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Dual targeting of cytokine storm and viral replication in COVID-19 by plant-derived steroidal pregnanes: An in silico perspective

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ABSTRACT

The high morbidity and mortality rate of Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) infection arises majorly from the Acute Respiratory Distress Syndrome and “cytokine storm” syndrome, which is sustained by an aberrant systemic inflammatory response and elevated pro-inflammatory cytokines. Thus, phytocompounds with broad-spectrum anti-inflammatory activity that target multiple SARS-CoV-2 proteins will enhance the development of effective drugs against the disease. In this study, an in-house library of 117 steroidal plant-derived pregnanes (PDPs) was docked in the active regions of human glucocorticoid receptors (hGRs) in a comparative molecular docking analysis. Based on the minimal binding energy and a comparative dexamethasone binding mode analysis, a list of top twenty ranked PDPs docked in the agonist conformation of hGR, with binding energies ranging between −9.8 and −11.2 kcal/mol, was obtained and analyzed for possible interactions with the human Janus kinases 1 and Interleukins-6 and SARS-CoV-2 3-chymotrypsin-like protease, Papain-like protease and RNA-dependent RNA polymerase. For each target protein, the top three ranked PDPs were selected. Eight PDPs (bregenin, hirundigenin, anhydroholantogenin, atratogenin A, atratogenin B, glaucogenin C and hirundigenin D) with high binding tendencies to the catalytic residues of multiple targets were identified. A high degree of structural stability was observed from the 100 ns molecular dynamics simulation analyses of glucocorticin C and hirundigenin complexes of hGR. The selected top-eight ranked PDPs demonstrated high druggable potentials and favourable in silico ADMET properties. Thus, the therapeutic potentials of glucocorticin C and hirundigenin can be explored for further in vitro and in vivo studies.

1. Introduction

Coronavirus disease 2019 (COVID-19) is a clinical syndrome, caused by Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) [1]. The clinical presentation of SARS-CoV-2 infections ranges from asymptomatic condition or mild symptoms (such as fever, cough, and generalized malaise) in the majority of the cases to severe respiratory failure. The early stage of infection, progresses to interstitial pneumonia and acute respiratory distress syndrome (ARDS) in nearly 10%–20% of the cases, especially in the elderlies and people with co-morbidities [2]. The pathophysiology of SARS-CoV-2 infection is a complex mechanism that is known to mobilize several biomolecules of the immune and hematologic systems [3].

Cytokines are a group of polypeptide signaling molecules responsible for regulating a large number of biological processes via cell surface receptors [4]. The term “cytokine storm”, a condition characterized by an exaggerated activation of the immune system was first associated with onset of the graft-versus-host disease [5] and later known to be involved in several viral infections [6]. The exaggerated cytokine release in response to viral infection, has emerged as one of the mechanisms leading to acute respiratory distress syndrome and multiple-organ failure in COVID-19 [7]. In this regard, recent studies have shown that patients with COVID-19 have higher levels of inflammatory cytokines, such as interleukin (IL)-1β, IL-2, IL-6 IL-7, IL-8, IL-9, IL-10, IL-18, tumor necrosis factor (TNF)-α, granulocyte colony-stimulating factor (G-CSF),

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granulocyte-macrophage colony-stimulating factor, fibroblast growth factor, macrophage inflammatory protein 1, compared to healthy in granulocyte-macrophage colony-stimulating factor, fibroblast growth signaling of the gamma common (with the other three JAKs. The pairing of JAK1 with JAK3 regulates the signaling of type I interferons (IFN\(\alpha\), IFN\(\beta\), IFN\(\gamma\)), type II interferons (IFN\(\gamma\)) and the IL-10 family of cytokines [16]. Inhibitors of the JAK-STAT pathway, such as baricitinib and Ruxolitinib, are used for suppressing proinflammatory cytokine production and systemic inflammation. Interleukin-6 (IL-6) is a pleiotropic cytokine. In general, IL-6 inhibitors prevent human IL-6 from binding to IL-6 receptors, thus impeding the formation of immune signaling complexes on cell surfaces [17].

Along with structural proteins, the SARS viral genomes encode non-structural proteins, including 3-chymotrypsin-like protease (3CL\textsuperscript{pro}), papain-like protease (PL\textsubscript{pro}), helicase and RNA-dependent RNA polymerase (RdRp) which are important target for the development of therapeutics [18]. The proteolytic processing of the polyproteins is performed by the viral cysteine proteases to yield 16 non-structural proteins; 3CL\textsuperscript{pro} cleaves and modifies the viral polyproteins at 11 sites while PL\textsubscript{pro} cleaves the first three sites at the N-terminus [4,19]. The RNA-dependent RNA polymerase (RdRp), is a central component of coronaviral replication/transcription machinery that catalyzes RNA-template dependent formation of phosphodiester bonds between ribonucleotides In our recent work, we have demonstrated the potential of some natural compounds as inhibitors of these proteins [20–22].

Recently, dexamethasone, a potent synthetic anti-inflammatory glucocorticoid was declared as the world’s first treatment proven effective in reducing the risks of death through cytokine storm among severely ill COVID-19 patients based on clinical trial results [23,24]. Through computational and biological comparison, few plant-derived steroidal compounds have been suggested as modulators of inflammation through interactions with GR (Dean et al., 2017; Morsy et al., 2019). Such plant-derived anti-inflammatory steroids like glycyrrhetinic acid [25], guggulsterone [26], boswellic acid [27], withaferin A [28] and diosgenin [29] have a common cyclopentanoperhydrophenanthrene steroid ring structure. Steroids are naturally occurring C_{21} steroidal compounds that have been documented with wide range of bioactivities including anti-inflammatory activity [22,30–32]. Due to the present COVID-19 pandemic, there is urgent need for such plant-derived steroids that may possess dual interference with cytokine storm and viral replication/transcriptase complex but with fewer side effects. Thus, the aim of this study was to screen an in-house library of plant-derived steroidal pregnanes for hGR agonist using a comparative molecular docking approach.

2. Materials and methods

2.1. Retrieval of protein structure

The three-dimensional (3D) structure of human glucocorticoid receptors in the agonist conformation (hGRag) (PDB ID: 4UDC), human glucocorticoid receptors in the antagonist conformation (hGRagt) (PDB ID: 1NHZ), human Interleukin-6 (hIL-6) (PDB ID: 1ALU), human Janus kinase 1 (hJAK1) (PDB ID: 6BU), SARS-CoV-2 3-chymotrypsin-like protease (3CL\textsuperscript{pro}) (PDB ID: 6Y84), SARS-CoV-2 papain-like protease (PL\textsubscript{pro}) (PDB ID: 6W9C) and SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) (PDB ID: 6MT1) were retrieved from the Protein Data Bank (http://www.rcsb.org).

2.2. Protein preparation

The crystal structures of the of proteins were processed by removing existing ligands and water molecules while missing hydrogen atoms were added according to the amino acid protonation state at pH 7.0 utilizing Autodock version 4.2 program (Scripps Research Institute, La Jolla, CA). Thereafter, non-polar hydrogens were merged while polar hydrogens were added to each protein. The process was repeated for each protein and subsequently saved into a dockable pdbqt format for molecular docking.

