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Al-Akidi Therapeutic Protocol for Severe Infections Associated with Covid-19: Potential and Effective Treatment by Levofloxacin and Vitamin D3 and Zinc (Part 1)

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Abstract: Background: The emergence of Coronavirus SARS-CoV-2 evoked an unprecedented threat globally. Ever since the spread of this pandemic research and clinical trials have concentrated on the repurposing of already exciting FDA drugs to find a successful candidate to combat Covid-19. **Objective**: The objective of this study is to propose a therapeutic protocol that may have a potential solution to treat the severe infections associated with coronavirus. The clinical application of this protocol (Al-Akidi Therapeutic Protocol, A-TP) is highly recommended, as there are several scientific evidences that support this trusted protocol to be of great potential. **Methods and Materials**: This A-TP includes the use of one of the respiratory Fluoroquinolones (Levofloxacin or Moxifloxacin) in doses of 500 mg twice daily for 7-10 days, together with high doses of Vitamin D3 (10000 IU/day) and Zinc (50 mg daily) for few weeks. This protocol is based on the previous antiviral activity of those Fluoroquinolones towards few viruses, the potent antibacterial activity on respiratory infections and high available concentrations in the lungs. It is also based on molecular docking of Levofloxacin and Moxifloxacin on various viral enzymes. **Results**: Molecular docking showed encouraging and very interesting docking scores and binding affinity of Levofloxacin and Moxifloxacin to certain viral enzymes, such as, RNA dependent RNA polymerase (RdRp), 3-Cysteine-Likeprotease, Neuraminidase, Replicase polyproteins and Trans-Membrane Protease Serine 2 inhibitor (TMPRSS2). The highly expected clinical results of using this protocol are; reduce infection, control of temperature, improve breathing with less dependent on supplemented oxygen, and remarkable reduction of the pro-inflammatory cytokine storm, and hence, reduce hospitalization and mortality. **Conclusion**: Levofloxacin is highly recommended in managing the severe infections associated with Corona virus and has a remarkable reduction of pro-inflammatory cytokine storm as an immuomodulating agent. Levofloxacin is superior in this protocol over Moxifloxacin, due to its high excretion (≤ 83%) as unchanged through the kidneys, while Moxifloxacin is only 20% is excreted unchanged. It is an extra advantage of Levofloxacin to manage coronavirus in the kidneys. High doses of Vitamin D3 and Zinc are very useful to provide additional effective measures to combat Corona virus. Therefore, the use of this A-TP is highly and strongly recommended, as it serves the full requirements for excellent and potential therapy for the severe infections associated with Covid-19.

Key words: Covid-19, Levofloxacin, Moxifloxacin, ACE2, docking, protocol, viral enzymes, 3CL pro-1, Camostat, Panobinostat, Oseltamivir acid.

1. Introduction

WHO declared Covid-19, caused by the virus SARS-CoV-2, a global pandemic. Coronavirus causes different diseases and complications. These may include: severe acute respiratory syndrome (SARS), middle east respiratory syndrome (MERS), Coronavirus pneumonia, multi-organ failure, septic shock, cardiovascular complications (heart failure, arrhythmias, heart inflammation, and blood clots), leading to severe disturbance of lung activities and breathing, and may lead to death [1-4].

It has been stated that the angiotensin-converting enzyme type 2 (ACE2) is the main host cell receptor of human pathogenic coronaviruses (SARS-CoV),

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HCoV-NL63, and Covid-19, and it plays a crucial role in the penetration of the virus into lung cells to cause the final infection [5]. ACE2 is over expressed by epithelial cells of the lungs, intestine, kidneys and blood vessels [6]. This may explain the high incidence of pneumonia and bronchitis in those with a severe Covid-19 infection. A recent study reported that ACE2 is also highly expressed on the mucosa of the oral cavity, granting the virus easy access to a new susceptible host [7]. Accordingly, it is now concluded that this site is a target for any novel antiviral agent that may block ACE2 and prevent Coronavirus from attacking those tissues, including the lungs. Viral enzymes are another important target for the proposed antiviral agents. These include RNA-dependent RNA polymerase (RdRp), Protease, Neuramidase and Priming Protease (TAMPRESS2), which is the main Protease that acts on the proteolysis of the replicase polyproteins. Therefore, the search for potential antiviral strategies for protection and treatment is particularly urgent.

