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DRUG REPURPOSING FOR SNAKE BITE: AN INSILICO INVESTIGATION

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***Abstract*—Snake bite is a major neglected public health issue within poor communities living in rural areas of several countries throughout the world. An estimated 2.5 million people are bitten by snakes each year and the cost and lack of efficacy of current anti-venom therapy together with the lack of detailed knowledge about toxic components of venom and their modes of action, and the unavailability of treatment in rural areas mean that annually there are around 125,000 deaths worldwide. In other to develop cheaper and more effective therapeutics, the toxic components of snake venom and their modes of action need to be clearly understood. One particularly poorly understood component of snake venom is phospholipase A2. These are enzymes which in mammals are involved in vascular inflammation correlating with coronary events in coronary artery disease and acute coronary syndrome and possibly leading to acute respiratory distress syndrome. Although phospholipase A2 activities have been reported in some snake venoms, no detailed analysis of any individual snake venom phospholipase A2 has been performed so far. Through the use of docking process, a tool in Insilico investigation, already approved drugs were tested for binding affinity to the active site of snake phospholipase A2, to detect which can be used to inhibit snake phospholipase A2. 25 of the sampled drugs exhibits higher affinity than the control (lupeol), thus, considered having anti-venom properties as such recommended for integration into health care.**

Keywords- **Drug, Repurposing, Snake bite, insilico investigation.**

I. INTRODUCTION

Most snake bites are caused by non-venomous snakes of the roughly 3,000 known species of snake found worldwide, only 15% are considered dangerous to humans (Kasturiratne *et al.*, 2008). Snakes are found in every continent except Antarctica (Kasturiratne *et al.*, 2008). There are two major families of venomous snakes, Elapidae and Viperidae. Three hundred and twenty five species (325), in sixty one (61) genera are recognized in the family Elapidae and two hundred and twenty four (224) species in twenty-two genera are recognized in the family Viperidae (integrated taxonomic information system, 2006). In addition, the most diverse and widely distributed snake family, the colubrids, has approximately 700 venomous species (Mackessy, 2002), but only five (5) genera- boomslangs, twig snakes, keel back snakes, green snakes and slender snakes have caused human fatalities (Mackessy, 2002).

Since reporting is not mandatory in regions of the world, snake bites can go unreported. Consequently, no accurate study has ever been conducted to determine the frequency of snake bites on the international level. However, some estimates put the number at 5.4 million snake bites, 2.5 million Envenoming, resulting in perhaps 125,000 deaths (Kasturiratne *et al.*, 2008). Others estimated 1.2 to 5.5 million snake bites, 421,000 to 1.8 million envenomings and 20,000 to 94,000 deaths (Karir *et al.*, 2015).

Many people who survive bites nevertheless suffer from permanent tissue damage caused by venom leading to disability (Gutierrez *et al.*, 2007). Most snake envenomings and fatalities occur in South Asia, South East Asia and sub-Sahara Africa, with India reporting the most snake bite deaths of any country (Kasturiratne *et al.*, 2008).

Although, Africa is home to four venomous snake families – Atractaspididae, colubridae, Elapidae and Viperidae – approximately 605 of all bites are caused by vipers only Mackessy, 2002). In drier regions of the continents, such as sahels and Savannas, the saw-scaled vipers inflict up to 90% of all bites (Mackessy, 2002). The black mamba, although responsible for far less snake bite incidents, is the species which has the highest mortality rate in Africa and in the world (Van Der Vlies, 2010).

In sub-Sahara Africa, over 50% of snake bite injuries are not appropriately treated (Mackessy, 2002). Between 40% and 80% of victims, depending on the country, exclusively rely on traditional medicine for treatment (Mackessy, 2002). In many sub-Sahara countries, poor availability of expensive antivenom contributes to morbility, and snake bites continue to remain a neglected health problem (Mackessy, 2002).

Sequel to the poor availability of antivenom and it expensive nature, there comes the need to initiate a conventional means of handling snake bite, and this can be best achieved through drug repurposing. Drug repurposing is one such strategy, many agents approved for other uses already have been tested in humans, so detailed information is available on their pharmacology, formulation and potential toxicity. Because

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repurposing builds upon previous research and development efforts, new candidates therapies could be ready for clinical trials quickly, speeding their review by the food and drug administration and if approved, their integration into health care.

