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RESEARCH ARTICLE

IDENTIFICATION OF FDA APPROVED DRUGS WITH ACTIVITY FOR HIV- A COMPUTATIONAL DRUG REPOSITIONING APPROACH

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ABSTRACT

This study was aimed at identifying generic drugs with activity against HIV. It has two components; computational and *in-vitro* studies. The computational study entails sequential screening of all FDA approved drugs (1491) against three protein targets; through structural- and ligand- based pharmacophore screening followed by molecular docking of the selected drugs against the viral targets. Two (2) drugs with the best binding affinities against the viral targets were chosen for an *in-vitro* confirmation of activity. The non-toxic concentrations used for the study were established from MTT cytotoxicity study using C_{max} of the drugs as a guide. Iodixanol and sirolismus had the best binding affinities against the three viral targets. Again,iodixanol and sirolismus produced a concentration dependent viral killing in the antiviral studies. Iodixanol produces significant anti-HIV virucidal effect (%VI =43.3) at 1000 µg/ml not different from the effect by Zidovudine (%VI = 45.3).

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INTRODUCTION

Infectious diseases are becoming more alarming with high morbidity and mortality in developing countries like Africa (Yamani et al., 2017). HIV for instance is often regarded as incurable and fatal disease (Yamani et al., 2017). Again, the emergence of drug resistant strains have compromised the efficacy of most antiretroviral agents; some have troublesome and unbearable side effects while some are less efficacious (Kühnert et al., 2018; Wang et al., 2018). The high cost of developing drugs has limited the number of antiviral agents into a short list (Bhandari, 2017). Again, available anti-retroviral agents are both costly and of limited efficacy. The burden of HIV/AIDS for instance is of great public healthy importance. Globally, 1.8 million people became infected with HIV in 2017 alone – that is three people every minute (Zazzi et al., 2018). It is also the second leading cause of death next to lower respiratory tract infections in Africa (Awofala and Ogundele, 2018). The prevalence of HIV in Nigeria has increased from 1.8% in 1991 to 3.2% in 2014 (Awofala and Ogundele, 2018). If no intervention, this value was predicted to increase exponentially in another twenty years (Awofala and Ogundele, 2018). Nigeria was rated second HIV most prevalent country worldwide (Awofala and Ogundele, 2018). Currently available anti-retroviral agents include nucleosides reverse transcriptase inhibitors (NRTIs), non-nucleosides reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors, integrase inhibitors and CCR5 receptor antagonist (Barnabas et al., 2017). Gibson et al. (2017) reported more than 50% of treatment failures as due to development of drug resistance strains. Antiretroviral toxicity and resistance rank high on the list of problems that must be overcome to gain the long-term benefits associated with highly active anti-retroviral therapy (Barnabas et al., 2017). Toxicity is inherent to all the known anti-retroviral drugs. For example; protease inhibitor has been associated with hyperlipidaemia, hyperglycemia, gastrointestinal symptoms, and body-fat distribution abnormalities. For non-nucleoside reverse transcriptase inhibitors, rashes and hepatotoxicity have been reported while nucleoside reverse transcriptase inhibitors are associated with lactic acidosis, hypersensitivity reactions, neuropathies, pancreatitis, anaemia, and neutropenia (Glynn and Bhikha, 2017). Drug repositioning approach might be a better alternative for discovering more effective and less harmful agents. Several scientist have attempted to reposition approved drugs for the treatment of HIV. For instance, Dai et al. (2017) reported the novel anti-HIV effect of seven (7) approved drugs- cetrorelix, dalbavancin, daunorubicin,

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doxorubicin, epirubicin, idarubicin and valrubicin. This was on the basis of computational screening of approved drugs using anti-HIV predictor software followed by *In-vitro* confirmation of activity. Again, in an attempt to re-position clinically approved antimetabolites for HIV infection, seven (7) antimetabolites were tested against HIV infected cell lines (Clouser et al., 2010).

Aim of the Study: To identify generic drugs with activity against HIV through *in-silico* screening and wet laboratory confirmation.

Objectives of the Study

To develop a local database for FDA-Approved drugs and three (3) HIV viral protein targets.

- To screen commercially approved drugs using structural-based and ligand based pharmacophores as templates.
- To run a docking simulation of the selected drugs against the three viral targets and select best two (2)
- To run an in-*vitro*- antiviral assay of the two selected drugs against HIV infected 3T3 cell line.

