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Review Article

Adamantane as the potential candidate for conjugation along with natural compounds as a potential drug for Sars-CoV-2

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Abstract

We need to develop antiviral drugs against Sars-CoV-2 main protease (M^{pro}) because it is essential for viral replication. The M^{pro} enzyme has separate catalytic and dimerisation domains and operates as a homodimer. Being peptidic in nature, Cys145 and His41, two catalytic residues, are essential for inhibition. Current inhibitors are GC-376, PF-00835231. Non-peptidic inhibitors like Wu04, R, and S-216722 are also promising candidates as less harmful substitutes. FDA-approved medications like Remdesivir and Nirmatrelvir have drawbacks that include decreased efficacy and interactions. Non-peptidic inhibitors, such as AF 399/40713777 and AI-942/42301830, exhibit modest M^{pro} inhibition. The use of virtual screening, molecular dynamics coupled with computer-aided drug design (CADD) has made it possible to find 17 new M^{pro} inhibitors. Furthermore, substantial M^{pro} inhibition has been demonstrated by natural compounds such flavonoids and glycosides, suggesting their potential as therapeutic agents. In preclinical settings, AMA derivatives—especially 3F4—show notable antiviral activity and decreased toxicity among new drugs. It suggests potential of combinatorial therapy and the significance of various inhibitor types for efficient Sars-CoV-2.

Keywords: Sars-CoV-2, M^{pro}, Adamantane. Remdesivir, Nirmatrelvir.

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1. Introduction

COVID-19 is infectious disease transmissible from human to human, however, there are few reports of its transmission from animal to human. It is commonly caused by severe acute respiratory syndrome corona virus 2, popularly known as Sars-CoV-2. It led to pandemic like situation in the entire world in late 2019 and early 2020 leading to lockdown and causing ≥6000000 deaths with huge disruption of public life and economic losses. It has made significant impact on global public health emergency after Spanish flu that spread in 1918. Sars-CoV-2 and Middle East respiratory syndrome corona virus (Mers-CoV) are reported as two highly contagious diseases that are known to the public and scientific community in 2002 and 2012 respectively. These deadly respiratory disease, have made corona viruses a new public health concern in the 21st century. The patients suffering from SARS and MERS, showed symptoms of viral pneumonia, which includes fever (high or low), cough and chest congestion (discomfort), and if the case is severe dyspnoea then bilateral lung infiltration which may lead to several complications including fatality.[1]

First case of corona virus with scientific proven record, was reported on 8th December 2019, in Wuhan, a province of Hubei in China. Municipal Health Commission of Wuhan on 31 December, 2019 informed a pneumonia outbreak by an unidentified causal organism and reported to the World Health Organization (WHO). The virus was isolated from the broncho-alveolar lavage fluid collected from large number of patients. Using metagenomic RNA sequencing methods, the causal agent was identified as corona virus and the strain that was never seen before.[2-4] On January 10, the novel corona virus's initial genome sequence was made available on the Virology website. Two days later, on January 12, other nearly

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full genome sequences of the corona virus were made available through the GISAID database by various research institutions. WHO still did not declare it as pandemic for the reasons still not known to the public and the scientific community.

Later, other patients were discovered who had not previously been exposed to Seafood Wholesale Market in Huanan. Numerous reports of familial clusters of infection have been made, and nosocomial infections have also been documented in healthcare settings. Each of these cases offered convincing proof that the novel virus spreads from person to person.[3-5] Traveling between cities prior to the celebration of the Lunar New Year helped the spread of the virus in China, as the outbreak fell around that time. Furthermore, it was observed that the unique corona virus pneumonia was spreading to about 34 Chinese provinces after initially affecting the entire metropolis of Hubei province. There was a sharp rise in the number of confirmed new cases, that tallied thousands in number identified every day in late January. WHO finally on January 30, declared an outbreak, a public health emergency of international concern. The novel corona virus was named as Sars-CoV-2 by the International Committee on Taxonomy of Viruses (ICTV).[6] The disease was named 'COVID-19' by WHO. February marked the apex of the COVID-19 epidemic in China and an average of 3,000 cases were diagnosed each day worldwide as per the reports of Chinese National Health Commission.