II. MATERIALS AND METHOD

During the course of this project, certain materials were used. They include;

Hardware (Computer System)

This is the major material needed for this research and was bought from Obejor communications Nnewi, Anambra State, Nigeria.

Software

Series of software were used for the research work and were gotten from Obejor communications Nnewi, Anambra State, Nigeria.

These softwares include:

Linus operating system: This is operating system software that serves as the platform for the molecular docking simulation.

Auto dock tool: used in the preparation of ligands.

Chimera: used in preparing the receptor.

Pymol: used in viewing of the binding pockets during validation.

Auto dock Vina: used for the molecular docking simulation.

Bioinformatics Data Mining Tools

These are banks of information through which already established facts were obtained. They include:

Google: A research tool which serves as an interface for sourcing all internet based information.

Protein data bank: A bioinformatics data mining tool from which the receptor was obtained.

Drug bank: A bioinformatics data mining tool from which the ligands were obtained.

Molinspiration: Used to obtain the biological activities of the ligands.

Zinc data bank: A bioinformatics data mining tool from which the physiological parameters and structures of ligands were obtained.

III. METHODS

Several steps and techniques were employed for the success of this research work.

Selection of Ligands (Approved Clinical Drugs)

The drugs were generated from drug bank (www.drugbank.com). Approved drugs were filtered and used to query zinc data base to obtain there physiological parameters (Smiles file format, X log P, H-bond donor, H-bond acceptor, rotatable bonds, topological polar surface area).

Using the smiles file format, molinspiration was opened and the smile file was pasted to obtain the biological activities (G-coupled protein receptor ligand, Ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor, enzyme inhibitor).

The structures of these approved drugs were gotten from zinc data base in mole file extension.

Preparation of Ligands (Approved Clinical Drugs)

The downloaded structures of drugs were prepared using an Autodock tool.

The ligand (drug) of study was called into the autodock interface by clicking on “file” icon and “read molecule”. This gives an interface to browse through to locate the ligand of interest and was right clicked on to open.

Hydrogen was added to the ligand by clicking “Edit”, “hydrogen”, “add hydrogen” and “Ok”.

The number of rotatable bonds was set to ensure it does not exceed the maximum autodock tool can handle, that is 32 rotatable bonds.

The root detected by clicking “ligand” “Torsion tree” and “detect root” after which the torsion is being chosen and the icon “Done” is clicked.

The prepared ligand is saved by clicking on “ligand”, “Output” and the “Save as PDBQT”.

Selection of the receptor (Phospholipase A2)

Receptors are gotten from protein data bank (PDB). Google interface was opened and “Protein Data bank” typed into the search panel. This launched the PDB where the receptor was generated from.

Each receptor is given a four Alpha numeric PDB ID e.g. 1YXL and 3NJU. Knowing that the PDB ID of the receptor of interest is 3NJU, 3NJU was typed into the search panel, this brought forward the structure of phospholipase A2 needed.

The structure with a lower resolution is preferred because the smaller the resolution, the better the structure.

NOTE: It is better to use a receptor with a ligand because it will serve as control.

The structure of interest was downloaded as PDB file (Text).

Preparation of Receptor (Phospholipase A2)

The downloaded receptor was prepared using chimera and Autodock tool

a. Using Chimera

The icon “file” was clicked and an interface was opened to enable browsing through the documents where the receptor saved as 3NJU was called in by clicking “open”.

To Determine the Number of Chain

“Select” icon was clicked and then “chain”, one chain was detected, making it acceptable, then “OK” was clicked. If the chain happens to be two or above, the extra chain(s) will be deleted leaving only one.

Deletion of the Residues

To delete the residues present, “select” icon was clicked, then “residue”, calcium, water and ANN was detected. “HOH” was selected followed by CA by clicking on them, followed by “Action”, “Atoms/Bonds” and then “Delete”. Having deleted water and calcium, the receptor with ANN was saved by clicked on “file”, “save PDB” and was saved as 3nju-ann.

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To delete the ligand ANN, the receptor with the ligand only i.e. 3ju-ann was called into the chimera interface, "select" was clicked on, then "residue", "ANN", "Action", "Atoms/"Bond" and "Delete". The resulting structure was then saved as 3nju-edited.

b. Using Autodock Tool

The edited receptor was called into the autodock tool by clicking "file", "Read molecule", this gave an interface where edited receptor was opened.