1.1 Respiratory Fluoroquinolones

(Levofloxacin and Moxifloxacin) are potent antibacterial agents and broad-spectrum antibiotics that are active against both (G+ve) and (G-ve) bacteria. They function by inhibiting the DNA gyrase and Topoisomerase types II & IV. These have been indicated for several cases including; respiratory tract infections, community acquired Pneumonia (CAP), acute bacterial sinusitis, pneumonia, tuberculosis, urinary tract infections, chronic prostatitis, anthrax, and other diseases [8]. Noteworthy, Levofloxacin and Moxifloxacin constitute the first line therapeutic agents for the treatment of severe community-acquired pneumonia. They are characterized by advantageous pharmacokinetic properties, higher concentration in the lungs and an excellent safety profile comparable to other antibiotics, such as, macrolides (Azithromycin) and β-lactam (Ceftriaxone or Meropenem) [9]. Levofloxacin and Moxifloxacin have clinical benefits in the treatment of chronic obstructive pulmonary disease (COPD) exacerbations, including a longer infection-free period and a reduction in the number of hospitalizations after treatment compared with other antibiotics [10, 11]. The overproduction of early response pro-inflammatory cytokines, known as cytokine storm, tumor necrosis factor (TNF), IL-6, and IL-1β) leading to an increased risk of vascular hyper-permeability, multi-organ failure, and eventually death when the high levels of cytokine are unabated in time [12]. Therefore, any therapeutic strategy should target the overactive cytokine response with anti-cytokine therapies or immunomodulators.

Based on the present situation, drug repurposing offers great opportunity to rapidly nominate a drug to treat, manage or eradicate the SARS-CoV-2 virus, although an intensive clinical trials and validation are required. WHO has launched scientific cooperation, under the term “Solidarity”, which is one of the largest international cooperative clinical trials aiming to find successful treatment against Covid-19 [13].

1.2 The Role of Vitamin D3 in Covid-19

Vitamin D3 has great potential in reducing the effects of coronavirus.

Newer evidence suggests that vitamin D3 plays a major role in regulating the immune system, including the immune responses to viral infection. Interventional and observational epidemiological studies provide evidence that vitamin D deficiency may confer increased risk of influenza and respiratory tract infection [14].

Vitamin D can reduce the risk of infections, by several mechanisms, such as induction of Cathelicidin and defensins that can interrupt viral replication rate and reduce the concentration of pro-inflammatory cytokines. This leads to the inflammation of the lining of the lungs, which develops pneumonia [15]. The potential role of Vitamin D in fighting viral respiratory infections was previously reported [15]. Lung epithelial cells express high basal levels of CYP27B1 and low level of CYP24A1, favoring the

conversion of vitamin D to its active metabolite [15]. Treatment of those cells with vitamin D leads to increasing the level of the TLR c-receptor CD-14 and cathelicidin [15]. Several reviews have considered the ways in which vitamin D reduces the risk of viral infections [16-19].

Supporting evidence of the role of vitamin D in reducing the risk of Covid-19 was included during the outbreak, when 25-hydroxyvitamin D (25(OH)-D) concentrations are lowest. It was found that Calcitriol (1, 25-Dihydroxyvitamin D3) exhibited pronounced impact on ACE2/Ang (1–7)/MasR axis with enhanced expression of ACE2, MasR and Ang (1-7) generation [20].

Vitamin D deficiency has participated in developing acute respiratory distress syndrome. This is the case-fatality rate that increased with age and chronic disease co-morbidity. Both of these cases are associated with lower 25(OH)-D level. In order to reduce the risk of infection of influenza and/or Covid-19, it is recommended that people should consider taking 10,000 IU/day of vitamin D3 for a few weeks to rapidly raise 25(OH)-D level, followed by 5000 IU/day. The objective of this dose is adjusted to increase the level of 25(OH)-D above 40–60 ng/mL (100–150 nmol/L). For treatment of infected people with Covid-19, higher vitamin D3 doses should be used and this might be useful [21]. There is a significant crude relationship between physiological levels of vitamin D and the recorded cases of Covid-19, particularly, the mortality rate. Vitamin D has already been proved to protect against acute respiratory infections and reduces the risk of viral infections [16, 22].

1.3 The Role of Zinc in Covid-19

Zinc ions modulate the antiviral and antibacterial activities towards individuals' immunity and manage the inflammatory response. Certain indications may suggest that modulation of Zinc status is beneficial in Covid-19 infections. Zinc ions may lower the activity of angiotensin-converting enzyme 2 (ACE2), which is known to be the receptor for SARS-CoV-2 [22].

In vitro experiments demonstrated that Zinc possesses antiviral activity through inhibition of SARS-CoV RNA polymerase [23].

The improved immunity by Zinc may also occur through up-regulation of interferon α production and consequently, increasing the antiviral activity. Zinc ions have anti-inflammatory activity by inhibiting NF-κB signaling and modulation of regulatory T-cell functions, which limit the cytokine storm in Covid-19 [24, 25].

