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AN *IN SILICO* APPROACH FOR COMPUTING THE EFFICACIES OF COMPASSIONATELY PRESCRIBING ANTIVIRALS FOR SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS COV-2) INFECTED PATIENTS

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ABSTRACT

The 2020 year began with a news of neglected/mishandled viral outbreak of the last year 2019 that turned to a pandemic zoon which caused many casualties and lead to a complete lock down all over the world. In this present research paper the results of in silico evaluation of the otherwise proven antiviral drugs using computational methodologies against the published main protease (6yb7.pdb) of novel SARS CoV-2 virus. The selection of drug moleccules was achieved by randomely selecting them from the contemperory medical literatures.

KEYWORDS: SARS CoV-2, COVID 19, Antivirals, Computational Studies, Docking.

INTRODUCTION

The human kind is passing through one of the toughest periods ever faced since its existance as an epedemic outbreak caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which has been reported in China since November 2019.[1] This outbreak turned to a pandemic within next two months and termed novel Coronavirus Disease (COVID 19). At present most of the countries of the world are individually or collectively taking different measures to control it. This SARS CoV-2 (COVID 19) outbreak immediately became the subject matter for not only the workers of community medicine but also for the entire scientific community during the first quarter of the year 2020. So far there exists no single well accepted error proof treatment protocol with clinically proven medicine/vaccine for this novel Corona virus. This research work effectively incorporates the Artificial

Intelligence (AI) to the field of contemporory medical research by evaluating various antivirals using in-silico methods. This complicated challenge should also be taken by all the medicinal scientists for the sake of not only the human society but also for the entire world. For many years the strategy for discovering new drugs consisted of concentrating plaussible lead structures, designing a probably active lead candidate and developing or selecting a suitable computational program for screening these bio-analogue molecules for their targeted biological or therapeutic effects. These processes involve several trial and error cycles patiently adopted by the computational medicinal scientists utilizing their skill and experience based intelligent intutions. The incorporation of these methodologies have considerably reduced the laborious and expensive traditional methods often adopted in the field of drug research. It is well known that the molecular modelling has become a new discipline in drug research that has grown very rapidly since the 1980s and has contributed to the discovery of not only many lead structures but also helped to evaluate the efficacies of the otherwise proven drug molecules against new challenging targets.[2] Usually the more selective a drug to its target the lesser will be the chances for undesirable side effects. Antivirals are useful only in tackling viral diseases if there exists no effective vaccine or where the infection has already outbroken. The life cycle of a virus means that for most of its time within the host cells and is effectively or intelligenly disguised both from the immune system and from circulating drug molecules. A virus is capable enough for utilizing the biochemical mechanisms of the host cells itself for its own multiplication. This causes the antiviral molecules to aim intelligently the targets that are unique to the virus which are very limited. Hence the search for effective antivirals has proved more challenging and time consuming ones than that for antibacterial drugs.

Coronaviridae and SARS CoV-2: The coronaviruses belong to the family of Coronaviridae, order Nidovirales and realm Riboviria. They are named from their appearance in the electron microscope that reveals moderately pleomorphic, spheric or elliptic virions covered with distinctive club shaped projections. The viral envelope consits of a lipid bilayer where the membrane, envelope and spike structural proteins are anchored. Inside the envelope there is the nucleocaspid that is formed from the multiple copies of the nucleocapsid protein boud to the positive sense single stranded RNA genome in a continuous beads on a string type conformation. Coronaviridae are associated with harmless common cold and severe respiratory diseases to the deadly coronavirus disease like COVID-19. The animal coronaviruses were isolated in 1940s and human coronaviruses were first discovered in

1960s. The important human coronaviruses include Severe Acute Respiratory Syndrome Coronavirus (SARS CoV), Human Coronavirus NL63(HCoV NL63), Human Coronavirus HKU1 (HCoV HKU1), Middle East Respiratory Syndrome-related Coronavirus (MERS-CoV) and the recent Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2). Most of these viruses have involved serious respiratory tract infections. The SARS CoV-2 causes COVID-19 is also an RNA virus belong to the genus Betacoronavirus that infects mammals.