To add charges:

"Edit" was clicked, then "Charges", "Add Kollman charges" and finally "Ok".

To add Hydrogen

"Edit" was clicked, then "Hydrogen", 'Add", "polar only" and "Ok".

Treating the Receptor as a Rigid Molecular

"Grid" icon was clicked followed by "Macromolecule", "choose", the molecule was selected and "OK" and "Save" was clicked.

The spacing was changed to 1 and sizes of X-dimension, Y-dimension and Z-dimension were changed to 25, 28 and 25 respectively, and center value of the dimension noted.

This information was used to prepare a configuration file which was used to write a script for the docking process.

Configuration file

Receptor = 3nju-edited pdbqt
Center - x = -1.922
Center - y = -11.229
Center - Z = -0.271
Size – x = 25
Size - y = 28
Size - Z =25

Validation of molecular Docking simulation

The original receptor obtained from Pdb (Protein Data Bank) containing Ann was visualized using Chimera, to obtain the amino acids present.

The receptor was edited, removing the Ann, a free Ann was obtained from Drug bank and was docked to the edited receptor. The new receptor was also visualized using Chimera to ensure that the amino acids were duplicated.

Molecular Docking Simulation

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Lengaver and Rarey, 1996).

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs (Kitchen *et al.*, 2004).

Using the configuration file above, a script was written, which was used to predict the affinity of the ligands to the receptor on autodock Vina software using a Linus platform.

The docking process was done three times to obtain three values for each ligand, this is to ensure consistency and accuracy and the values tabulated.

IV. RESULT AND DISCUSSION

From the insilico investigation conducted on 213 clinically approved drugs (ligands) to determine their affinity level to snake phospholipase A2, the following results were obtained.

Selection of Clinically Approved Drugs Result

Table 1: Drugs with higher affinity than the control (lupeol)

Name of Drug	Zinc code	Average±Std.Dev(Kcal/mol)
Dutasteride	14880001	-9.60±0.082
Ciclesonide	36047107	-9.50±0.000
Formestane	3977753	-8.80±0.000
Norelgestromin	34142545	-8.80±0.000
Fulvestrant	26265317	-8.63±0.150
Stanozolol	3873362	-8.63±0.050
Masoprocol	1539579	-8.60±0.346
Conjugated estrogens	3830788	-8.60±0.000
Nabilone	1542930	-8.53±0.050
D-Norgestrel 3-Oxime	3973186	-8.50±0.000
Bexarotene	1539579	-8.45±0.300
Nandrolone Phenylpropionate	3881613	-8.35±0.058
Estrone	13509425	-8.23±0.050
Norgestimate	3938695	-8.18±0.050
Estriol	3815418	-8.10±0.000
Finasteride	3830838	-8.08±0.050
Ecabet sodium	3779720	-8.03±0.050
Mestranol	71789838	-8.00±0.000
Sulindac	16694163	-8.00±0.000
Megestrol Acetate	12655011	-7.93±0.050
Dihydrotachysterol	4212953	-7.90±0.000
Methylprednisolone	3881946	-7.90±0.000
Oxaprozin	1863	-7.83±0.050
Lupeol	**6303891**	**-7.80±0.000**

From Table 1 above, Dutasteride and Ciclesonide have been shown to have greater affinity to phospholipase A2.

The result above is presented in average of four dock values ± standard deviation

Validation of Molecular Docking Simulation Result

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Table 2: Amino acids present in original receptor and in docked receptor

Original receptor (3nju_Ann)	Docked receptor (3nju_ann)
Tyrosine 64	Tyrosine 64
Glycine 30	Glycine 30
Aspartate 49	Aspartate 49
Tyrosine 52	Tyrosine 52
Leucine 2	Leucine 2
Histidine 48	Histidine 48
Phenyl alanine 5	Phenyl alanine 5
Tryptophan 19	Tryptophan 19
Phenyl alanine 22	Phenyl alanine 22
Phenyl alanine 101	Phenyl alanine 101
Cysteine 29	Cysteine 29
Tyrosine 28	Tyrosine 28
Lysine 31	Lysine 31
	Isoleucine 9
	Lysine 6

Table 2 above shows the result obtained in validating the docking process, it shows that all the amino acid present in the original ligand-receptor complex was duplicated in the docked complex. With this it can be said that the protocol was near 100% efficient.