MATERIALS AND METHODS

Criteria for Viral Targets Selection: The HIV viral targets were selected based on validated selection criteria and scoring (Ansari et al., 2018). The criteria include: involvement in a critical pathway necessary for the survival and replication of the viruses, confirmed or putative targets of known Antiviral agents, absence of significant cross talk from NCBI blast search, drugability of the target (easily accessible binding site) and site or location of the protein target within virus; either on the cell surface/cytoplasm or inside the nucleus (Ansari et al., 2018; Sliwoski et al., 2014). Targets with score of at least 80 out of 115 were selected and considered as critical in the survival and multiplication of the viruses (Sliwoski et al., 2014).

Development of Local Database of Viral Protein Targets from Protein DataBank: Protein Data Bank (PDB) is an archive of 3D structures of about 35,000-50,000 biological molecules. Three (3) HIV viral targets (in PDB text format) necessary for their survival and multiplication were selected and downloaded from the PDB website (www.rcsb.org) and saved in PDB text format. A local Database was created for the viral targets in my personal computer (Smart et al., 2018).

Development of Local Database for FDA-Approved Drug from DrugBank: A DrugBank is a drug Database that contains more than 4,000 compounds linked to about 14,000 molecular targets. All (1491) FDA-Approveddrugs were downloaded from DrugBank website (www.drugbank.ca) and saved in structural data format (SDF) (Wishart et al., 2017).

Structure-based Pharmacophore Screening: Ligand Scout advanced molecular design software was used to generate structure-based pharmacophore for each viral targets using the target- co-crystalized ligand complex as a template (Ansari et al., 2018).

Ligand-Based Pharmacophore Screening: Ligand-based pharmacophore was generated using all the co-crystalized ligands for the nine different viral targets as templates. The generated ligand-based pharmacophore can be merged or shared similarity pharmacophore (Ansari et al., 2018).

Screening of FDA-Approved Drugs using the Structural and Ligand-Based Pharmacophores as Templates: The structure and ligand-based pharmacophores were copied to the screening perspective using the copy board widget. Approved drugs downloaded from the DrugBank were loaded to the screening Database using the "create and load screening database". The generated pharmacophores were screened against the approved drugs and the drugs with similar pharmacophores were displayed in the tabular form compatible with excel (Ellingson and Baudry, 2014).

Docking Simulation of the Selected Drugs from Pharmacophore Screening against three (3)Viral Targets using PyRx Virtual Screening Tool

Importation of macromolecules from the local Database: To import macromolecule from local Database, File > Import molecule was selected, this displays "import molecule wizard" carrying different options. Workspace Tarball> local File was then selected and "Next" button clicked followed by Finish button. Shortly an "Import Completed Successfully" dialog appears, then OK button was clicked. The 3D structure of themacromolecule was displayed in the workspace and the protein ID appears in the "molecule tab" of the navigator panel. Atoms of the macromolecule were viewed in the workspace by deselecting and selecting them in the "molecule tab" of the navigator panel. The macromolecule was inspected in the workspace by right clicking and holding the mouse. The binding site of the co-crystallized area examined, in shape, size, polarity and accessibility (Ellingson and Baudry, 2014).

Importation of ligands from the local database: To import ligands from the local Database, select open babel button in the control panel of the PyRx tool. Clicking the insert new item tab on the upper left hand corner of the open babel panel, a "choose open babel supported file" box appears that takes you to the ligand Database in my personal computer. The ligand of interest was then selected and imported into the PyRx. The selected ligands appear in the open babel results table displaying the drugs ID, formula, weight and LogP. Minimized atomic coordinates of the ligand was created using "the minimize all" widget. The minimized coordinate of the ligands right clicked and different options displayed. The option "Covert all to autodock ligand PDBQT" was selected. The PDPQT format of the ligand appears in the ligand compartment of the autodock navigator area (Ellingson and Baudry, 2014).

Running the molecular docking simulation: The ligands of interest were selected from the autodock widget and "select ligand" button pressed, followed by the forward button. This automatically input the ligands into the ligand list in the control panel of PyRx software. Again, the macromolecules were selected from the autodock widget and "select macromolecule" button pressed followed by the forward button and this automatically input macromolecules into the macromolecule list in the control panel (Ellingson and Baudry, 2014). To run vina, the "run vina" was clicked, then forward button pressed. Finally "analyze result" was selected then forward button. This displays the binding affinities of the various poses against the ligands. The lower the binding affinities the better the protein-ligand interaction, since

molecules interact to conserve energy (Dallakyan and Olson, 2015). The Analyze results page is where the final docking results were presented. The table was sorted according to the values of the binding energies. The table row was selected one by one to see the corresponding docking pose for each ligand-protein complex in the 3D scene. The numerical results were exported as a Comma-Separated Values (CSV) file compatible with excel (Dallakyan and Olson, 2015).