2.3. Ligand preparation

PDPs (117) were compiled from literature search. The Structure Data Format (SDF) structures of the reference compounds: dexamethasone (Dex), mifepristone, methotrexate, ruxolitinib, ritonavir, disulfiram, remdesivir and some of the compounds were retrieved from the PubChem database (www.pubchem.ncbi.nlm.nih.gov); other compounds not present on the database were drawn with Chemeval version 19. All the compounds and reference compounds were converted to mol2 chemical format using Open babel (O’Boyle et al., 2011). The non-polar hydrogen atoms were merged with the carbons, polar hydrogen charges of the Gasteiger-type were assigned and the internal degrees of freedom
2.4. Molecular docking study

2.4.1. Competitive molecular docking to the human GRs approach combined separate molecular docking models for a structure based identification of agonist of the agonist and antagonist conformations. True agonists and antagonists Interleukin-6 (hIL-6) human Janus kinase 1 (hJAK1), SARS-CoV-2 3-chymotrypsin-like protease (s3CLpro), SARS-CoV-2 papain-like protease (sPLpro) and SARS-CoV-2 RNA-dependent RNA polymerase (sRdRp).

and torsions were set to zero. The protein and ligand molecules were further converted to the dockable pdbqt format using Autodock tools.

Human glucocorticoid receptors in the agonist conformation (hGRag), human glucocorticoid receptors in the antagonist conformation (hGRagt), human Interleukin-6 (hIL-6) human Janus kinase 1 (hJAK1), SARS-CoV-2 3-chymotrypsin-like protease (s3CLpro), SARS-CoV-2 papain-like protease (sPLpro) and SARS-CoV-2 RNA-dependent RNA polymerase (sRdRp).

2.4.2. Active site targeted molecular docking to other proteins targets

Using the same protocol above, the top twenty PDPs with the lowest binding energies to the hGRag in the agonist conformation and the reference inhibitors were docked to the active region of five proteins: human interleukin-6, human Janus kinases, SARS-CoV-2 3-chymotrypsin-like protease, SARS-CoV-2 papain-like protease and SARS-CoV-2 RNA-dependent RNA polymerase as defined by the grid boxes (Table 1b). The molecular interactions of the top three PDPs with the highest binding affinities to each of the proteins and the reference compounds were viewed with Discovery Studio Visualizer version 16.

2.5. Molecular dynamics simulation

Molecular Dynamics Simulation (MDS) was performed on the hGR in the agonist conformation (apo protein), Dex and top-two PDPs complexed with hGRag protein using NAMD software version 2.13 [38]. Necessary files for MDS were generated using CHARMM-GUI webserver [39,40]. For each complex or apo protein, the system was minimized for 10,000 steps in constant number of atoms, constant volume and constant temperature (NVT) ensemble then a production run for 100 ns in NVT ensemble was performed. Temperature was set to be 310 K and salt concentration was set to be the physiological concentration 0.154 M NaCl. Afterwards, calculations of Backbone-Root Mean Square Deviation (RMSD), Per residue Root Mean Square Fluctuations (RMSF), Radius of Gyration (RoG), Surface Accessible Surface Area (SASA) were performed using VMD TK console scripts [41].

2.5.1. Binding free energy calculations

Molecular Mechanics – Generalized Born Surface Area (MM-GBSA) implemented in Ambertools 17 is utilized to calculate the binding free energy in Dex and the top two-ranked pregnanes complexed with hGRag

| Protein | center_x | center_y | center_z | Size_x | Size_y | Size_z |
|---------|----------|----------|----------|--------|--------|--------|
| hGRag (4UDC) | 1.4 | 41.6 | 16.9 | 27.3 | 21.6 | 25.9 |
| hGRag (1NHZ) | -2.75 | 15.9 | 3.25 | 14.51 | 33.34 | 22.24 |
| hIL-6 (1ALU) | -8.8 | -14.5 | 4.7 | 24.4 | 22.2 | 17.9 |
| hJAK1 (6BBU) | 12.0 | 12.0 | -15.4 | 15.4 | 19.0 | 27.3 |
| s3CLpro (6Y84) | 9.4 | 19.2 | 19.2 | 16.0 | 18.9 |
| sPLpro (6W9C) | 19.5 | 28.0 | 18.3 | 26.8 | 26.8 | 34.7 |
| sRdRp (6M71) | 114.6 | 116.0 | 123.7 | 17.0 | 22.4 | 25.7 |

Table 1

Binding site coordinates of human GRs.

![Fig. 1. Binding energies of plant-derived pregnanes for selected protein targets in SARS-COV-2 and human: Human glucocorticoid receptors in the agonist conformation (hGRag); human Interleukin-6 (hIL-6), human Janus kinases (hJAK1); SARS-CoV-2 3-chymotrypsin-like protease (s3CLpro); SARS-CoV-2 papain-like protease (sPLpro) and SARS-CoV-2 RNA-dependent RNA polymerase (sRdRp).](image-url)
All frames (1000 frame) were used and igb parameter was set to 5, the default value. Saltcon parameter was set to 0.154 M while keeping the rest of the parameters as default. Binding affinity decomposition was used to know the contribution of each amino acid in the binding affinity.

2.5.2. Clustering of molecular dynamic trajectory
TTClust version 4.7.2 was used to cluster the trajectory automatically according to the elbow method, a representative structure for each cluster was produce [44]. These representative conformations were analyzed using Protein Ligand Interaction Profiler (PLIP) for pregnant atom-amino acid residue interactive analysis [45]. The images were created using PyMol V2.2.2 [46].

2.6. ADMET study
Eight compounds which are the top 3 compounds to the 6 protein targets were selected for evaluation of the drug-likeness and ADMET filtering analysis. The drug-likeness analysis which includes Lipinski, Veber, Ghose, Egan and Muegge were performed on the SwissADME (http://www.swissadme.ch/index.php) webserver. [47], while the predicted Absorption, Distribution, Metabolism, Excretion and toxicity (ADME/tox) study was analyzed using the SuperPred webserver (http://lmmd.ecust.edu.cn/admetsar1/predict/) [48]. The SDF file and canonical SMILES of the compounds were downloaded from PubChem Database or copied from ChemDraw to calculate ADMET properties using default parameters.

3. Results
3.1. Molecular docking
The binding affinities from the docking analysis of the proteins for the PDPs (117) and the reference compounds are shown in Table S1 (supplementary material). Based on the minimum binding energies and interactions with catalytic residues, the top twenty PDPs with binding energies ranging from –9.8 to –11.2 kcal/mol for hGRag was compared to the binding energies of the controls (positive control – dexamethasone = –12.2 kcal/mol and negative control – mifepristone = –6.0 kcal/mol; Fig. 1). Using competitive docking approach, these results were compared with the results obtained from the docking analysis of the top twenty PDPs with the antagonist conformation of the same protein (hGRag). The binding energies of the positive control (mifepristone: 11.5 kcal/mol), negative control (dexamethasone: 8.7 kcal/mol) and the top twenty PDPs (between –7.7 and –8.8 kcal/mol) are presented in Table S1 (supplementary material) and Fig. 1. It was also observed that the binding affinities of hGRag for glaucogenin C, hirundigenin and bregenin (–11.2, –10.8 and –10.6 kcal/mol respectively), the top three PDPs, were higher compared to those of hGRagt for them (–8.8, –8.7 and –8.7 kcal/mol respectively).