The physiological Zinc level is also strongly associated with risk factors for severe Covid-19, such as ageing, immune deficiency, obesity, diabetes and atherosclerosis. Because of its immunomodulatory effect and direct antiviral activity, zinc is considered to be a potential supportive treatment for Covid-19 infection [26, 27]. Zinc supplementation decreases incidence of infections in the elderly, as it has great effect on generation of cytokines and oxidative stress [28].

After Zinc supplementation, the incidence of infection in the zinc supplementation group was significantly lower than that in the placebo group, the plasma Zinc was significantly higher than that in the placebo group, and the production of tumor necrosis factor and oxidative stress markers was significantly lower than that in the placebo group [29].

Zinc ions inhibit RNA Polymerase of Coronavirus and Arterivirus in *in vitro* experiments and Zinc ionophores impair the replication of these viruses in cell culture [26]. Increasing the concentrations of intracellular Zinc ions can effectively impair the replication of a variety of RNA viruses, by interfering with the correct proteolytic process of viral polyproteins. More specifically, Zinc ions were found to block the initiation step of equine arteritis virus (EAV) RNA synthesis, whereas in the case of the SARS-CoV RdRp the elongation step was inhibited and the template binding was reduced [26].

2. Virus-Based Targets

2.1 Targeting SARS-CoV-2 Enzyme

2.1.1 RNA dependent RNA polymerase, RdRp

The RdRp of SARS-CoV-2 represents an ideal target for antiviral drugs, due to its vital role in virus replication and the absence of an enzymatic counterpart in the host cell. Importantly, inhibitors of viral polymerases represent the cornerstone of antiviral agents [30]. Most of the approved antiviral drugs, including HIV and HCV, belong to this class. Inhibitors of viral polymerases are classified into two main categories, based on their mode of action and structure. Nucleoside inhibitors (NIs) act at the substrate site, while the non-nucleoside inhibitors (NNIs) interact with allosteric binding sites. Based on the previous evidences and achievements these approaches might lead to strategies that effectively control SARS-CoV-2 infection. In fact, Remdesivir was recently granted EUA [31] for the treatment of SARS-CoV-2, following the encouraging results from the National Institute of Allergy and Infectious Diseases (NIAID) and Gilead clinical trials and from the compassionate use programs [32-34]. GS441524 was used in this study representing RdRp inhibitors, as it is the parent drug of Remdesivir [35].

2.1.2 Proteases (3-Cysteine-Like Protease, 3CLpro) and (Papain-Like Protease, PLpro)

The targeting of proteases represents an essential and solid route for antiviral drug discovery as demonstrated by the therapeutic success of HIV and HCV proteases inhibitors. Homologous proteins of related CoVs, such as, SARS and MERS-CoVs have represented the main targets for the search of potent and selective inhibitors of these viruses [36, 37].

SARS-CoV-2's genome encodes two large polyproteins, pp1a and pp1ab, as in like most of the Coronaviridae genome. The polyproteins are cleaved and transformed by the main proteases (3CLpro) and PLpro. Both proteins are crucial for virus replication and controlling the host cell response; therefore, they are considered as the key targets in the development of antiviral drugs. 3CL pro-1 is one of the most potent inhibitors of 3CL-pro target site of SARS-CoV-2 [38].

2.1.3 Histone Deacetylase (HDAC, 2VQQ)

HDACs act by deacetylation of amino terminal lysine residues of histone and non-histone proteins and are known for their great roles in regulating the transcriptional activity of targeted genes cellular homeostasis and other fundamental cellular processes, such as differentiation, progression and apoptosis [39]. Several HDAC inhibitors could bind to human angiotensin-converting enzyme 2 (ACE2) on the cell surface, which in turn resulted in overall structural changes of ACE2. Since SARS-CoV-2 recognizes human ACE2 receptor by its spike protein during viral infection, such alternations inhibited the ACE2-S protein binding and prevented host cell entry of SARS-CoV-2. They have found that four inhibitors, such as, Panobinostat, Givinostat hydrochloride monohydrate, CAY10603 and Sirtinol are noticeably effective [40]. Accordingly, Panobinostat is used in this study for comparison.

2.1.4 Viral Neuraminidases

Viral Neuraminidases are glycoside hydrolase enzymes found on the surface of influenza viruses that enables the virus to be released from the host cell. Neuraminidases are enzymes that cleave sialic acid (also called neuraminic acid) group from glycoproteins. Neuraminidase inhibitors are antiviral agents that inhibit influenza viral neuraminidase activity and are of major importance in the control of influenza [41, 42]. Oseltamivir acid is used in this study as a representative drug for comparison.