Selection of the Best two (2) Performing Drugs for In-vitro Antiviral Studies: Two (2) drugs with overall best binding affinities against the nine viral targets were selected for confirmation of activity in the wet laboratory using *in-vitro* cell line based assay.The analytical grade of the selected drugs were purchased from sigma Aldrich.

In-vitro Model for HIV: Ten thousand cells/well were cultured in a 24-well plates for 24 hours to achieve 80-90% confluency. Media changed and the cells washed with phosphate buffer solution before addition of viral particles.

For 3T3 cell lines, 1ml of serum infected with HIV (containing 3.6 Log IU/ml of virus) was added to each well. The infected cells were maintained and propagated in DMEM (Dulbecco's Modified Eagle Medium) medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/ streptomycin and incubated at 37°C in a humidified and 5% CO_2 chamber(Farag and Mansour, 2016).

In-vitro Antiviral activity of the Two Selected Drugs: Twenty four hour post infection, the three distinct cell lines were treated with three graded concentrations of the two selected drugs (in duplicates), DMSO treated and positive controls (in duplicates). The viral RNA were released following lyses of theinfected cell lines at 24, 48 and 72 hours post treatment. The viral loads for each of the treatment groups were quantified using real time PCR.

Determination of percentage viral inhibition (%VI): The percentage viral inhibition for each of tested group was calculated using the formula(Kati et al., 2015):

$$\% \text{ VI} = \frac{\text{VC - VTG}}{\text{VC}} \times 100$$

Where VC is the viral load of the DMSO treated group
VTG is the viral load of the treated group.

Determination of IC_{50}: Nonlinear regression analysis curve generated by graph pad prism was used to extrapolate the IC_{50} of each drug against each virus. The IC_{50} is the concentration that produces 50% viral inhibition (Lo et al., 2016).

Determination of CC_{50}: Nonlinear regression analysis curve generated by graph pad prism was used to extrapolate the CC_{50} of each drug against each cell line. The CC_{50} is the concentration that produces 50% cell line viability (Lo et al., 2016).

Determination of Selectivity Index (SI): The selectivity index for each drug against a particular cell line was calculated using the formular(Lo et al., 2016).

$$SI = CC_{50}/IC_{50}$$

Data Analysis: Data were presented in tables and graphs and were expressed as mean ±SEM.

The half- maximal inhibitory concentration (IC_{50}) and half-maximal toxic concentrations (CC50) were extrapolated from a sigmoidal dose-response curve. Statistical differences between the viral loads for the different drug groups and distinct post treatment time points were analyze using a two-way ANOVA followed by Dunnet's multiple comparison tests. The p values of < 0.05 wereconsidered to be statistically significant. All analyses were performed using Graph Pad Prism version 7.

RESULTS

Three (3) Selected Protein Targets and their Scores: Three viral protein targets were identified and validated. The total score for each viral target was greater than 80, hence validated (Table 1).

Selected Drugs from Structure and ligand-based Pharmacophores: Three hundred and eight (308) and fourty three (43) FDA drugs were selected from the structure-based pharmacophore and ligand-based pharmacophore screening respectively.

Pharmacophores for the three (3) Viral Targets and the Two Selected Drugs: Hydrogen bond donor (HBD), Hydrogen bond acceptor (HBA), Negative ionisable areas (NI) and hydrophobic interactions (H) were the common structure and ligand-based pharmacophore features, as shown in Figures 1 to 6.

Best Two (2) Drugs and there Binding Affinities: Iodixanol and sirolismus had the best binding affinities against the three HIV targets. The binding affinities for each drug against the viral targets were greater than that for the respective co-crystalized ligands (Table 3).

Three Graded Nontoxic Concentrations of the Two Selected Drugs for the In vitro Antiviral Studies: The three graded non- toxic concentrations for iodixanol and sirolismusfrom MTT study were used for the antiviral study. The concentrations of the stock solution for each drug and the volume of stock to make the highest concentrations were calculated. This is to allow easy serial dilution during drug treatment (Table 4).

In-vitro Virucidal Effects of the Two (2) Selected Drugs against HIV at three Graded Concentrations and at three Post-treatment time points: Table 5 showed significant difference in the virucidal effect of iodixanol and sirolismus against HIV when compared to the negative control in all the post-treatment groups at the three graded concentrations. The virucidal effect of iodixanol was not significantly different from zidovudine in the 48 and 72 hours post treatment groups at non-toxic lowest concentrations. Again iodixanol had statistically the same virucidal effect with zidovudine in all the post treatment groups for mid non-toxic concentrations and the 24 and 48 hours post treatment groups at highest non-toxic concentration. Iodixanol produced a high viral killing effect than sirolismus across the post treatment groups of the three graded concentrations.