From the interaction of the top twenty ranked PDPs with hGRag, hIL-6, hJAK1, sSCLpro, sPLpro and sRdRp, top three PDPs with the lowest binding energies for each of the proteins were obtained, yielding a combined list of eight pregnanes: bregenin, hirundigenin, anhydroholantogenin, atratogenin A, atratogenin B, glaucogenin A, glaucogenin C and glaucogenin D. From this list, glaucogenin C, hirundigenin, glaucogenin A and glaucogenin D were part of the top three ranked PDPs with least binding energies for at least two proteins, while glaucogenin C, with the least binding energy for hGRag, was listed among the top three ranked PDPs for hIL-6, hJAK1, sPLpro and sRdRp, thereby exhibiting multiplicity of binding properties. It was also observed that apart from GR and JAK, the three top pregnanes for each protein had binding energies for other proteins that were lower than those of the reference compounds.

3.2. Amino acid interaction of selected pregnanes with target proteins
The amino acid interactions of hGR in the agonist conformation, hIL-6, hJAKsSCLpro, sPLpro and sRdRp with reference inhibitors and the top
Fig. 3. Amino acid interactions of pregnanes in binding cavity of human Interleukin-6. Stick representation of ligands are coloured green while interacting amino acids are in grey colour. (a) L(+) tartaric (reference inhibitor) (b) atratogenin A (c) glaucogenin C (d) anhydroholantogenin. Types of interactions are represented by green-dotted lines: H-bonds; light purple-dotted line: hydrophobic interactions (Pi-Alkyl, Alkyl & pi-stacking); purple-dotted line: Pi-Pi T Shaped; yellow-dotted lines: Pi-sulphur interactions, pi-stacking interactions. Three-letter abbreviations of amino acids are in black colour.

Fig. 4. Amino acid interactions of pregnanes in binding cavity of human Janus kinase 1. Stick representations of ligands are coloured orange while interacting amino acids are in grey colour. (a) ruxolitinib (reference inhibitor) (b) atratogenin B (c) hirundigenin (e) glaucogenin C. Types of interactions are represented by green-dotted lines: H-bonds; light purple-dotted line: hydrophobic interactions (Pi-Alkyl, Alkyl & pi-stacking); purple-dotted line: Pi-Pi T Shaped; yellow-dotted lines: Pi-sulphur interactions, pi-stacking interactions. Three-letter abbreviations of amino acids are in black colour.
three ranked PDPs that demonstrated the highest binding tendencies are represented in Figs. 2–7. The interacting residues of the proteins with respective ligand groups were majorly through H-bond, hydrophobic interactions and few other bonds (Table 2). The revalidation of the docking pattern of the native ligand (dexamethasone) co-crystalized with hGRag showed that dexamethasone was docked into ligand-binding domain (LBD) of hGRag. The A-ring of dexamethasone was positioned adjacent to the β-strands 1 and 2 while the D ring was close to helix 12 of hGRag. The 3′-carbonyl oxygen of the A ring formed a hydrogen bond to the guanidinium group of ARG611 of hGRag. On the C ring, the 11α-hydroxyl group formed a hydrogen bond with LEU563 while the 18 and 19-methyl groups displayed an alkyl interaction with CYS736 and MET604 of hGRag respectively (Fig. 2a). A Pi-alkyl interaction was observed between the D ring and the remaining amino acid residues of hGRag. Glaucogenin C, the topmost ranked PDP for hGRag was also docked into the LBD of hGRag. The 3-hydroxyl and 15, 20α-diepoxy groups of glaucogenin C interacted via hydrogen bonds with GLN570 and THR739, while the double bond between carbons 13 and 18 formed 2 hydrogen bonds with ASN564 and MET604. An alkyl interaction was observed between TRYP725 and 19-methyl moiety, while several Pi-alkyl interactions were observed between the A, B and C rings and the remaining amino acid residues of hGRag (Fig. 2b). In a similar manner to the 3′-carbonyl oxygen of dexamethasone, the 2-hydroxyl group of hirundigenin formed a hydrogen bond with ARG611, while the other hydrogen bond was formed between ASN564 of hGRag and 20-oxahexacyclo group. Numerous alkyl interactions were formed by 5- and 19-methyl groups while the pi-alkyl interactions were formed by the rings (Fig. 2c). Bregenin was also docked into the LBD of hGRag, interacting with the amino acid residues of the active site. The 3-, 16- and 17-hydroxyl groups of bregenin formed 3 hydrogen bonds with GLN570, GLN642 and LEU732 of hGRag. The 10- and 13-methyl groups formed alkyl interactions with MET601, CYS736 and MET604 of hGRag. The remaining residues interacted via Pi-alkyl interaction with the B and C rings of bregenin (Fig. 2d). L(+)-tartaric acid, the reference inhibitor, and the native molecule bound to the crystallographic structure of hIL-6 were docked into the “site 1” binding site. Five hydrogen bonds were observed between tartarate and IL-6. Direct hydrogen bonds to which ARG179 and ARG182 of hIL-6 served as the donors of four pairs of hydrogen atoms were formed with α-carboxyl moiety, while the α-hydroxyl group of the tartarate donated the hydrogen atom for the hydrogen bond with GLN175 (Fig. 3a). ARG30 and GLN175 of hIL-6 donated the hydrogen atoms for the hydrogen bonds formed with the carbonyl group of atratogenin A, while a carbon-hydrogen bond was formed between the furan ring and LEU33 (Fig. 3b). Alkyl interactions were formed between the 4β-methyl moiety and ARG30 and LEU33 while Pi-alkyl interactions were formed by the B and furan rings with LEU178 and LUE33 of hIL-6 respectively. Glaucogenin C was docked into the
same binding site and interacted with some of the amino acid residues as methotrexate (Fig. 3c). A conventional hydrogen bond and carbon-hydrogen bond were formed with ARG$^{182}$ and GLN$^{175}$ of hIL-6 respectively while most of the alkyl interactions were formed with 5- and 19-methyl groups. ASP$^{34}$ of hIL-6 donated a hydrogen atom to form hydrogen bond with 7-hydroxyl group, while the alkyl interactions were formed by the four rings of anhydroholantogenin (Fig. 3d). The amino group of the pyrimidine ring of roxolitinib (reference inhibitor) contributed two hydrogen atoms to form hydrogen bonds with GLU$^{957}$ and SER$^{963}$ of hJAK1, while that of pyrazole ring formed two hydrogen bonds with ARG$^{1007}$ and ASN$^{1008}$ of hJAK1. Two Pi-sigma bonds were formed between the pyrrole ring of roxolitinib and LEU$^{881}$ and LEU$^{1010}$. An alkyl interaction was observed between the cyclopentane ring of roxolitinib and ARG$^{1007}$ (Fig. 4a). The 6-hydroxyl group of atratogenin B donated the hydrogen atom for the only hydrogen bond formed with ARG$^{1003}$, while the 2- and 4-methyl groups and the methyl group attached to the furan ring interacted via alkyl interactions with VAL$^{889}$, ALA$^{906}$ and LEU$^{1010}$ of hJAK1 respectively (Fig. 4b). Hirundigenin was docked into the same active site as roxolitinib; 8- and 16-hydroxyl groups and 20-oxahexacyclo ring of hirundigenin formed hydrogen bonds with LEU$^{959}$ and ASN$^{1008}$, while the alkyl interactions were formed between the A and B rings and VAL$^{899}$, LEU$^{1010}$ and LEU$^{881}$ of hJAK1 (Fig. 4c). For glaucogenin C, the 21-carbonyl group formed two hydrogen bonds and the 8-hydroxyl group formed a hydrogen bond with ARG$^{1007}$, SER$^{963}$ and GLY$^{1020}$ of hJAK1 respectively. The alkyl interactions were majorly contributed by 5-methyl group of glaucogenin C (Fig. 4d). Ritonavir was docked into the receptor-binding site and interacted with amino acid residues that form the catalytic dyad (Cys-145 and His-41) of s3CL$^{pro}$ via a conventional hydrogen bond to LEU$^{141}$ while the remaining interactions with HIS$^{164}$, THR$^{24}$ and CYS$^{45}$ were via carbon-hydrogen bonds. It further interacted via Pi-alkyl, Pi-Pi T-Shaped and Pi-sulphur with LEU$^{27}$, HIS$^{41}$ and MET$^{165}$ of s3CL$^{pro}$ respectively (Fig. 5a). The three top ranked PDPs for s3CL$^{pro}$ were docked in the same binding site as the reference compound (ritonavir). Glaucogenin D interacted via conventional hydrogen and carbon-hydrogen bonds with GLY$^{143}$ and HIS$^{41}$ of s3CL$^{pro}$, while it interacted with MET$^{165}$ and THR$^{24}$ via alkyl interactions (Fig. 5b). A conventional hydrogen bond was formed between the 8- and 16-hydroxyl groups of hirundigenin and the catalytic residues (THR$^{24}$ and CYS$^{45}$) of s3CL$^{pro}$, while the 19-methyl group formed an alkyl interaction with MET$^{165}$ (Fig. 5c). The 7-hydroxyl group of anhydroholantogenin formed hydrogen bond with THR$^{24}$ of s3CL$^{pro}$ while the remaining hydrophobic interactions were formed by 10- and 17-methyl groups and the rings (Fig. 5d). Disulfiram, a known inhibitor of PL$^{pro}$, was docked into the binding cavity of SARS-C0V-2 PL$^{pro}$. It interacted with the amino acids HIS$^{272}$ and TRP$^{106}$ via a pi-sulphur interaction in the binding cavity of sPL$^{pro}$ (Fig. 6a). In the same vein, glaucogenin D, glaucogenin A and glaucogenin C were docked into the same binding site. The carbonyl and 19-methyl groups interacted via conventional hydrogen and Pi-alkyl interaction with HIS$^{272}$, while an alkyl interaction was observed with...
TRP$_{106}$ of sPL$_{pro}$ (Fig. 6b–d). The 7- and 8-hydroxyl groups of glaucogenin A formed two hydrogen bonds with ASP$_{286}$, 8-hydroxyl group interacted via alkyl interaction with HIS$_{272}$, while the pentacyclo ring formed multiple Pi-Sigma bonds with TRP$_{106}$ (Fig. 6c). Two hydrogen bonds were formed between the carbonyl group of glaucogenin C and HIS$_{272}$ and TRP$_{106}$, while multiple Pi-alkyl interactions were formed with the same amino acid residues of sPL$_{pro}$ (Fig. 6d). The 4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl, 5-cyno and carbonyl groups of sRdRp served as hydrogen donors for all of the conventional hydrogen bonds with the catalytic residues. The alkyl end of the 2-ethylbutyl moiety of sRdRp interacted via alkyl interaction with CYS$_{622}$, while an electrostatic force was formed between the phosphoryl group and APS$_{618}$ (Fig. 7a). In case of glaucogenin A, 7- and 8-hydroxyl groups of sRdRp formed two hydrogen bonds with GLUS$_{811}$ and TRP$_{617}$ respectively, while a conventional hydrogen bond and an alkyl interaction were formed by the double bonds at positions 21 and 10 with ASP$_{760}$ and LYS$_{798}$ respectively (Fig. 7b). The 12-hyroxyl group of Glaucogenin D formed the only two hydrogen bonds with APS$_{760}$ and TRY$_{615}$ of sRdRp while Glaucogenin D exhibited similar binding pattern with that of Glaucogenin A (Fig. 7 c & d) (see Table 3).