China took stringent public health measures to contain COVID-19. All forms of transit and movement within Wuhan were suspended, and the city was closed and the people were strictly restricted to all outdoor activities, community gatherings, public and social meetings, all over the country to restrict the spread of the virus from human to human. The incubation period of the virus was known 14 day, hence, the isolation protocol was advised. The International Committee on Taxonomy of Viruses' Coronaviridae Study Group classified and named it as 2019-nCoV corona virus, which is connected to severe acute respiratory syndrome.[7] The patients were advised to remain inside home, follow distance of 6 feet, wearing of mask, washing of hands and eat healthy and immune boosting diet. The daily number of cases began to decline with increasing observance of COVID-19 protocol. But it was during late February that spread of COVID 19 spread from China in different countries and cases now started to be reported from there as well. Uninterrupted frequent international travel facilitated transmission of the virus and enabled rapid worldwide spread of COVID-19. On March 11, 2020, the WHO officially declared the global COVID-19 outbreak as a pandemic (World Health Organisation. 2020). Interestingly, there was a noticeable decrease of cases in China, but the number increased in the US, Italy, and other European countries of the world. All six continents reported more than 20 million COVID-19 cases with >733,000 deaths as of August 11, 2020, as per COVID-19 dashboard of the Centre for System Science and Engineering at Johns Hopkins University. As of August 11, 2020, that number of cases had jumped to over 229 countries [8]. A high number of COVID-19 cases were reported in the United States of America.

2. Discussion

2.1. Origin and phylogenetic analysis of SARS-CoV-2

It was just after the isolation and identification of the causative agent behind Sars-CoV-2 it was clear that the, SARS- COV and/or anti- SARS-COV antibodies were found in masked palm civets (*Paguma larvata*) and animal keepers in a marketplace.[9,10] Examining the geographic distribution and molecular development of severe acute respiratory syndrome virus, in palm civets from farms and livestock markets.[11] It was observed that SARS-COV2 strains were found in market civets that were transmitted to them by other animals. The relation between the novel corona virus and human its transmission in humans as different strains were reported in two different studies in the year 2005, which were named Sars-CoV-related viruses or SARS- like corona viruses, in horseshoe bats (genus *Rhinolophus*).[12] There are evidences that suggest that SARS-CoV-2 might have originated from multiple handling of animals in Wuhan city market.

The genomic analysis has revealed that bats could be the natural hosts for SARS-CoV-2 and the civets are the intermediate reservoirs. Several phylogenetically related variants of corona virus were discovered in bats from various provinces of China and also from Europe, Africa and Southeast Asia.[13-15]

The phylogenetic analysis of RNA-dependent RNA polymerases (Pol) of different 10 corona viruses and their complete genome sequences facilitated the ICTV to suggest that the strains identified in the *Rhinolophus* bats are Sars-CoV's variants while the strains from *Hipposideros* bats in Africa are lesser related to Sars-CoV, hence we suggest that these should be classified as a new corona virus species as advocated by Tong et al. also.[15]

Most diverse forms of SARS-CoV's were found in a caves of Yunnan province in China. This has become a hotspot wherein all the genetic diverse forms of SARS-COV's have been identified.[16-18] The viral strains isolated from this location have all genetic elements for SARS-CoV. Despite extensive searching, no direct progenitor was found, suggesting that SARS-CoV have emerged from the recombination in unidentified bat populations.[19] Recombination analysis also suggests that the civet SARS-CoV strain SZ3 has emerged from the recombination of two existing bat strains, namely WIV16 and Rf4092.WIV16. It may be safely concluded that the closest relative to Sars-CoV is found in bats.[20] Based on the analysis of data about the prevalence, high genetic variation of bat Sars-CoV and their close coexistence and high recombination frequency, it is

evident that in future numerous novel variants may emerge.[21,22] The climate change and use of various anti-viral drugs may further complicate the emergence of these viruses in near future.