DISCUSSION

In this study, sirolismus demonstrated a concentration-dependent viral killing effect against HIV statistically different from the vehicle-treated group in the three post-treatment time points.

Table 1. List of three (3) Selected Protein Targets and their Scores

S/N	Viral protein targets	Critical in the pathway (50)	Putative Antiviral drug target (30)	No signi-ficant crosstalk (15)	Drug ability of the target (10)	Location of target in virus (10)	Total Score (115)
1	HIV Reverse transcriptase (1rev)	50	30	15	10	Nucleus(5)	110
2	HIV protease (1XL2)	50	30	15	10	Nucleus(5)	110
3	HIV GP41 (3L36)	50	30	15	10	Surface(10)	115

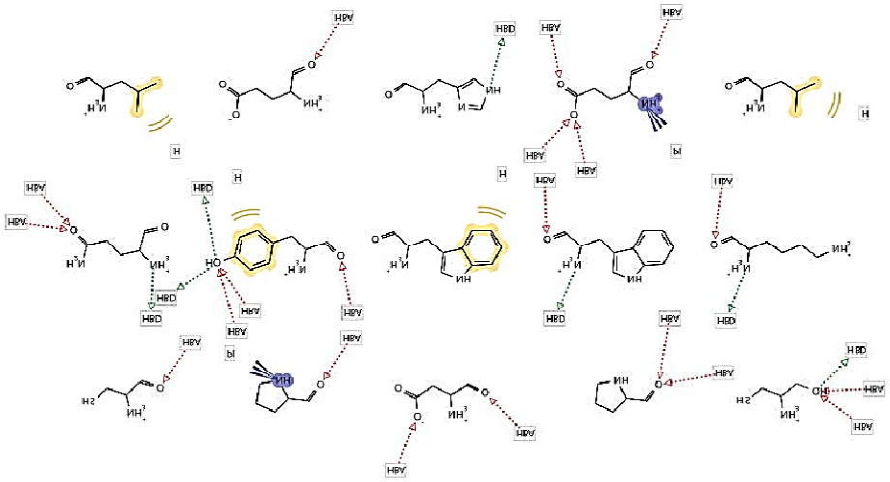

Figure 1. Structure-based Pharmacophore for HIV Gp41 (3L36) –Co-crystalized Ligand Complex

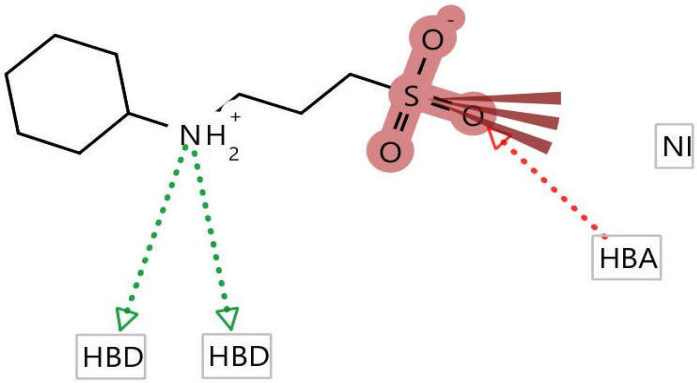

Figure 2. Structure-based Pharmacophore for HIV Gp41 (3L36) –Co-crystalized Ligand Complex

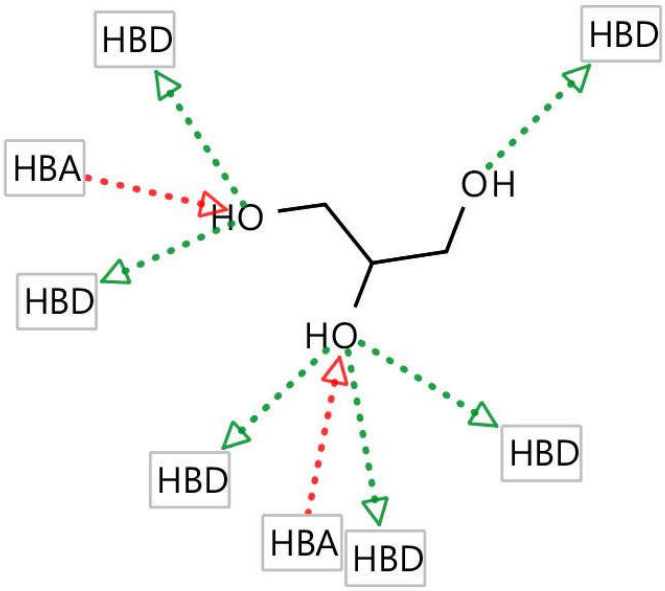

Figure 3. Structure-based Pharmacophore for HIV Protease (1XL2) –Co-crystalized Ligand Complex