3.3. Results for molecular dynamics

The stability, structural/conformational fluctuations that occurred in the hGRag (apo protein), PDPs-hGRag and Dex-hGRag systems were monitored in a simulated dynamic environment. The apo form and two complexes of hGRag with glaucogenin C and hirundigenin were used in a MDS study for 100 ns in NVT ensemble. The results were analyzed using VMD Tk console scripts to calculate RMSD, RMSF RoG, SASA, and H-bond.

The RMSD is a plausible measure of protein stability. The RMSD plots indicate how much each frame is deviated from the initial conformation as a function of time. The averages RMSD values of the apo protein (unbound), hGRag-glaucogenin C, hGRag–hirundigenin and hGRag–Dex complexes are 11.58 Å, 1.67 Å, 1.82 Å, and 1.68 Å, respectively (Fig. 8). The RMSF values give insights on the flexibility of amino acids with high values corresponding to high fluctuations. RMSF was calculated for C$_{\alpha}$ atoms and the average values are 0.9 Å, 1.05 Å, 1.06 Å, 0.88 Å for the apo protein (unbound), hGRag–glaucogenin C, hGRag–hirundigenin and hGRag–Dex complexes respectively. The RMSF results show spikes at both the start and the end, which corresponds to the motion of the terminals. Few fluctuations were observed at amino acid residue number 27, 90, 180 and 243 in both the unbound and complexed hGRag proteins. Fig. 9 shows that residue: D21: D26, S89:N91, and Q104:R127 and R176:N183 have a larger fluctuation relative to the apo protein, this region are involved in the binding of the PDPs and Dex. The RoG plots gives information on the folding/unfolding properties while SASA plots indicate the surfaces exposed to solvent taking. Therefore, increasing values of SASA and RoG indicates that the protein have undergone unfolding. The averages of RoG and SASA are 18.67 Å and 14293 Å$^2$, for the apo protein (unbound); 18.75 Å and
Table 2
Combined list of the top three ranked plant-derived pregnanes with the lowest binding energies for each of the protein targets in human and SARS-COV 2.

| S/No | Plant Pregnanes | Plant species                  |
|------|-----------------|-------------------------------|
| 1    | Bregenin        | **Sarcostemma brevistigma**   |
| 2    | Hirundigenin    | **Vincetoxicum officinale**   |
| 3    | Anhydroholantogenin | **Holarrhena antidysenterica** |
| 4    | Atratogenin A   | **Cynanchum Atratum**         |
| 5    | Atratogenin B   | **Cynanchum Atratum**         |
| 6    | Glaucogenin A   | **Cynanchum glaucesens hand-maazz** |
| 7    | Glaucogenin C   | **Cynanchum glaucesens hand-maazz** |
| 8    | Glaucogenin D   | **Cynanchum glaucesens hand-maazz** |

*Protein Targets: Human glucocorticoid receptors in the agonist conformation (hGRag); human Interleukin-6 (hIL-6), human Janus kinases (hJAK1); SARS-CoV-2 3-chymotrypsin-like protease (s3CL\textsuperscript{pro}); SARS-CoV-2 papain-like protease (sPL\textsuperscript{pro}) and SARS-CoV-2 RNA-dependent RNA polymerase (sRdRp).*
### Table 3

Interaction of reference inhibitors and plant derived pregnanes with amino acid residue of various targets.