2.2. History of corona virus outbreaks

Corona viruses have changed their nature during the previous millennium on multiple occasions.[23] Animal infections were identified as the initial corona virus recovery, and in 1937 the infectious bronchitis virus (IBV) was isolated from chickens,[24] [murine hepatitis viruses (MHV) from mice in 1949[25] and the transmissible gastroenteritis virus (TGEV) in pigs in US in 1946.[25] Human corona viruses were first genetically characterised from the respiratory tract infections in late 1960s and were named as B814 and 229E.[27,28] Since then, several other corona virus strains have been isolated and characterised from humans using cell and tissue culture techniques (OC16 and OC43).[29,30] The number of corona viruses that have been genetically identified have increased dramatically over the time, and now includes viruses from a variety of different animal species, including bats, sparrows, rabbits, calves, cats, dogs, and turkeys.[31] SARS-CoV caused serious outbreak in the year 2002-2003 with reported 8,096 cases and 774 deaths (9.6% fatality rate), primarily in China and Hong Kong. Genome sequencing suggests that it is closely related to a virus from Himalayan palm civets, potentially its source.[9] Later, civets have been widely considered as intermediate host for SARS-CoV, with bats as their natural host.[20,32] Genome analysis has revealed high genetic diversity in several genes (S, ORF3, and ORF8). Despite marked differences in the S protein sequence, all 11 SARS-like CoVs used the same human angiotensin-converting enzyme-2 (hACE2) receptor to cause infection. It shows its close relationship with SARS-CoV. Therefore, SARS-CoV are considered likely to be emerged by recombination of bat SARS-like CoVs before infecting the civets. This virus might have later spread to humans, causing the SARS epidemic in recent years, compelling the lock down world over. Middle Eastern countries showed widespread of MERS-CoV from where it was transmitted to humans from dromedary camels.[33] It has been reported that human and camel MERS-CoV strains share >99% genetical similarity with variations/substitutions located only in the S, ORF3, and ORF4b genes.[34]

The genetic evidence reveals that MERS-CoV is very close to bat corona viruses HKU4 and HKU5 phylogenetically[13] and might have emerged from the bats through recombination within ORF1ab and S genes.[35] Various studies have revealed that MERS-CoV uses the human dipeptidyl peptidase 4 (DPP4) receptor to infect the host cell. MERS-related CoVs isolated from bats in China with spike proteins to possess the capacity to bind to the same receptor as MERS-CoV, further confirm the possibility of a bat origin for MERS-CoV.[14] Sars-CoV-2 was reported in Wuhan City, of China in December 2019, causing severe respiratory illness, human to human spread with high mortality (see the epidemiology section) throughout the world. Various studies from different laboratories have reported that it might have evolved from bats[36] with its high identity (96.3%) with corona virus RaTG13 of bat.