MATERIALS AND METHODS

This study being based on purely in silico methodologies neither chemicals nor standard strains of microorganisms were needed. The selected antivirals are Danoprevir, Darunavir, Favipiravir, B-DN^4- Hydroxycytidine, Lopinavir, Remdesivir, Ritonavir, Simeprevir and Umefenovir. The efficacy of Baricitinib a rheumatoid arthritis drug was also included as it was reported with some effectiveness in respiratory diseases. The anitimalerial drugs like Chloroquine and Hydroxychloroquine were also included. The main protease deposited to protein data bank as 6yb7.pdb was downloaded and used for the computational docking studies. The software like GAMESS, ArgusLab 4.0.1 a freeware and ACD ChemSketch 2015 Freeware installed in a computer having Windows10 Enterprise 32 bit Operating System with intel core 2.10GHz processer and 2GB RAM was used for docking and computational studies. The details of the antivirals and antimalerials selected for the computational studies are briefed below.

Baricitinib (MF:$C_{16}H_{17}N_7O_2S$; MW: 371.42 g mol^{-1}): The Baricitinib manufactured by Eli Lilly sold uder the brand name Olumiant is an oral drug approved for the rheumatoid arthritis in adults that act as an inhibitor of janus kinase JAK-1 and JAK-2. In additon to its anti-inflammatory activity it has also showed immunomodulating and antineoplastic activities. This drug due to its anti-inflammatory and immunomodulating activities is selected for the screening with the main protease of SARS CoV-2. Certain recent research studies also reported the effectiveness of Baricitinib to treat respiratory diseases.[3]

Danoprevir (MF:$C_{35}H_{46}FN_5O_9S$; MW: 731.83 g mol^{-1}): It is an orally available macrocyclic peptidomimetic inhibitor of NS3/4A HCV protease and hence has been used in trials studying the treatment of chronic Hepatititis C. Danoprevir was initially developed by Array BioPharma and further development was licenced to Ascletis by Roche. The HCV NS3/4A protease is an effective drug target because of its potential involvement in viral

replication and suppressive effects on host responses to viral infections. There are reported clinical studies for the therapeutic effects of Danoprevir in a combination therapy.[4]

Darunavir (MF:$C_{27}H_{37}N_3O_7S$; MW: 547.66 g mol^{-1}): Darunavir developed by Tibotec in 1998 is a synthetic non peptidic protease enzyme (HIV protease) inhibitor by forming a complex thereby preventing cleavage of the gag-pol polyproteins resulting immature, non infectious viral particles. The drug was licensed in United States (2006) and then in European union (2007) by the pharmaceutical company Tibotec. The efficacy and safety of the Darunavir alone and in combination therapy with Ritonavir have been demonstrated in many studies.[5]

Favipiravir (MF:$C_5H_4FN_3O_2$; MW: 157.10 g mol^{-1}): It is a broadspectrum inhibitor of viral RNA polymerase and was developed by Toyama Chemical Co., Ltd.. Favipiravir undergoes an intracellular phosphoribosylation to be an active form, favipiravir-RTP (favipiravir ribofuranosyl-5B-triphosphate), which is recognized as a substrate by RdRp, and inhibits the RNA polymerase activity. This mechanism of action underpins a broader spectrum of anti-viral activities of Favipiravir. It is effective against a wide range of various types of influenza viruses, including many drug resistant strains.[6]

B-DN^4- Hydroxycytidine (MF:$C_9H_{13}N_3O_6$; MW: 259.22 g mol^{-1}): It is an antiviral ribonucleoside analogue that can act as a competitive alternative substrate for virally encoded RNA-dependent RNA polymerases. It is well known that nucleoside analogues can play a critical role in antiviral therapy begenning with the first antiviral drug Idoxuridine for the treatment of Herpes Simplex Virus (HSV).[7]

Lopinavir (MF:$C_{37}H_{48}N_4O_5$; MW: 628.81 g mol^{-1}): It is an antiretroviral HIV protease inhibitor that is rapidly and exlusively metabolized by cytochrome P450 3A isoenzymes (CYP3A). Its design is based on peptidomimetic principle and mimics the peptide linkage typically targeted by the HIV-1 protease enzyme that itself cannont be cleaved. Thus Lopinavir effectively prevent the activity of the HIV-1 protease. Recent study also proves that it is effective against human papilloma virus (HPV). It was patented in 1995 and license was granted in 2000 for use as a full drug. Usually Lopinavir use in combination with Ritonavir.[8]

Remdesivir (MF:$C_{27}H_{35}N_6O_8P$; MW: 602.58 g mol^{-1}): Remdesivir is an adenosine analogue that inserts into viral RNA chains plausibly causing their premeture termination. It

has broadspectrum activity against various viruses like filoviruses and previously reported coronaviruses. The compassionate use of Remdesivir for Critical COVID-19 cases was suggested and clinical studies have been planned as evident from the published media.[9]