| Compounds          | Protein | Numbers | Hydrogen bonds (Bond distance Å) | Hydrophilic Interaction (Bond distance Å) | Other interactions (Bond distance Å) |
|--------------------|---------|---------|----------------------------------|------------------------------------------|-------------------------------------|
|                    |         |         | Interacting residues             | Interacting residues                     |                                    |
| Dexamethasone       | hGRag   | 2       | ASN#H (2.14) THR# (3.07) LEU# (3.07) ARG# (2.21) | TYR# (4.46) MET# (5.44) LEU# (3.68) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Glauconin C         |         | 4       | THR# (3.07) MET# (3.07) ASN# (3.07) GLN# (3.07) | TYR# (3.07) PHE# (3.07) LEU# (3.07) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Hirundigenin        |         | 2       | ASN#H (1.64) ARG# (3.17)         | TYR# (3.07) PHE# (4.72) LEU# (5.41) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Bregerin            |         | 4       | GLN# (2.03) GLN# (2.24) LEU# (2.57) | CYS# (3.71) PHE# (4.72) LEU# (5.42) | LYS# (4.08) (2)                    |
| Methotrexate        | hIL-6   | 5       | ARG# (2.76, 3.04) ABC# (2.76, 2.74) GLN# (2.80) | LUE# (4.67) ARG# (4.20) LUE# (4.28) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Atratogenin A       |         | 5       | GLN# (2.01) ARG# (2.79) LUE# (3.68) LUE# (2.89) | LUE# (4.67) ARG# (4.20) LUE# (4.28) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Glauconin C         |         | 8       | GLN# (3.29) ARG# (2.44)          | ARG# (3.92) LUE# (4.05, 3.82) LUE# (5.27) ARG# (4.40) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Anhydroholantogenin |         | 4       | GLN# (2.78)                      | LUE# (5.22, 4.50) ARG# (5.49) | none                                |
| Ruxolitib           | hJAK1   | 4       | ARG# (2.03) ASN# (2.16) GLU# (2.69) GLU# (2.23) | LEU# (3.77) LEU# (3.82) ALA# (4.80) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Atratogenin B       |         | 1       | ARG# (2.85)                      | LEU# (4.63, 4.63) LEU# (3.46, 4.81) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Hirundigenin        |         | 3       | ARG# (1.95, 3.77) LEU# (4.65) ASN# (2.37) | LEU# (3.50, 4.09) LEU# (3.46, 4.28) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Glauconin C         |         | 4       | ARG# (3.02) SER# (2.81) GLY# (3.62) GLY# (4.20) | LEU# (3.56, 4.88) LEU# (4.46) | none                                |
| Ritonavir           | 3CLpro  | 4       | CYS# (3.84) HIS# (3.07) LEU# (2.88) THR# (3.67) | LEU# (3.77) HIS# (5.24) MET# (4.82) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Glauconin D         |         | 2       | GLY# (2.74) HIS# (3.36)          | MET# (5.07) THR# (3.67) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Hirundigenin        |         | 2       | CYS# (3.57) THR# (2.50)          | MET# (5.07) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Anhydroholantogenin |         | 2       | ASN# (3.67) THR# (2.78)          | CYS# (3.49) HIS# (3.36, 5.36, 4.14) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Disulfiram          | 3BP    | 4       | HIS# (2.82)                      | HIS# (3.84, 4.01) TRP# (1.06) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Glauconin D         |         | 1       | HIS# (7.5) ASP# (2.32)           | TRP# (4.21, 5.05, 4.30) HIS# (3.54, 5.09) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Glauconin A         |         | 3       | HIS# (7.5) ASP# (2.32)           | TRP# (3.86, 3.81, 4.44, 4.60, 4.95) TRP# (4.27) HIS# (5.31) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Glauconin C         |         | 2       | HIS# (7.6) TRP# (2.72)           | HIS# (3.54, 5.08) TRP# (4.23, 5.28, 4.30, 4.70) | none                                |
| Remdesivir          | sRdRp   | 5       | TYR# (2.11) ASN# (2.72) CYS# (2.72) SER# (2.13) | CYS# (3.94) ASP# (5.27) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Glauconin A         |         | 3       | GLU# (2.48) TRP# (3.45) ASP# (2.42) | LYS# (4.95) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Glauconin D         |         | 2       | TRP# (3.45) ASP# (2.17)          | LYS# (3.98) | none                                |
|                    |         |         |                                  |                                          |                                    |
| Glauconin D         |         | 2       | GLU# (2.18) ASP# (3.36)          | LYS# (3.98) | none                                |

* Targets: Human glucocorticoid receptors in the agonist conformation (hGRag); human Interleukin-6 (hIL-6); human Janus kinases (hJAK1); SARS-CoV-2 3-chymotrypsin-like protease (3CLpro); SARS-CoV-2 papain-like protease (3BP); and SARS-CoV-2 RNA-dependent RNA polymerase (sRdRp).

14,670 Å² for the hGRag-glauconin C; 18.82 Å and 14710 Å² for the hGRag-hirundigenin; and 18.64 Å and 14,353 Å² for the hGRag-Dex complexes respectively (Figs. 10 and 11). The average H-bonds for the apo protein (unbound), hGRag-glauconin C, hGRag-hirundigenin and hGRag-Dex complexes respectively are 69.33, 65.81, 68.42, and 67.2 respectively. The PDPs and Dex complexes had slightly lower number of hydrogen bonds than the unbound hGRag (Fig. 12). The binding free energy (ΔGbind) measures the affinity of a ligand to its target protein. The free energy difference between the ligand-bound state (complex) and the corresponding unbound states of proteins and ligands are also employed in the calculations. Calculation of binding free energy ΔGbind for hGRag-glauconin C, hGRag-hirundigenin and hGRag-Dex complexes using MM-GBSA implemented in Amber tools 17 shows the binding free energies of −30.86 ± 2.8 kcal/mol, −35.22 ± 4.23 kcal/mol, and −39.83 ± 3.1 kcal/mol. The results of the binding free energy contribution per residue are shown in Fig. 13. Putting the three graphs in perspective, it was observed that in all the complexes, residue number 30–46, 71–81, around 120 and 201–210 corresponding to 558–574, 599–609, around 648 and 279–738 participated in the interactions to the PDPs and Dex. Table 4 shows the results of the number of clusters that were generated and the interactions at different clusters, using a representative conformer. The most common interactions in the hGRag-glauconin C and hGRag-hirundigenin complexes are hydrophobic with few hydrogen bonds. The hGRag-Dex complex indicated more hydrogen bonds. The most amino acid that commonly participates in the interactions of hGRag-glauconin C is LEU# while in hGRag-hirundigenin complex there are two amino acids, which are LEU# and LEU#. Figs. 13 and 14 show the first and last cluster of the hGRag-glauconin C complex while Figs. 15 and 16 show the first and last clusters of protein-hirundigenin complex. The amino acids that participated in interactions in hGRag-Dex complex are ASN#H, GLN#H, and THR# (see Fig. 16).
3.4. Results for in silico drug-likeness and ADMET properties of top docked plant derived pregnanes to selected human and SARS-CoV-2 proteins

From the docking analysis, eight plant pregnanes (bregenin, hirundigenin, anhydroholantogenin, atratogenin A, atratogenin B, glaucogenin A, glaucogenin C and glaucogenin D) with high binding tendencies to hGRag with corresponding high binding tendencies to hIL-6, hJAK1, s3CL\textsuperscript{pro}, sPL\textsuperscript{pro}, and sRdRp were subjected to the predictive drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) filtering analyses. The results for the predictive filtering analyses are presented in Table 5.