2.3. Clinical and epidemiological features

The people of nearly all ages are susceptible to SARS- CoV-2 infections, but the middle aged 50 years were more prone to infection. Old age people took extra care to escape the infections but they were equally susceptible to the infections as the clinical manifestations depend on age. In general terms, we can say that older people (>60 years old) with co-morbidities are more susceptible to severe respiratory disease that may require hospitalisation or may eventually die, whereas it has been seen that most young people and children have only mild symptoms of SARS- CoV-2 infections (non-pneumonia or mild pneumonia) or are asymptomatic.[37-39] The risk of disease in pregnant ladies was not higher. However, in an isolated case there was an evidence of transplacental transmission of SARS- CoV-2 from an infected mother to a neonate.[4] The common symptoms of infections are fever, fatigue, dry cough. Less common symptoms of Sars-Cov-2 include sputum production, headache (severe or mild), haemoptysis, diarrhoea, anorexia, sore throat, chest pain, chills and nausea and vomiting reported in various studies conducted in China. Self- reported olfactory and taste disorders in patients in Italy are also available in scientific literature[18,38,40-42] with an incubation period of 1–14 days, usually about 5 days, the majority of participants began to exhibit symptoms of their diseases; dyspnea and pneumonia emerged within a median of 8 days from the commencement of illness. Eighty-one per cent of the 72,314 cases reported in China were categorised as mild, 14% as severe, requiring ventilation in an intensive care unit (ICU), and 5% as critical, meaning the patients had multiple organ failure, septic shock, respiratory failure, or both. When a patient was admitted, the most frequent radiologic finding on a chest CT scan was ground-glass opacity along with noticeable lymphopenia, as observed in SARS and MERS patients, most patients also experienced progressive lymphopenia over the time in non-survivors. ICU patients showed greater plasma cytokine levels than non-ICU patients, which may be an indicator of immune- pathological process induced by a cytokine storm.[4,41,43] About 2.3% of the patients in this group passed away within a median of 16 days after the sickness started. Irrespective of a suffering from cardiovascular disease, men over the age of 68 were more likely to experience respiratory failure, acute cardiac damage, and heart failure that resulted in death. Most patients made enough progress to be discharged from the hospital within two weeks. Initial reports of early SARS-CoV-2 transmission in Wuhan in December 2019 indicated that Huanan Seafood Wholesale Market was the point of origin of the outbreak of COVID-19, however, the community transmission might have occurred earlier.[37,44]

Humans of all ages are susceptible to the infection from corona virus. However, the individuals aged ≥60 and patients having medical co-morbidities like obesity, diabetes, chronic kidney and lung diseases, smoking associated diseases, cardiovascular related diseases including cancer, or hematopoietic stem cell transplant patients, have higher risk of developing severe COVID-19 infections as observed by various doctors during recent years.

As per observations of CDC, age, health, immunity, lifestyle still, exposure still remains the strongest grounds for development of severe illness in patients with COVID-19. The data available on National Vital Statistics System (NVSS) reveals that patients with COVID-19 aged between 50 to 64 years have 25 times greater risk of death than the adults with less than 30 years. The risk further increases to 60 fold in the patients between 65 to 74 of age. The study reveals that the patients older than 85, have 340 times risk for death. These findings are based on the data collected in United States during the pandemic in between February 2020 to July 1, 2022, including the deaths among non-vaccinated individuals. A particular race of the people in US did not prefer the vaccination in spite of several medical advisories.

The data analysis made by Stokes *et al.* from the confirmed COVID-19 cases only that were reported to the CDC during January 22 to May 30, 2020, has revealed that the probability of COVID-19 patients that required hospitalisation was 6 times higher in those individuals that were suffering from pre-existing medical conditions than healthy (45.4% vs. 7.6%). A study involving 42604 patients in USA with confirmed diagnosis of Sars-CoV-2 infection, reported that the mortality rate in male patients was 12.5% than 9.6% in females. Gender-based differences suggests that male patients have a higher risk of COVID-19 infections with increased mortality as compared to females patients, that may possibly because of their greater exposure to the external infectious environment.

CDC analysis involving 300,000 COVID-19 patients over a period of 10 months in the year 2020 (March to December) showed that racial and ethnic minority groups have higher percentage of hospitalisations than white. Data analysis of 50 studies from USA and UK further confirmed that Black, Hispanic, and Asian ethnic minority groups are at greater risk of contracting the infection and death from COVID-19. Corona virus related death rates were the highest among Hispanic persons with medical comorbidities and were more prevalent in sexual minority than heterosexual individuals within the general population and also within specific racial/ethnic groups.[45-50]

The virus has substantially progressed and mutated in various variants and continues cause certain outbreaks in many countries. Although there have been several variants of the virus known since then, a few have been declared as variants of concern (VOC) by WHO of which following five have been identified since the beginning of the pandemic:

1. Alpha (B.1.1.7): First variant, identified in the United Kingdom (UK) in December 2020[63]
2. Beta (B.1.351): First identified in South Africa in December 2020.
3. Gamma (P.1): First identified in Brazil in January 2021.
4. Delta (B.1.617.2); First known case in India in December 2020.
5. Omicron (B.1.1.529): First reported in South Africa in November 2021.