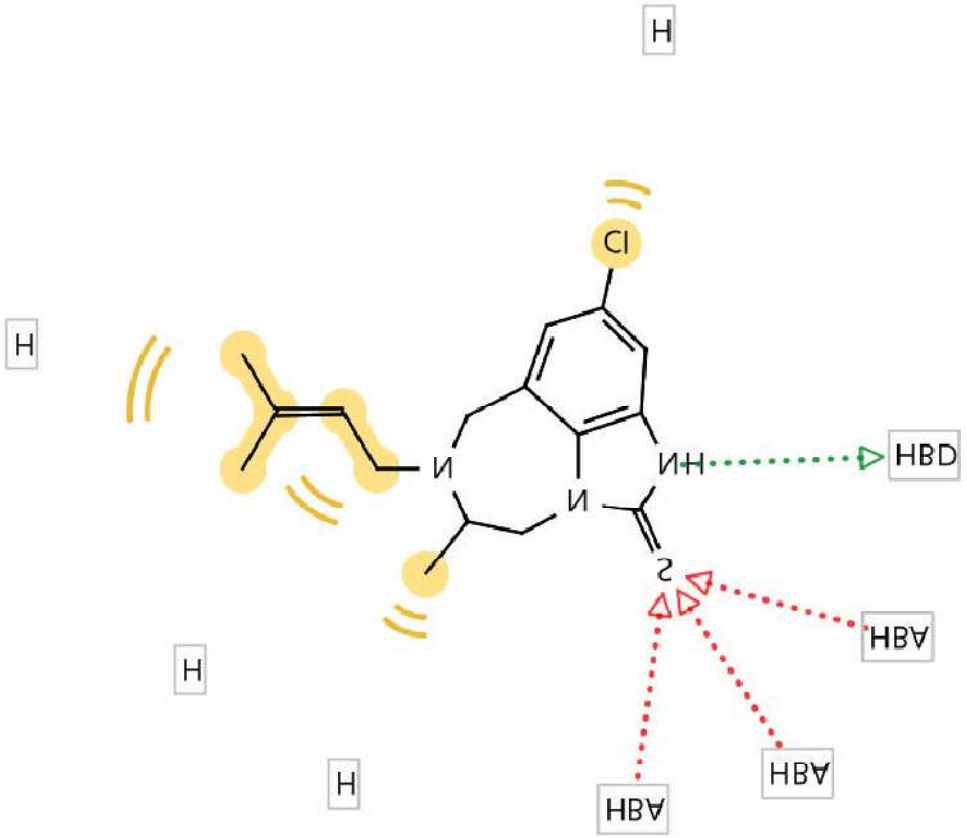

Figure 4. Structure-based Pharmacophore for HIV Reverse Transcriptase (1REV) –Co-crystalized Ligand Complex