4. Discussion

Parallel advances in protein crystallography and various virtual-screening software for the modeling of ligand-receptor interactions have enhanced computer-aided drug design [49]. In this study a structure based virtual-screening of PDPs was employed via competitive docking approach for hGR agonist with a dual inhibitory potential against cytokine storm syndrome and viral replication in COVID-19. The top potential agonists were further analyzed for multiplicity of inhibitory tendencies against the hIL-6 and hJAK1 (used as anti-inflammatory targets), and s3CL\textsuperscript{pro}, sPL\textsuperscript{pro} and sRdRp (used as SARS-CoV-2 therapeutic targets). The docking of the PDPs to the hGRs identified the top ranked twenty PDPs with dexamethasone binding mode to hGR agonist. A total of eight PDPs were selected. From these eight PDPs, top three ranked PDPs (glaucogenin C, hirundigenin and bregenin) for hGR agonist were competitively and selectively docked to the hGRag. They were docked into the hydrophobic ligand binding pocket (LBP) which is located in the bottom half of the GR ligand binding domain, LBD [50,51]. The polar residues on the LBP interacted with the dexamethasone and the top ranked PDPs via several hydrogen bonds [51]. The binding of the amino acid residues on helix 12 and the loop preceding helix 12 have been earlier hypnotized to stabilize the helix in the active conformation that could serve as the molecular basis for the ligand-dependent activation of GR [52]. Among the several amino acids involved in the interactions, Cys-736 has been implicated in
Fig. 10. Surface Accessible Surface Area plots of molecular dynamics (MD) simulation of human glucocorticoid receptors in the agonist conformation (hGRag), hGRag-hirundigenin, hGRag-glaucogenin and hGRag-dexamethasone complexes generated over 100 ns.

Fig. 11. Radius of gyration plots of molecular dynamics (MD) simulation of human glucocorticoid receptors in the agonist conformation (hGRag), hGRag-hirundigenin, hGRag-glaucogenin and hGRag-dexamethasone complexes generated over 100 ns.

Fig. 12. Hydrogen bonds plots of molecular dynamics (MD) simulation of human glucocorticoid receptors in the agonist conformation (hGRag), hGRag-hirundigenin, hGRag-glaucogenin and hGRag-dexamethasone complexes generated over 100 ns.
the interactions with heat shock proteins [52], Tyr-735, has been shown to be important for transactivation [53], while Gln-642 have been reported to play a unique role in steroid recognition [52]. In addition to the steroid structures of glucocorticoids, the 3′-carbonyl oxygen, 2′-carbonyl oxygen, double bonds between C4 and C5, 17′ hydroxyl group and 21′ hydroxyl group, are critical for anti-inflammatory potency and glucocorticoid receptor affinity [54]. The identified PDPs contained similar and analogous functional groups that interacted with GR; thus, the binding of these plant steroidal pregnanes may initiate the ligand-dependent activation of GR since they share similar binding patterns with dexamethasone. The activated glucocorticoid-receptor complex can: (i) bind the promotor responsive elements (RE) of key pro-inflammatory transcription factors (e.g. AP-1, NF kappa B) to inactivate them; (ii) upregulate the expression of cytokine inhibitory proteins, e.g. I kappa B, via glucocorticoid RE; and (iii) reduce the half-life time and usefulness of cytokine mRNAs [11]. IL-6 is a major causative factor of inflammatory disease and it is a promising target, as well as its signaling pathways; however, orally available small-molecule drugs specific for IL-6 have not been developed [55]. From the PDPs with high binding tendencies to hGRag, three PDPs (atrategeninA, glaucogenin C and anhydroholantogenin) exhibited the lowest binding energy poses for hIL-6 in the same binding site as observed for the co-crystallized ligands (tartaric acid) of hIL-6. In a similar study, furosemide exhibiting the same binding mode as tartaric acid was further

Fig. 13. Molecular mechanics/Poisson-Boltzmann surface area (MM/PBSA) plot of binding free energy contribution per residue of (A) hGRag-dexamethasone complex, (B) hGRag Glaucogenin C complex and (C) hGRag Hirundigenin complex.
found to inhibit hIL-6 activity in vitro [56]. From the X-ray crystal diffraction of hIL-6 structure, it was shown to contain four alpha helices (helices A, B, C, and D), which were linked with loops. The receptor-binding domain is located at the C-terminus (residues 175–181) [57], in which ARG179 is known to be the key residue [57]. AB loop and helices A and D is important in receptor binding and signal transduction [58]. Compounds that interact strongly with residue ARG179 may interfere with the binding of the receptor to its ligands [59], thus these PDPs may proffer anti-inflammatory activity via hIL-6 inhibition. The Janus kinases (JAK) family of proteins function as critical mediators of cytokine signaling from membrane receptors to various signal transducers and activators of transcription (STAT) family of proteins [60]. Activation of STATs by the JAK kinases promotes the transcriptional activation of target genes controlling cell proliferation and survival, angiogenesis, and immune function [61]. Some JAK family inhibitors such as tofacitinib [62] and ruxolitinib [63] have progressed

### Table 4

| PROTEIN- COMPOUND COMPLEX | CLUSTER NUMBER | HYDROPHOBIC INTERACTIONS | H-BONDS |
|---------------------------|----------------|--------------------------|---------|
| AGRag - glaucogenin C     | Cluster 1      | L563 L566 M604 L608      | 1       |
|                           | Cluster 2      | W600 M604 L608           | 0       |
|                           | Cluster 3      | L563 (2) M604 L608       | 1       |
|                           | Cluster 4      | L563 Q570 W600 T739     | 2       |
|                           | Cluster 5      | L563 (2) L566 M604 L608 (2) Y735 | 2 |
| AGRag - hirundigenin      | Cluster 1      | L563 L566 W600           | 0       |
|                           | Cluster 2      | L563 L566 M604 F740 F749 | 1       |
|                           | Cluster 3      | L563 L566 Q570 M604 I757 | 1       |
|                           | Cluster 4      | L563 L566 Q570 T739 F749 | 1       |
| AGRag - Dexamethasone      | Cluster 1      | Q642 - L732 – Y735      | 5       |
|                           | Cluster 2      | L566 – M604 – L608 – L732 – Y735 | 4 |

The most common amino acids are in **bold**, amino acid residues are represented in one-letter format.

**Fig. 14.** Interactions in human glucocorticoid receptors in the agonist conformation (hGRag)-Glaucogenin C complex (a) cluster 1 and (b) cluster 5. Grey dotted dashed lines: hydrophobic interactions; Blue solid line: Hydrogen bond. Amino acids are in one-letter format and blue colour. Glaucogenin C is in orange stick representation.

**Fig. 15.** Interactions in human glucocorticoid receptors in the agonist conformation (hGRag)-hirundigenin complex (a) cluster 1 and (b) cluster 4. Grey dotted dashed lines: hydrophobic interactions. Blue solid line: hydrogen bond. Amino acids are in one-letter format and red colour. Hirundigenin is in orange stick representation.
into clinical trials, and FDA approvals. In comparison with the reference inhibitor, ruxolitinib, the top-three ranked PDPs (atratogenin B, hirundigenin, glaucogenin C) with the best binding modes, for which hJAK1 had the highest affinities, interacted with the hinge residues LEU189, GLU167 and the side chain of ASN199 and the backbone carbonyl oxygen of ARG100 of the catalytic residues. These residues are involved in the inhibitory activities of selected compounds in both in silico and in vitro analyses [64–66].