Eventually, a globally dominant variation, D614G, was shown to have greater transmissibility but not the capacity to induce serious illness.[51] A different variation was linked to infection from Danish farmed mink, but it was not linked to higher transmissibility.[52] Since then, other Sars-CoV-2 variations have been identified; some of these are regarded as variants of concern (VOCs) since they may result in increased virulence or transmissibility. A categorization method for differentiating the developing variants of Sars-CoV-2 into variants of interest (VOIs) and variants of concern (VOCs) has been separately developed by the WHO and the US Centre for Disease Control and Prevention (CDC).

2.4. The structural aspect of the Sars-CoV-2

The spike or S protein is a glycoprotein of the virus fuses that with the ACE-2 receptors of the host that helps in the entry and infection of the virus. S protein is a homotrimer and adhere in multiple copies to the phospholipid bilayer of the virion that is viewed as crown like structure of the virus. It has two binding sites S1 which interacts non-covalently with ACE-2 receptors and S2 which helps in attachment with the viral membrane. The + sense RNA of the viral genome is translated into polyprotein that is then cleaved into 16 non-structural proteins which are cleaved majorly by M protease. These non-structural protein are important for the viral replication and is therefore the most prominent target for the antiviral drugs.[53]

2.5. The most potent inhibitor of the virus

The M^{pro} of the virus is a homodimer that helps in the processing of the functional protein. The homodimer has two major domains, the catalytic domain and dimerisation domain. The catalytic domain has Cyt 145 and His 41 known as the major sites of inhibition and are ideal for drug design and development. The interaction of inhibitors with the M^{pro} takes place via hydrogen bonds with Glu 166, His 163, Ser 144, Gly 143 residues of the M^{pro}. There are several potent inhibitors under final trials like GC-376, PF-00835231and 11athat are known to inhibit cathepsins and stimulate the endogenous substrate of M^{pro}. They can covalently interact with the CYS145 residues of M^{pro} but they are peptidic in nature and show off target side effects. Certain non- peptidic inhibitors like R, S-216722 and Wu04 are now known to potentially inhibit the M^{pro} and are potential inhibitor of drugs for treating the infection.

There are two main antiviral drugs namely, Remdesivir and Nirmatrelvir which have been approved by FDA for urgent use but Remdesivir, although is a novel nucleoside inhibitor of RNA dependent RNA polymerase enzyme of the Sars-CoV-2 virus but shows a weak potency against it and the other, Nirmatrelvir is an oral active ligand in combination with CYP3A inhibitor Ritonavir but is only prominent against severe symptoms only. Recently, two compounds, AF 399/40713777 and AI-942/42301830 have been identified, that are non-peptidic inhibitors and show moderate level of inhibition against the M^{pro}. High preference is given to non-peptidic inhibitors over peptidic inhibitors as they show low off-target side effects, high bioavailability, and are less toxic as compared to peptidic inhibitors. The computer aided drug design (CADD) is used for the designing of the novel drugs like Glivec. In this context, virtual screening- based techniques are used to discover novel chemotype of Sars-CoV-2 M^{pro} inhibitor. There were 17 compounds that were isolated and identified using virtual screening, pharmacophore models, and QSAR techniques that also help to guide the structure, optimise M^{pro} inhibitors and molecular dynamics of the identified inhibitors.[54,55]