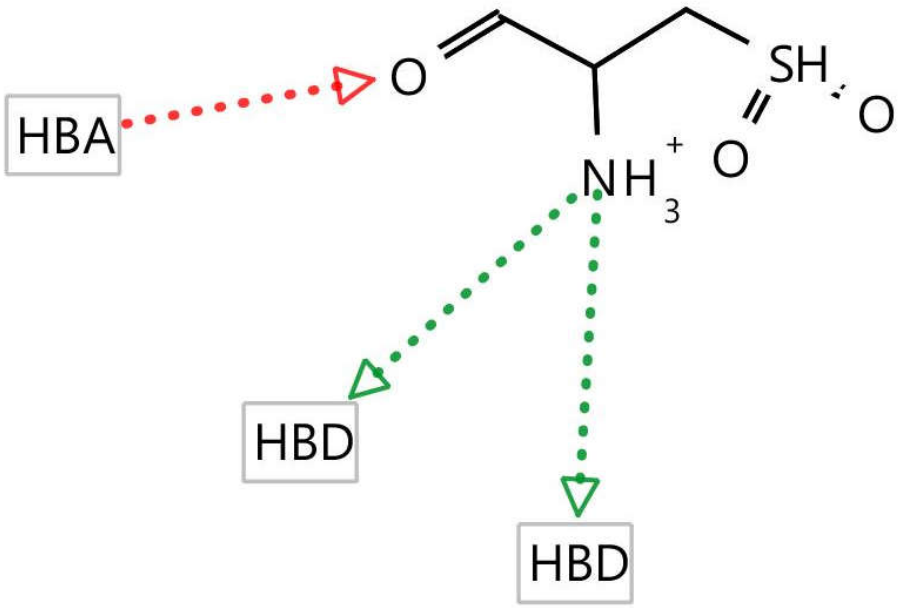

Figure 5. Structure-based Pharmacophore for HIV Reverse transcriptase (1REV) –Co-crystalized Ligand Complex

Table 3. List of Best two (2) Drugs and there Binding Affinities

Ligand	HIV-RT	HIV Protease	HIV GP41
Co-crystalized ligand	-5.5	-12.3	-7.6
Iodixanol	-19.5	-20.8	-12.8
Sirolismus	-7.7	-22.8	-13.5

Keys: HIV-RT- Human immunodeficiency virus Reverse transcriptase, HIV Protease- Human immunodeficiency Protease, HIV GP41- Human immunodeficiency virus Glycoprotein 41,

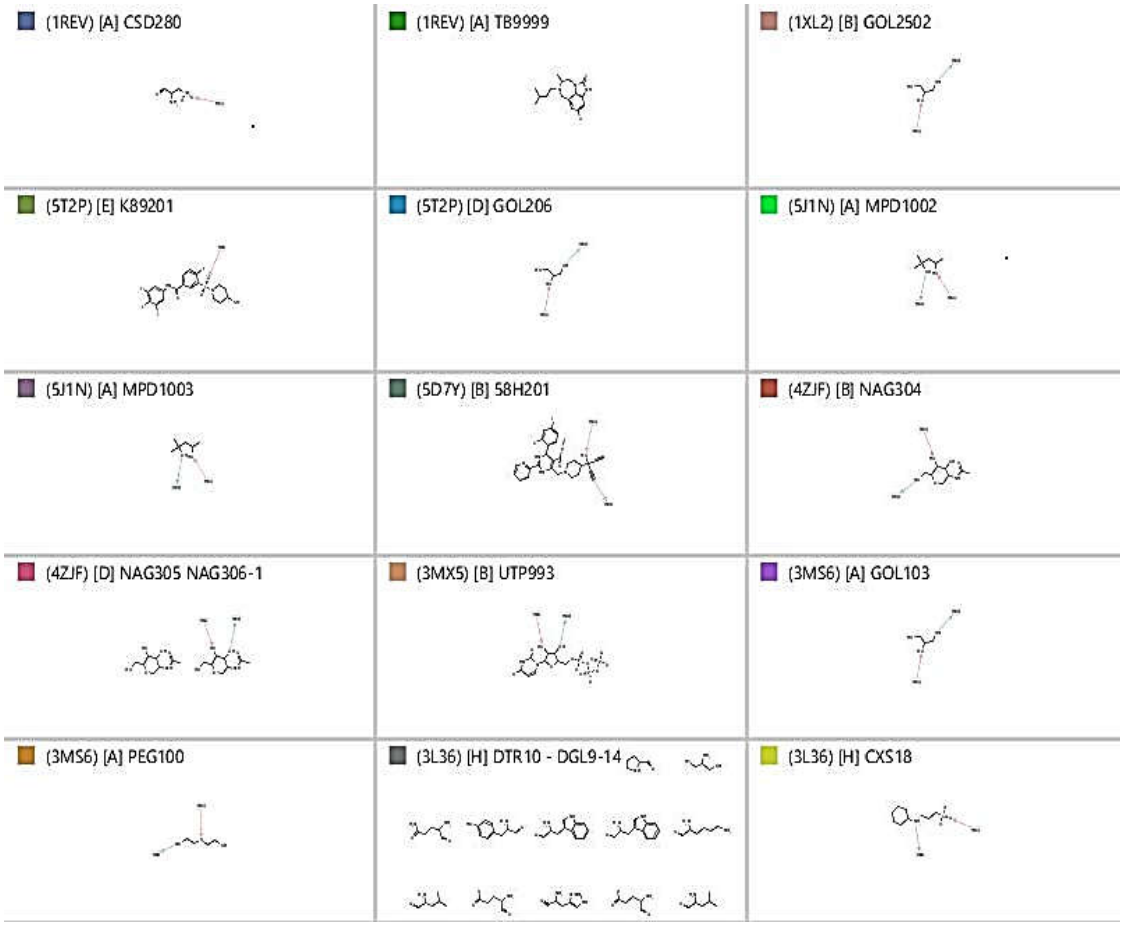

Figure 6. Ligand-based Pharmacophore for the Nine (9) Viral Targets

Table 4. Three Graded Nontoxic Concentrations of the Two Selected Drugs for the *In vitro* Antiviral Studies

