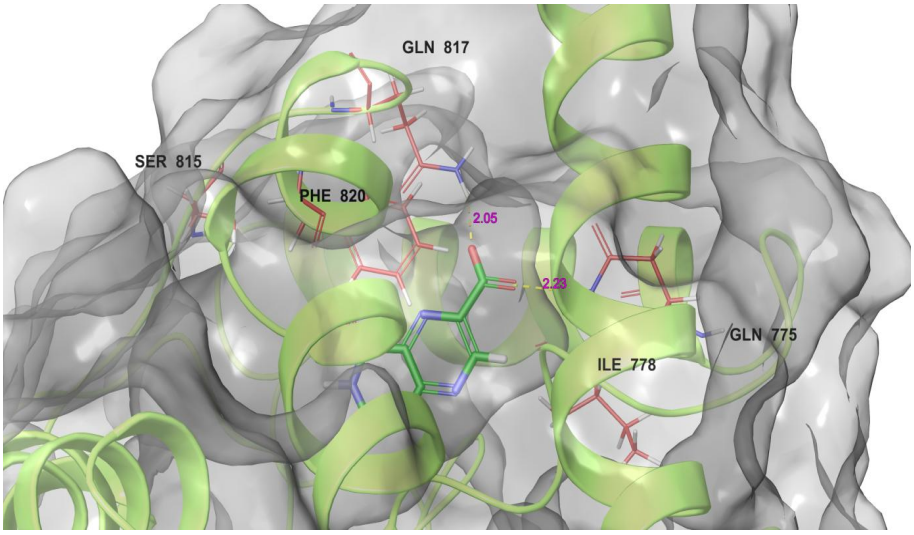

Plate 2. 3D interaction of the pterin 6 carboxylic acid and PDE5

Table 3. Docking result of plant extracts on phosphodiesterase 5 (PDE5)

Plant GC-MS examination part	Docking score
Crystallized Ligand	--8.7
(2-Aziridinylethyl)amine	--6.8
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	--5.4
1-Deoxy-d-mannitol	--5.7
1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol	--4.1
1-Pentanol, 4-amino-	--3.1
2-Methoxy-4-vinylphenol	--5.8
4-(acetyloxy)-2-butanone	--4.6
9-Octadecanoic acid	--6.9
9-octadecenoic acid, Methyl ester (E)	--6.3
Actinobolin	--6.4
Benzeneethanamine, 2,5-difluoro-.beta.,3,4-trihydroxy-N-methyl-	--6.3
Benzeneethanamine, 2-fluoro-.beta.,5-dihydroxy-N-methyl	--5.8
Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid	--6.1
Docosanoic acid	--6.4
(E)-9-Octadecanoic acid	--6.4
E-9-Tetradecenoic acid	--6.5
Eicosanoic acid	--6.3
Ethanol, 2,2'-oxybis-, diacetate	--5.1
Hentriacontane	--6.1
Hentricontane	--5.9
Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	--5.7
L-Glucose	--5.6
l-Guanidinosuccinimide	--5.5
N-DL-Alanylglycine	--5.0
n-Hexadecanoic acid	--5.8
N-Serylserine	--5.4
Octadecanoic acid	--5.9
Oleic acid	--6.2
Phenethylamine, p,a-dimethyl	--6.2
Pterin-6-carboxylic acid	--7.1
Tetradecanoic acid	--6.3
zMethy 11-methy-dodecanoate	--5.6

5. CONCLUSION

The results from the present *In silico* study indicates that Pterine-6-caboxylic acid can inhibit phosphodiesterase 5 enzyme which could be a potential drug candidate for the treatment of erectile dysfunction.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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