2.6. The importance of natural products in antiviral drug treatment

Further studies show that the Sars-CoV-2 M^{pro} is one of the prominent targets for the development of antiviral drug treatment and there are various natural products or resources that can be exploited for their pharmacological importance. A combination of computational biology, MD simulation, pharmacophore based virtual screening molecular docking, MM GBSA may be used to identify a potential inhibitor of Sars-CoV-2 M^{pro}. There are various natural products that possess potential anti-COV activity and can be easily identified using virtual screening against the natural product databases. Therefore, a generated pharmacophore models may be used as filter for screening the selected natural product databases- SNP, MNP and zinc natural product database for the identification of potential inhibitors against SARS COV-2 M^{pro}. There have been at least six natural products reveled after docking analysis that show strong affinity with the SARS COV-2 M^{pro}. Further stability analysed using MD simulation, showed that there are six potential inhibitors which belong to different classes like anthocyanin, amylogycosides, glycosides, flavonoid, FOC's and, glycosides.These are known to occur in various plants and bacteria. The plants have been used as traditional Chinese medicine for various purposes and these compounds have at least one sugar molecule (Pentose or hexose) which helped in hydrogen bond formation with the surrounding amino acid residues and hence, shaped significant docking scores. Among these compounds, flavonoids have been one of the proven inhibitors of M^{pro} of Corona virus[64] that are widely known to be present in *Aloe vera*, *Ocimum sanctum*, giloy and ginger.

2.7. Potential candidates for Sars-CoV-2

Several existing drugs, previously tested during other viral outbreaks, were evaluated for COVID-19 treatment, including ACE2 inhibitors, anti-spike monoclonal antibodies, RdRp inhibitors, endosome maturation inhibitors, and inhibitors of viral protein synthesis, maturation, and shedding.[56] The first effective antiviral approved for Sars-CoV-2 was remdesivir, an adenosine analogue initially developed for Ebola and repurposed for RNA viruses. It improved COVID-19 patients' conditions, promoting faster recovery and reducing mortality in moderate cases.[57-59]

Two oral formulations namely molnupiravir and the ritonavir-boosted nirmatrelvir combination (Paxlovid™) were approved in late 2021 as antiviral drugs for the potential treatment of COVID-19. The former is a prodrug showing antiviral activity. It is metabolised to the cytidine nucleoside analog *N*-hydroxycytidine (NHC). It is taken up by the cells and phosphorylated to form the active ribonucleoside triphosphate (NHC-TP) that cause errors in the viral genome by incorporating into Sars-CoV-2 RNA using viral RNA polymerase, thus inhibiting the viral replication.

Nirmatrelvir, is a Sars-CoV-2 main protease inhibitor (M^{pro}), while ritonavir is the protease inhibitor of human immunodeficiency virus 1 (HIV-1). It is also the inhibitor of cytochrome P450 (CYP) 3A, and act as a pharmacokinetic enhancer for nirmatrelvir.[22,58] There are known clinical limitations for the use of Paxlovid™. It is detrimental drug-drug interactions (*e.g.*, ritonavir interference and nirmatrelvir efficacy reduction), and resistant strains to nirmatrelvir have also been reported, limiting its use. Therefore, other antivirals could be restructured or their activity may be enhanced against Sars-CoV-2, to find potential COVID-19 therapeutic options.[60-63]

Adamantane is a powerful antiviral drug with a tricyclic bridged hydrocarbon structure that has the capacity to disrupt the viroporin protein channel of RNA viruses, that are crucial for virus maturation/replication and its release. Amantadine, an aminoadamantane (AMA) derivative, was the first drug based on AMA that was used as an antiviral against the Influenza A virus. It was found that AMA has the potential to inhibit Sars-CoV-2 *in vitro*, and production of its derivatives have enhanced potency.[64-66] Five new AMA derivatives have been synthesised and evaluated for their anti-Sars-CoV-2 potency as potential COVID-19 treatments. AMA and its derivatives are broad-spectrum antiviral drugs known for their low toxicity and potent ADMET properties. Adamantane or AMA is synthesised through the condensation reaction of aldehyde with aminoadamantane.

AMA and some of its derivatives have demonstrated the ability to reduce viral genomic copies in Vero-CCL81 and Calu-3 cell lines, showing enhanced antiviral properties. Their increased selectivity index (SI) values make them strong candidates for Sars-CoV-2 antiviral drugs, with a 50%

chance of killing the virus. Among these derivatives, 3F4 exhibited the highest efficacy against virus-infected Calu-3 cells, a lung cancer cell line with specific genetic mutations like ErbB2/Her2, EGFR, K-Ras, TP53, and CDKN2A. Further analysis of these derivatives was done using the immunofluorescence staining technique by infecting Vero-cells with Sars-CoV-2. Vero CCL-81 cells exhibit green staining for protein S. The control cell (uninfected) shows no green staining for protein S and the nuclei are stained in blue. The impact of treatment may be verified with fluorescence visualisation to identify new AMA derivatives.