The catalytic dyad (His145 and Cys140) of SARS-CoV-2 3CLpro is domiciled between its domain I (residues 8–101) and domain II (residues 102–184) [67]. A long loop (residues 185–200) that connects domain II and domain III (residues 201–303) completes the 3CLpro monomer [68]. The enzymatic activity of 3CLpro resides in its catalytic dyad of Cys140 and His145 [69]. The catalytic dyad and the residue Glu166 have been reported to be involved in the protein dimerization and substrate cleaving through the catalytic activities present in the cleft between domains I and II. The substrate-binding pocket lies in the cleft consisting of residue 140–145 and 163, 166, 169 domain II [70,71]. The substrate-binding pocket is divided into a series of subsites (S1–S6), each accommodating a single but consecutive amino acid residue in the substrate. The key residues in the substrate binding pockets of 3CLpro are His141, Tyr254, Met49, Phe45, Cys145, His163–Pro168, His172, and Asp107–Glu192 regions [72]. In the same binding pattern as ritonavir (a known inhibitor of the protease), the top-three ranked pregnanes (glaucogenin D, hirundigenin and anhydroholantogenin) were docked into the substrate binding pocket, interacting with various catalytic residues listed above. Considering our results and references to existing literature, the strong interaction of these PDPs to the critical residues (most especially HIS41, GLY143, CYS145 and MET165) will greatly impair the dimerization and substrate binding of the SARS-CoV-2 3CLpro.

The catalytic triad of SARS-CoV-2 PLpro is formed by CYS111, HIS272 and ASP286 [73,74], while TRP108, GLY256, and LYS274 are catalytic residues (Li et al., 2020). LEU162, GLU167, ASP164 and TYR264 have been reported to be crucial for deubiquitinating activity of PLpro [75]. The host innate immune system is critical to controlling SARS-CoV-2 infection. Reverse post-translational modifications of immune proteins, such as interferon factor 3 and NF-κB via ubiquitination and the suppression of interferon-stimulated gene product 15 (ISG15), have also been implicated in the activities of PLpro of SARS-CoV-2. [73,76], these, in turn, assist SARS-CoV-2 to escape the host innate immune responses. Pregnanes (glaucogenin D, hirundigenin and anhydroholantogenin) interacted with the catalytic triad and residues that are involved in deubiquitination. Such interactions may alter the catalytic conformation of PLpro and inhibit its ability to reverse ubiquitination. SARS-CoV-2 RdRp plays a central role in coronaviral replication/transcription machinery; it is, therefore, accepted as an excellent target for new therapeutics for which lead inhibitors, such as remdesivir, have been approved by the FDA [77]. Glaucogenin A, glaucogenin D and glaucogenin C were docked into the Motif C of the enzyme, exhibiting the same binding pattern as remdesivir. Motif C, the region comprising amino acid residues 753 to 767, contains the catalytic residues SER759, ASP760, and ASP761 in the β-turn structure [77]. The stability of the complexes formed by the pregnanes with the enzyme stemmed from the vast number of interactions with the catalytic residues in the Motif C of the active site of the enzyme.

The several thermodynamics parameters (RMSD, RMSF, SASA and RoG) that were analyzed from the 100 ns full atomistic MDS trajectory files of the top two ranked PDPs-hGRag complexes revealed a high degree of stability throughout the period of the MDS run as compared to the apo receptor.

The RMSD plots showed that the binding of glaucogenin C and hirundigenin in the same manners as Dex to the active region of hGRag still preserved the structural integrity of the protein [78]. The

The RMSF plots indicates the flexibility of different regions of a protein and the amino acid residue along the trajectory, which can be related to crystallographic B factors [78]. The RMSF plots of the PDPs-hGRag complexes shows similar plot pattern with the Dex-hGRag complex. Though a lower amount of fluctuation occurred at with the interacting residues, it has been established that greater amounts of structural fluctuations usually occur in regions known to be involved in ligand binding and catalysis, notably the catalytic loop regions [79] The RoG and SASA was assessed to evaluate the compactness and the accessibility of hGRag upon the binding of the PDPs and Dex. The PDPs and Dex maintained a reasonably steady RoG and SASA over the simulation time, indicating a highly compacted hGRag - PDPs and Dex complexes and well folded protein structure with intact intermolecular bonds [80]. The approximately close H-bonds between the top two ranked PDPs and Dex - hGRag complexes as compared to the unbound hGRag protein further indicated that the structural integrity of the protein was preserved in each of the system. At a quantitative level, simulation-based methods provide substantially more accurate estimates of ligand binding affinities (free energies) [81]. These results are calculated based on the total binding free energy of the complex. In these calculations, the binding free energy (ΔGbind) measures the affinity of a ligand to its target protein. The free energy difference between the ligand-bound state (complex) and the corresponding unbound states of proteins and ligands are also employed in the calculations. Thus, the ΔGbind calculations are important to gain in-depth knowledge about the binding modes of the hits in drug design [82]. The result from the MMPBSA calculation further corroborated the docking studies. Though Dex a known inhibitor to the hGRag protein presented the highest ΔGbind, hirundigenin the top ranked PDP presented a very high and close ΔGbind to Dex. A further evaluation of the MDS trajectories through clustering analysis showed that for each of the representative

Fig. 16. Interactions in human glucocorticoid receptors in the agonist conformation (hGRag)-dexamethasone complex (a) cluster 1 and (b) cluster 2. Grey dotted dashed lines: hydrophobic interactions. Blue solid line: hydrogen-bond. Amino acids are in one-letter format and red colour. Dexamethasone is in orange stick representation.
conformers from the several clusters, the interactions (H-bonds and hydrophobic interaction) were preserved at different time frames, indicating that the interactions can be maintained in a dynamic environment, thus can be well adapted for experimental procedures.

Despite the various efforts to improve current glucocorticoids and anti-inflammatory drugs, they still pose significant side effects [83]. Hence the top-docked PDPs to various proteins were subjected to in silico physicochemical and ADMET analysis. The eight top-ranked PDPs fulfilled all the requirements for the five physicochemical filtering analysis as reported by Lipinski [84] Ghose [85], Veber [86], Egan [87] and Muegge [88] thereby suggesting favourable physicochemical/druggable properties. The top-eight ranked PDPs expressed positive and predicted to be well absorbed into the blood stream subverting the capability of P-gp to pump them back into the intestinal lumen, bile ducts, urine-conducting ducts and capillaries respectively [89]. The blood brain barrier (BBB) penetration descriptor, predicts the ability of the inhibitors of the permeability-glycoprotein (P-gp). These PDPs are predicated to have high probability of human intestinal absorption and non-substrate but druggable properties. The top-eight ranked PDPs expressed positive and thus can be well adapted for experimental procedures.

Despite the various efforts to improve current glucocorticoids and anti-inflammatory drugs, they still pose significant side effects [83]. Hence the top-docked PDPs to various proteins were subjected to in silico physicochemical and ADMET analysis. The eight top-ranked PDPs fulfilled all the requirements for the five physicochemical filtering analysis as reported by Lipinski [84] Ghose [85], Veber [86], Egan [87] and Muegge [88] thereby suggesting favourable physicochemical/druggable properties. The top-eight ranked PDPs expressed positive and predicted to be well absorbed into the blood stream subverting the capability of P-gp to pump them back into the intestinal lumen, bile ducts, urine-conducting ducts and capillaries respectively [89]. The blood brain barrier (BBB) penetration descriptor, predicts the ability of the
PDPs to penetrate the blood brain barrier. The top-eight PDPs displayed the properties that suggested their ability to cross the BBB. SARS-CoV-2 has been reported to infect the brain, thus indicating its ability to cross the BBB [90], these PDPs may cross the BBB for to exert an overall viral clearance.