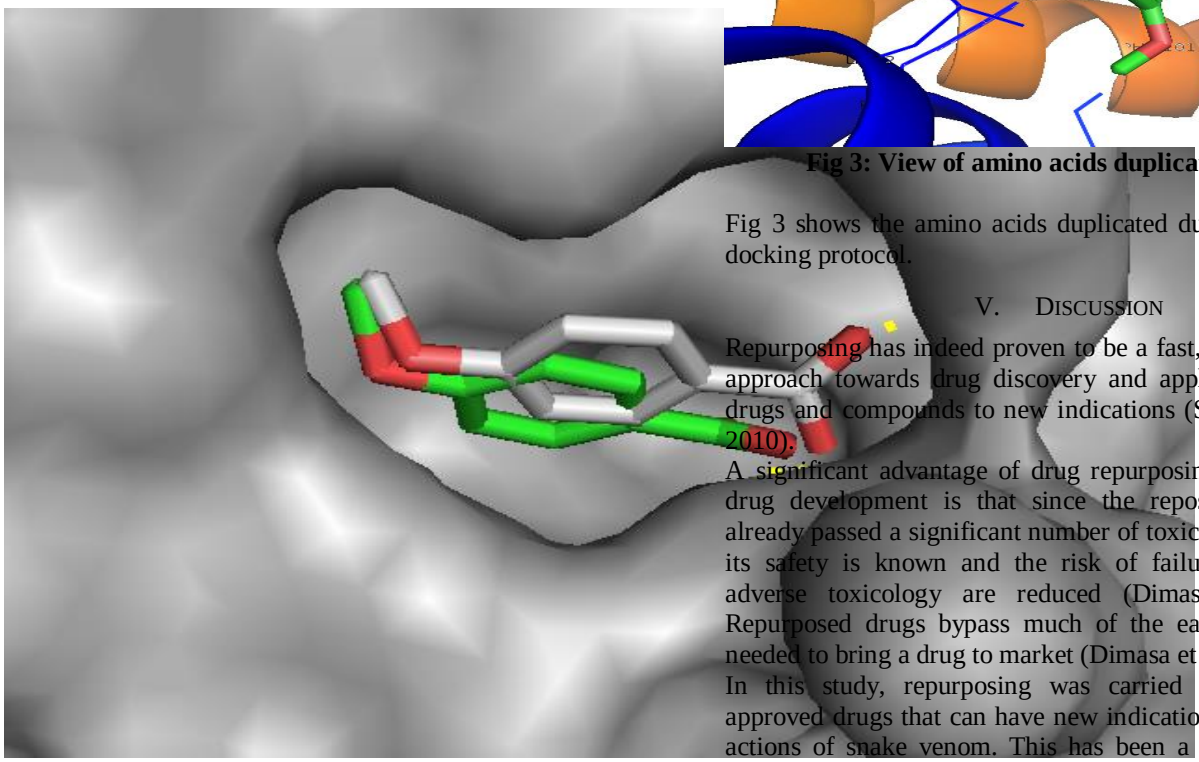

Fig 1: Validation binding pockets

Fig 1 shows that both the original (white) and the docked (green) bind at the same binding pocket.

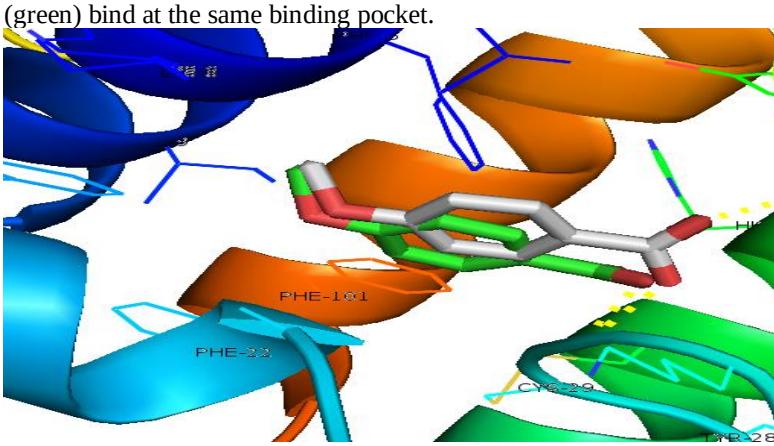

Fig 2: View of the binding pockets

Fig. 2 shows the result of the binding site of the original ANN and generated ann on phospholipase A2 when viewed using pymol, hence showing that the two targeted same binding site.