Transmission electron microscopy is commonly used to analyse viral morphogenesis for studies on treatment with AMA derivatives. The compounds showing best antiviral results (3F4, 3F5, and 3E10) are assessed that show a significant reduction ($p < 0.05$) in viral particles. It has been demonstrated that AMA and its derivatives, especially 3F4, effectively reduced vacuolisation, various cytoplasmic effects, and reduce viral particle size, suggesting greater efficacy when used in combination rather than single. Cathepsin and docking analysis-Cathepsins, especially human CatL is a cysteine protease enzyme are critical in various steps of entry of virion into host cells.[67] The virus may enter by receptor-mediated endocytosis, in which host endosomal/lysosomal cysteine peptidases [cathepsins B (CatB) and L (CatL)], are involved in the activation of S protein.[67,69] The main function of CatL and CatB is protein catabolism at intracellular level. Among host cysteine peptidases, CatL is normally associated with viral glycoprotein activation. Hence, amantadine was tested against the cathepsin enzyme and it has been found putative cathepsin inhibitor. The docking analysis of AMA with gold and cathepsin-L with an RMSD value of 0.77 angstrom reveals that AMA and its derivatives are not completely able to inhibit cat-L but follow a different pathway to inhibit its activity.

The toxicity predictions were done using two different servers that is Protox -11 which is based creating molecular fragments and fingerprinting to calculate the similarity in compounds based on the molecular analysis and another server is PKCSM which helps in creating graph-based structure to predict the ADMET properties of AMA and its derivatives and acute oral toxicity was tested and compared using both the methods. It was concluded that AMA and its planned analogues are less toxic and show a safer profile for consumption.

Antiviral activity of AMA- The model used for testing the antiviral activity is BALB-mice (10-12 weeks old). AMA was injected peritoneally after 12 to 24 hours of infection with Sars-CoV-2 MALO which is known to cause acute lung damage in mice. The results showed that the weight of the mice was certainly reduced after the exposure with the virus and after the AMA treatment it was significantly recovered. An increase in the efficacy was observed after the combinatorial treatment of both AMA and 3F4.

It was further detected that the viral titres were reduced to 4- fold after the treatment with AMA alone and it reduced to 16 folds when 3F4 and AMA were used together as compared to AMA alone. Using modern bioinformatics tools, homology modelling and molecular docking studies of spike protein in SARS-CoV-2 we have reported that the virus is more close to bat. We have further identified tetrandrine as a potential inhibitor of SARS-CoV-2, however, it needs further exploration for its development as antiviral drug in view of the recent reports of recurrence of corona infections with new variant.[71]

3. Conclusions

This study presents a promising approach to COVID-19 treatment by evaluating new AMA derivatives with potent antiviral properties against Sars-CoV-2. The derivatives effectively reduced viral genomic copies in cell lines and demonstrated increased selectivity index (SI) values, making them strong candidates for antiviral therapy. Notably, the 3F4 derivative exhibited the highest efficacy, suggesting that AMA derivatives, particularly in combination, could offer enhanced therapeutic potential against Sars-CoV-2. Further investigations, including docking analysis and toxicity predictions, support their low toxicity and safe profile. The recent reports of JN.1 variant of COVID-19 in May, 2025 in Asia particularly Singapore, China and some parts of India is a matter of great concern to scientific community but the possibilities of dangers are second wave of COVID-19 are eliminated. WHO and most countries are keeping a watch on spread and occurrence of new variants and the RNA based vaccines against new variants can be effective. Wide exposure of a larger population against COVID-19 has provided natural immunity besides the vaccination and the better understanding of COVID-19 management pose lesser health risk now than in early 2020.

4. Sources of Funding

None.

5. Conflict of Interest

None.

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