The top-eight PDPs displayed a probability of at least 65% ability to be bond to the plasma protein, suggesting their ability to be transported by these proteins. The estimated half-life time (less than 2 h) and clearance ratefall within the moderate range. The three phytochemicals presented a tolerable LD50 between (51–500 mg/kg). The hERG channel plays a vital role in the repolarization and termination stages of action potential in cardiac cells [91]. Compounds that block the hERG channel may cause cardiotoxicity [92]. The top-eight PDPs exhibit low probability of being a potential hERG channel blockers, suggesting that they may not cause hERG channel-related cardiotoxicity [92]. Using the mutagenicity and skin sensitization descriptors, the top-eight PDPs did not display the properties to be mutagenic in silico, thereby suggesting that they may not cause genetic mutations, which do initiate the pathophysiology of other diseases. The impact of the PDPs on the liver phase I drug metabolism was also analyzed using the various cytochrome P450 descriptors. The top-eight PDPs demonstrated no inhibitory potential for the various cytochrome P450, thus may not adversely affect phase I drug metabolism in the liver. ADME/tox analysis indicated high aqueous solubility, ability to pass the high human intestinal absorption, low acute oral toxicity with a good bioavailability score. Therefore this natural plant pregnane may be considered to be non toxic with druggable potential.

5. Conclusion

In this study we employed a competitive docking approach to screen 117 plant derived pregnanes (PDPs) for hGR agonist, with a dual inhibitory potential against SARS-CoV-2 infection and the accompanied cytokine storm syndrome. Eight PDPs (bregenin, hinridugenin, anhydroholantogenin, atratogenin A, atratogenin B, glaucogenin A, glaucogenin C and glaucogenin D) with high agonist binding tendencies to the hGR displayed different levels of multiplicity of inhibitory potentials to other pro-inflammatory targets (hIL-6, hJAK1) and three SARS-CoV-2 therapeutic targets (s3CLpro, SPIpro and sRDRP). The 8 PDPs fulfilled the requirements for various physiochemical and ADMET descriptors thereby suggesting favourable druggable properties. The top two ranked PDPs (glaucogenin C and hinridugenin) complexed to the hGRgag displayed the highest multiplicity of high binding tendencies to the catalytic residues of different targets. From this eight PDPs, glaucogenin C and hinridugenin having the highest agonist tendencies to the hGR were further subjected to a 100 ns atomistic molecular dynamics simulation. A high degree of structural stability was observed from molecular dynamics simulation analyses of glaucogenin C and hinridugenin complexes of hGRgag. A further clustering of the MDS trajectories of the complexes of glucocorticoid receptors (hGRs) in the agonist (hGRag) and antagonist (hGRagt) conformation, in a competitive molecular docking approach. Based on the minimal binding energy and a comparative dexamethason binding mode analysis, a hit-list of the top twenty ranked PDPs that were docked in the agonist conformation of hGR, with binding energies ranging between −9.8 and −11.2 kcal/mol, was defined. The top twenty ranked PDPs were further analyzed for interactions with the human Janus kinases 1 and interleukins-6 (hJAK1 and hIL-6 respectively), and SARS-CoV-2 3-chymotrypsin-like protease, Papain-like protease and RNA-dependent RNA polymerase (3CLpro, PLpro and RdRp respectively). For each of the 6 targeted proteins (3 humans and 3 SARS CoV-2), the top three ranked PDPs were selected, to give a sum of eight PDPs (bregenin, hinridugenin, anhydroholantogenin, atratogenin A, atratogenin B, glaucogenin A, glaucogenin C and glaucogenin D) with multiplicity of high binding tendencies to the catalytic residues of different targets. The selected top-eight PDPs demonstrate novel druggable properties over the Lipinski, Veber, Ghose, Egan and Muegge predictive filters. In the same vein the 8 PDPs displayed favourable druggable properties over the 100 ns simulated dynamics environment. These promising hGRgag agonists with anti-inflammatory and SARS-CoV-2 replication inhibitory potential is recommended for further in vitro and in vivo experiments.

Declaration of competing interest

The authors declare no conflicting interest.

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Appendix A. Supplementary data

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Summary

The high morbidity and mortality rate of Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) infection arises majorly from the Acute Respiratory Distress Syndrome (ARDS) and “cytokine storm” syndrome, which is sustained by an aberrant systemic inflammatory response and elevated pro-inflammatory cytokines. Thus, the identification of compounds which target multiple proteins in the virus and exhibit anti-inflammatory activity will enhance the development of effective drugs against the disease. In this study, we carried out an in silico evaluation of some plant-derived pregnanes for their activities against selected human pro-inflammatory and SARS-CoV-2 replication proteins targets. This was carried out by a virtual screening of an in-house library of steroidal plant-derived pregnanes (PDPs). One hundred and six (106) PDPs were docked into the active regions of human glucocorticoid receptors (hGRs) in the agonist (hGRag) and antagonist (hGRagt) conformation, in a competitive molecular docking approach. Based on the minimal binding energy and a comparative dexamethason binding mode analysis, a hit-list of the top twenty ranked PDPs that were docked in the agonist conformation of hGR, with binding energies ranging between −9.8 and −11.2 kcal/mol, was defined. The top twenty ranked PDPs were further analyzed for interactions with the human Janus kinases 1 and interleukins-6 (hJAK1 and hIL-6 respectively), and SARS-CoV-2 3-chymotrypsin-like protease, Papain-like protease and RNA-dependent RNA polymerase (3CLpro, PLpro and RdRp respectively). For each of the 6 targeted proteins (3 humans and 3 SARS CoV-2), the top three ranked PDPs were selected, to give a sum of eight PDPs (bregenin, hinridugenin, anhydroholantogenin, atratogenin A, atratogenin B, glaucogenin A, glaucogenin C and glaucogenin D) with multiplicity of high binding tendencies to the catalytic residues of different targets. From this eight PDPs, glaucogenin C and hinridugenin having the highest agonist tendencies to the hGR were further subjected to a 100 ns atomistic molecular dynamics simulation. A high degree of structural stability was observed from molecular dynamics simulation analyses of glaucogenin C and hinridugenin complexes of hGRgag. Further clustering of the MDS trajectories of the complexes of glucocorticoid receptors (hGRs) in the agonist (hGRag) and antagonist (hGRagt) conformation shows that the interactions of these PDPs with the active site residues of hGRgag were preserved in different representative structures of the clusters. The selected top-eight ranked PDPs demonstrated novel druggable properties over the Lipinski, Veber, Ghose, Egan and Muegge predictive filters. In the same vein the 8 PDPs displayed favourable druggable properties over a wide range of predictive molecular descriptors, such as, ability to pass the blood brain barriers, high intestinal absorption, non-substrate to the permeability glycoprotein, non hERG blockers, non inhibitors of the cytochrome p450 etc. Thus, these promising hGRgag agonists, especially glaucogenin C and hinridugenin, with potential anti-inflammatory and SARS-CoV-2 replication inhibitory activity is recommended for lead optimization for drug candidate and further evaluation in an in vitro and in vivo experiment.

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Authors contributions

G. A. Gyebi Conceived and designed the analysis.
O. M. Ogunyemi Performed molecular docking analysis.
I. M. Ibrahim Performed molecular simulations.
J. O. Adebayo Editing and review of manuscript
S. O. Afolabi Editing and review of manuscript.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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