Drugs	Lowest conc. (µg/ml)	Mid conc. (µg/ml)	Highest conc. (µg/ml)	Conc. Of stock (µg/ml)
Iodixanol	1000	2000	4000	16,000
Sirolismus	0.1	0.2	0.4	100

In-vitro Virucidal Effects of the Two (2) Selected Drugs against HIV at three Graded Concentrations and at three Post-treatment time points

Table 5. In vitro Virucidal effects of Iodixanol, Sirolismus and Zidovudine against HIV at three Graded Concentrations and at three Post- treatment time points

	Drugs	24 hours	%VI	48 hours	%VI	72 hours	%VI
Lowest Concentrati	Iodixanol	2.077± 0.074***	40.7	1.981 ± 0.003*	43.3	1.987 ± 0.003*	43.3
	Sirolismus	2.16 ± 0.002***	38.3	2.118 ± 0.003***	39.4	2.070 ± 0.051***	40.9
	Zidovudine	1.954 ± 0.010*	44.2	1.911 ± 0.014*	45.3	1.902 ± 0.017*	45.7
	DMSO	3.500 ±0.000		3.496 ± 0.004		3.502 ± 0.002	
Mid concentrati	Iodixanol	1.836 ± 0.010*	47.6	1.808 ± 0.021*	48.6	1.783 ± 0.004*	49.1
	Sirolismus	2.079 ± 0.003***	40.6	2.058 ± 0.055***	41.5	2.005 ± 0.002***	42.7
	Zidovudine	1.807 ± 0.000*	48.4	1.811 ± 0.004*	48.6	1.807 ± 0.000*	48.4
	DMSO	3.501 ± 0.001		3.519 ± 0.019		3.500 ± 0.000	
Highest concentrati	Iodixanol	1.824 ± 0.002*	47.9	1.847 ± 0.021*	47.3	1.793 ± 0.006***	48.8
	Sirolismus	2.002 ± 0.001***	42.8	1.992 ± 0.021***	43.2	2.036 ± 0.052***	41.8
	Zidovudine	1.808 ± 0.001*	48.4	1.798 ± 0.011*	48.7	1.693 ± 0.036*	51.6
	DMSO	3.500 ± 0.000		3.503 ± 0.003		3.500 ± 0.000	

Values are expressed as mean ± SEM, n=2.
Two-way ANOVA followed by Dunnet's multiple comparison tests was used. Values of the group with superscript * are statistically significant ($p<0.05$) compared to negative control group. Values of the group with superscript ** are statistically significant ($p<0.05$) compared to positive control group. Values with superscript *** are statistical significant ($p<0.05$) compared to both negative and positive control groups. The higher %VI the more the antiviral activity of the drug.
Keys: HIV- Human Immunodeficiency Virus, DMSO = Dimethylsulfoxide (Negative control), Zidovudine (Positive control)
%VI = VC-VTG/VC X 100 %VI = Percentage viral inhibition VC = viral load of the DMSO treated group VTG = Viral load of the drug treated group

Table 6. Selectivity Index of Iodixanol and Sirolismus of HIV Cells

	Drugs	CC_{50} (µg/ml)	IC_{50}(µg/ml)	SI
HIV	Iodixanol	18778	3251	5.80
	Sirolismus	1.536	3.445	1.40
	Zidovudine	39.07	8.859	4.40

Keys:
HIV- Human Immunodeficiency Virus; 3T3 cells- Mouse embryonic fibroblast cells; CC_{50} is the concentration that produces 50% cell line viability; IC_{50} is the concentration that produces 50% viral inhibition; SI = CC_{50}/IC_{50} SI= Selectivity Index
Both IC_{50} and CC_{50} were extrapolated using nonlinear regression analysis generated by graph-pad prism. The higher the SI the more effective and safe a drug would be during treatment

Additionally, the maximal effect was seen in the 48 hours post-treatment group indicating that 48 hours is the optimal effective period for sirolismus against HIV. At C_{max} (0.1 µg/ml), the virucidal effect was only 40.9%, which is statistically different from the DMSO treated group. However, at the highest non-toxic concentration of 0.4µg/ml, a significant virucidal effect of 43.2% was recorded, though statistically different from 48.7% for zidovudine. Conversely, the IC_{50} of sirolismus against HIV was 1.4µg/ml, by far greater than the C_{max} (0.1 µg/ml), indicating that sirolismus is less potent for HIV. Again, the selectivity index for sirolismus against HIV infected 3T3 cells was 1.4 indicating a narrow margin between the safe and toxic concentrations. However, the selectivity index of the zidovudine on the other hand, was 4.4, implying that zidovudine was more selective for HIV.