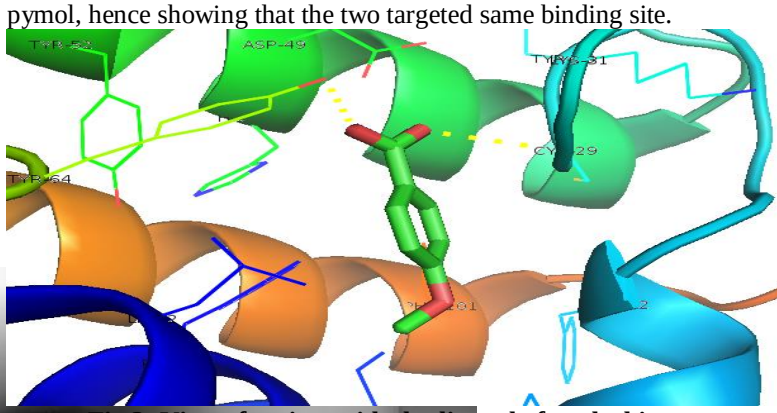

Fig 3: View of amino acids duplicated after docking

Fig 3 shows the amino acids duplicated during validation of docking protocol.

V. Discussion

Repurposing has indeed proven to be a fast, reliable and good approach towards drug discovery and application of known drugs and compounds to new indications (Sleigh and Barton, 2010).

A significant advantage of drug repurposing over traditional drug development is that since the repositioned drug has already passed a significant number of toxicity and other tests, its safety is known and the risk of failure for reasons of adverse toxicology are reduced (Dimasa et al., 1991). Repurposed drugs bypass much of the early cost and time needed to bring a drug to market (Dimasa et al., 1991).

In this study, repurposing was carried out to determine approved drugs that can have new indication of inhibiting the actions of snake venom. This has been a world problem as anti-venom are rare and expensive if not unavailable.

213 approved drugs were analyzed insilico using a type of molecular modeling known as Docking. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Lengaver and Rarey, 1996). Using Lupeol as a standard or control, 25 of these approved drugs showed greater affinity for phospholipase A2 thus showing their potentiality in the treatment of snake bite. Dutasteride and Ciclesonide have been shown to be leading in affinity to phospholipase A2. These drugs can be integrated into health care if proper investigations are done on them Invivo and Invitro.
Dutasteride is a triple 5α-reductase inhibitor that inhibits conversion of testosterone to dihydrotestosterone (DHT) (Yamana *et al.*, 2010). It is used in the treatment of benign prostatic hyperplasia (BPH); colloquially known as an "an enlarged prostrate" (Slater *et al.*, 2012). It increases the risk of erectile dysfunction (Gur *et al.*, 2013) and decreased sexual desire (Traish *et al*., 2011). Dutasteride has 99% protein binding affinity (Wilt et al., 2008), this is considered to possibly be the reason for the high affinity to phospholipase A2.
Ciclesonide is a glucocorticoid used to treat asthma and allergic rhinitis. The drug was approved for adults and children 12 and over by the US Food and Drug Administration in October 2006 (FDA News Release, 2006). Side effects of the medication include headache, nosebleeds, and inflammation of the nose and throat linings (Mutch *et al.*, 2007).

VI. CONCLUSION

Conclusively, snake bite should as a matter of urgency be seen as a world medical emergency. As such, these 25 potential anti-snake venom obtained especially the leads; Dutasteride and Ciclesonide should be incorporated into health care since their pharmacokinetic parameters have been known.
There is no end to research, science is evolving and there is no absolute truth in science. Bearing this in mind, further study on the half-life, adverse effects, bioavailability, toxicity and other parameters should be carried out for subsequent screening to obtain the best out of these potential 25 anti-snake venom. In vivo and Invitro experiments is encouraged to further determine the efficacy of these potential anti-snake venom.

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