Ritonavir (MF:$C_{37}H_{48}N_6O_5S_2$; MW: 720.95 g mol^{-1}): It is an oral antiretroviral medication used along with other medications to treat HIV/AIDS in low dosages. It is using along with other medicaments for hepatititis C. It belongs to the protease inhibitor class and was patented in 1989 and came into medical use in 1996. The Ritonavir in lower dosages found enhancing other protease inhibitors considerably.[10]

Simeprevir (MF:$C_{38}H_{47}N_5O_7S_2$; MW: 749.94 g mol^{-1}): Simeprevir is a macrocyclic NS3/4A protease inhibitor preventingg viral replication in infected cells and is hence found effective against HCV-1. The FDA had also approved the use of simeprevir in combination with PEG-IFN and RBV for HCV genotype 1 infection in patients with noncirrhotic and compensated cirrhotic disease.[11]

Umifenovir (MF:$C_{22}H_{25}BrN_2O_3S$; MW: 477.41 g mol^{-1}): It is a broad-spectrum antiviral drug that inhibits medically important flaviviruses. The Umifenovir developed at the Russian Research Chemical and Pharmaceutical Institute can ihibit various DNA and RNA viruses. It is also known as Arbidol and its mode of action is based on its intercalation into membrane lipids leading to the inhibition of membrane fusion between virus particles and plasma membranes, and between virus particles and the membranes of endosomes. In addition to its broad-sepectrum antiviral activity it has been proved as an immunomodulant and hence it represents a prime candidate for treatment of several viral infections in humans.[12]

Chloroquine (MF:$C_{18}H_{26}ClN_3$; MW: 319.87 g mol^{-1}): The Chloroquine is an antimalerial agent that can attack the malerial parasite by blocking DNA transcription as part of its action. A flat heteroaromatic structure is present that can itercalate DNA. This was introduced in 1950s and unfortunately the parasite has developed resistance against the drug since 1961 and was replaced by Artemisinin. Some recent studies report that Chloroquine has been showing some measurable activities against Dengue Viruse Type 2. Recently it had showed some preventing activity against the SARS CoV-2 and hence widely prescribing.[13]

Hydroxychloroquine (MF:$C_{18}H_{26}ClN_3O$; MW: 335.87 g mol^{-1}): The Hydroxychloroquine (HCQ) had been showing many immune-modulating effects including interference with

antigen processing and antibody production. It also showed some novel antiviral properties against the entry of influenza virus and adenovirus into the cells. Recently it showed certain predominent effect in SARS CoV-2 and hence widely prescribing in combination with other antivirals.[14]

These antivirals understudy were selected as they are presently prescribing/under clinical study for various viral ailments and are already understudies for COVID-19 also. The rheumatoid anthritis drug Baricitinib was selected as it reports some activity for respiratory diseases apart from its anti inflammatory and immunomodulant activities. The antimalerial Hydroxychloroquine is being prescribed as a preventive medicine for COVID-19.

Experimental

In this research work certain otherwise approved or understudy drugs belong to different categories were selected viz. a rheumatoid arthritis drug, various antivirals and antimalerials. These were screened against the SARS CoV-2 using in silico methods and the results were compared. The chemical structures of these selected drugs were drawn and the geometry optimized using various well described methods of moleccular mechanics, semiempirical calculations and Ab Initio method of Quantum Mechanics etc. For the computational studies free software like ChemSketch and ArgusLab were used.[15,16] The apt computational methods were selected wherever found well suited to the molecule under screening. The SARS-CoV-2 main protease with unliganded active site (6yb7.pdb) file was downloaded from www.rcsb.org and various possible binding sites were defined in different regions.[17] Each of the drug molecules was individually docked to the defined binding sites of the 6yb7.pdb file.

Table 1: The computed average of best ligand pose energies (BLPE) and total number of unique pose configurations (UPC) of each drug candidate under study.

Sl.No.	Drug Under Computational Study	Best Ligand Pose Energy (BLPE) (kcal mol^{-1})	Total Number Unique Pose Configurations (UPC)
1	Baricitinib	-4.894155	109
2	Danoprevir	NA	No acceptable ligand poses were found
3	Darunavir	-5.594363	309
4	Favipiravir	-5.202556	752
5	B-DN^4- Hydroxycytidine	-5.313585	543
6	Lopinavir	-5.630745	237
7	Remdesivir	-4.907787	224
8	Ritonavir	-5.816277	109

9	Simeprevir	NA	No acceptable ligand poses were found
10	Umifenovir	-5.106507	304
11	Chloroquine	-5.034458	628
12	Hydroxychloroquine	-5.508616	540

The average BLPEs were computed along with the UPCs from the obtained BLPEs of individual docking trials with the different binding sites defined. The average of best ligand pose energies (BLPE) and total number of unique pose configurations (UPC) were tabulated (Table 1).