Comparably, sirolismus shared similar pharmacophore features (2 HBA, 2 HBD and 2 hydrophobic interactions) with all the structure and ligand-based pharmacophores generated from the HIV targets. This might be a possible molecular reason for its activity against HIV. Again, Sirolismus had the highest (22.8) predicted binding affinity against HIV protease, hence its mechanism of action against HIV might be related to high affinity for HIV protease. Additionally, the 3D structure of sirolismus superimposes with the 3D of the respective co-crystalized ligands for three HIV targets, again this might further explain its *In-vitro* anti-HIV activity. Similar study revealed that sirolismus; a rapamycin, downregulates CCR5 density on lymphocytes(Heredia et al., 2003). In another study, sirolismus greatly enhances Vicriviroc activity against HIV-1 (Donia *et al*., 2010). Sirolismus/Vicriviroc combinations demonstrated a considerable synergistic activity that translated into Vicriviroc dose reductions of up to 65-fold (Donia *et al*., 2010). Theoretically, it may not seem appropriate to use immunosuppressive drugs in HIV-1 infected individuals. A possible explanation is that hyperactivation of the immune system may favour HIV-1 infection and that HIV infection of human T cells is favoured by T cell activation (Corbeau and Reynes, 2011). The fact that immune activation in HIV patient upregulates CCR5 expression further buttresses our argument (Kottilil *et al*., 2004). Iodixanol on the other hand, produced the maximum virucidal effect (49.1%) at mid-concentration of 2000µg/ml suggesting that the maximum efficacy of iodixanol against HIV infected 3T3 cell is achieved at 2000µg/ml. Again, the maximal effect was seen in the 72 hours post-treatment group indicates that 72 hours is the optimal effective period for iodixanol against HIV infected cell line. Excitingly the virucidal effect of iodixanol at C_{max} (1000µg/ml) was 43.3%, statistically the same with the effect produced by zidovudine (45.7%). Again, the highest virucidal effect of iodixanol (49.1%) produced at 2000µg/ml was statistically not different from the effect produced by Zidovudine (48.4%).

Conversely, the IC_{50} of iodixanol against HIV was 3251µg/ml, three times greater than the Cmax(1000µg/ml), indicating that iodixanol is less potent for HIV. Interestingly, the selectivity index for iodixanol against HIV infected 3T3 cells in this study was 5.8 (greater than 4.4 for zidovudine), signifying that the drug is more selective for HIV with minimal cytoxicity on the 3T3 cell line. Comparably, iodixanol shared similar pharmacophore features (2 HBA, 2 HBD and 2 hydrophobic interactions) with all the structure and ligand based-pharmacophores generated from the HIV targets. This might be a possible molecular reason for its activity against HIV. Again, Iodixanol had the highest (19.5) predicted binding affinity against HIV reverse transcriptase, hence its mechanism of action against HIV might be related to high affinity for HIV reverse transcriptase. Additionally, the 3D structure of iodixanol superimposes with the 3D of the respective co-crystalized ligands for three HIV targets, again this might further explain its *In-vitro* anti-HIV activity. From the literature search using different search parameters, no known study have reported anti-HIV effect of iodixanol. However, copper iodide, an iodine-containing compound exert Antiviral activity against H1NI influenza by generating hydroxyl radicals (Vincent *et al*., 2016). Similarly, Povidone iodinesolution showed good efficacy against both enveloped and non- enveloped viruses including adenovirus and polyomaviruses (Eggers *et al*., 2015). Conflictingly, an antimicrobial study revealed iodixanol not to impede bacteria growth in a culture media (Klimentová and Stulík, 2015).

Conclusion

The result of the study showed that iodixanol and sirolismus produced a concentration dependent viral killing against HIV.

Recommendations

Further evaluation of the iodixanol and sirolismus against the three viruses using different cell lines followed by in vivo studies at biosafety level IV is recommended. Combination analysis of iodixanol and sirolismus with known anti-retroviral drugs might yield fruitful result.

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