RESULTS AND DISCUSSION

The results of the computational studies can be expressed in the increasing order of either BLPEs or UPCs and as given below.

BLPE Series: Ritonavir (-5.816277 k cal mol^{-1}) <Lopinavir (-5.630745 k cal mol^{-1}) < Darunavir (-5.594363k cal mol^{-1}) <HCQ (-5.508616 k cal mol^{-1}) <BDNH (-5.313585 k cal mol^{-1}) < Favipiravir (-5.202556 k cal mol^{-1}) <Umifenovir (-5.106507 k cal mol^{-1}) <CQ (-5.034458 k cal mol^{-1}) < Remdesivir (-4.907787 k cal mol^{-1}) < Baricitinib (-4.894155 k cal mol^{-1}).

UPCs Series: Baricitinib= Ritonavir (109)< Remdesivir (224) < Lopinavir (237)< Umifenovir (304) < Darunavir (309) < HCQ (540) < BDNH (543)< CQ(628) < Favipiravir (752).

Note: The abbreviations used BDNH, CQ and HCQ represent B-DN^4- Hydroxycytidine, Chloroquine and Hydroxychloroquine respectively.

The Ritonavir has shown the least value for the BLPE (-5.816277 k cal mol^{-1}) with only 109 unique pose configurations (UPCs). The Favipiravir showed highest UPCs (752) with a moderate value for BLPE (-5.202556 k cal mol^{-1}). Unfortunately Danopravir and Simeprevir have not shown any acceptable ligand poses during docking. The Lopinavir has shown a second least value for the BLPE (-5.630745 k cal mol^{-1}) but with higher number of UPCs (237) than the Ritonavir (109 UPCs). The B-DN^4- Hydroxycytidine showed an average BLPE (-5.313585k cal mol^{-1}) with a comparatively high UPCs (543). According to the BLPE Series the antiviral Retinovir should have the highest activity and the Baricitinib be the one with least activity. But as per the UPCs Series the Favipiravir should have the highest and the

Ritonavir be the least active one. Here the results of these two series should be correlated in such a way that they shall not be contradictory but shall be complementary to each other. For this the available reported clinical studies of these drugs understudy and a tool of chemical intution based on Natural Intelligence was used. The Hydroxychloroquine HCQ was reported as more potent than Chloroquine at inhibiting SARS-CoV-2 in vitro. This computational study showed values of average BLPE and total UPCs for the Hydroxychloroquine HCQ(-5.508616 k cal mol^{-1}; 540) and Chloroquine (-5.034458 k cal mol^{-1}; 628) respectively. Hence the computed value obtained for Hydroxychloroquine HCQ is arbitrary fixed as a threshold value for the minimum antiviral activity against SARS CoV-2. Hence the rounded threshold values logically set for BLPE and UPC are -5.00 k cal mol^{-1} and 500 respectively as there is prime importances for five and its multiples in drug designing.[18] Based on these set threshold values, only the drug molecules understudy HCQ (-5.508616 k cal mol^{-1}; 540), BDNH (-5.313585 k cal mol^{-1}; 543), CQ(-5.034458 k cal mol^{-1}; 628) and Favipiravir (-5.202556 k cal mol^{-1}; 752) can show atleast a moderate activity against SARS CoV-2. Both Ritonavir and Lopinavir in lesser quanties are proved enhancers for other antivirals when used in combination. The combination therapies of these drugs might be considered as a compassionate prescription for the time being.

CONCLUSION

This computational study showed that the present antivirals of modern medicine cannot act on SARS CoV-2 to cure completely. We have to search for better antivirals as expedite as possible to overcome the present dilema. This computational study showed Favipiravir, Hydroxychloroquine HCQ, B-DN^4- Hydroxycytidine BDNH and Chloroquine CQ might have moderate activities. The combination therapy of these drugs along with Ritonavir/Lopinavir might be the only best possible compassionate treatment as per the present situation. The research for better antivirals and a suitable vaccine shall be boosted. This computational study also showed that so far there is no completely effective antiviral drug in the modern medicine for treating the pandemic COVID-19 caused by SARS CoV-2. Presently I am concentrating on various ligand molecules of phyto origin for a better alternative based on *Ayurvedic* principles without any prejudice.

Note: The computational works were statrted with 6LU7.pdb file but later revised with downloaded 6YB7.pdb file.

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