2.2 The Corona Virus Structural Proteins

The coronavirus structural proteins that form virus particles are: spiculate S-glycoprotein, membrane, envelope, and nucleocapsid proteins. These proteins are considered to be an interesting pharmacological target that merits further attention due to their critical function in viral RNA transcription and replication

[43]. The spike (S) glycoprotein is a structural transmembrane protein situated on the outer envelope of the virion. As has been observed for other viruses of the Coronaviridae family [44], the spike glycoprotein mediates the virus entry by interacting with specific host-receptors located on the surface of cells. Host−guest recognition is a vital process, which determines the specificity and selectivity of the virus tropism and pathogenesis [45, 46].

3. Host-Based Draggable Targets

3.1 Angiotensin-converting Enzyme 2 (ACE2) Receptor

Coronavirus inter the human body mainly by the virus binding to the cell surface of different host receptors. In SARS-CoV-2, ACE2 has been confirmed as the main target for the virus [47]. Accordingly, inhibition or modulation of ACE2 receptor represents one of the proposed host-based approaches for treatment of SARS-CoV-2 [48]. Olmesartan is an angiotensin II receptor antagonist and works by blocking the effects of angiotensin II and is used as a representative of this class of drugs [49].

3.2 Transmembrane Serine Protease 2 (TMPRSS2)

The host cell surface enzyme TMPRSS2 activates S protein present in the highly pathogenic human coronaviruses SARS-CoV and MERS-CoV. Human TMPRSS2 is expressed in the epithelia of the gastrointestinal, urogenital, and respiratory tracts [50, 51].

Recent research has confirmed that SARS-CoV-2 entry is facilitated by TMPRSS2 and the viral infection is decreased by the use of the protease inhibitor Camostat [50].

Covid-19 may find a potential therapy among different repurposed drugs with inhibitory activity against TMPRSS2. Nowadays, only Camostat has shown *in vitro* activity against SARS-CoV-2. Camostat is used in this study for comparison.

4. Experimental Work

Materials: GS441524 is the active metabolite of Remdesivir; Oseltamivir acid is the active metabolite of Oseltamivir (Tamiflu); Camostat, Trans-membrane Protease Serine 2 inhibitor (TMPRSS2); Olmesartan is an ACE2 blocker; 3CLpro-1 is an inhibitor of 3-Cysteine-Like protease (3CL pro), the main protease of Coronavirus; Panobinostat is an HDAC inhibitor; Levofloxacin and Moxifloxacin are the respiratory Fluoroquinolones and the suggested drugs for this protocol. Chemical structures of the investigated drugs: Moxifloxacin, Levofloxacin, Panobinostat, Camostat, 3CLpro-1, GS441524, Oseltamivir acid and Olmesartan are illustrated on Figure 1.

Al-Akidi Therapeutic Protocol (A-TP) for COVID-19:

- Levofloxacin (500 mg) infusion, twice daily for 7-10 days.
- Aspirin coated tablets (100 mg) once daily.
- Ceftriaxone 1G (IV administration) should be given once a day in very severe cases of inflammation. In case of hard breathing, one ampoule of Dexamethasone could be used once daily for few days (Optional).
- Vitamin D3 (10000 iu) once-twice daily for 14-21 days.
- Zinc tablets (50 mg) once daily for 14-21 days. Scientific

Scientific support for the use of this suggested protocol (A-TP)

A. Levofloxacin and Moxifloxacin are potent antibacterial agents and broad-spectrum antibiotics and are called (Respiratory Fluoroquinolones) that are used for several severe types of respiratory infections including, Pneumonia and Community-Acquired Pneumonia (CAP) [9, 10].

B. Levofloxacin and Moxifloxacin are transported to the lung tissue in an approximate concentration of two-to-five fold higher than plasma concentration [52].

C. Levofloxacin can induce a dose-related reduction in the production of the pro-inflammatory cytokines [53-55].

D. Levofloxacin is previously used by the WHO for TB [56, 57].
E. Levofloxacin has already proved to have antiviral activity against a number of viruses [58-61].
F. Levofloxacin is a safe drug with a high margin of safety for the periods that is recommended to be used in this protocol.
G. Based on our computer-aided drug design calculations of molecular properties and molecular docking, Levofloxacin and Moxifloxacin showed good binding affinity to the ACE2 receptor with binding scores of 54.15 and 64.04 respectively, when compared with Olmesartan binding score of 81.97 and also showed reasonable binding to Coronavirus genomic subunits (Table 1).
H. Levofloxacin and Moxifloxacin have also showed reasonable binding affinities to viral enzymes (Table 1), particularly, 1DMP (main protease), 2Q6F (3CL-Like protease), 1YVF (RdRp), 3NZI (TAMPRSS2) and 1A4Q (Neuraminidase).
I. Aspirin is a well-known anticoagulant drug.
J. Ceftriaxone is a potent broad spectrum antibacterial agent that may be useful for many types of microbial infections including, pneumonia and urinary tract infection.

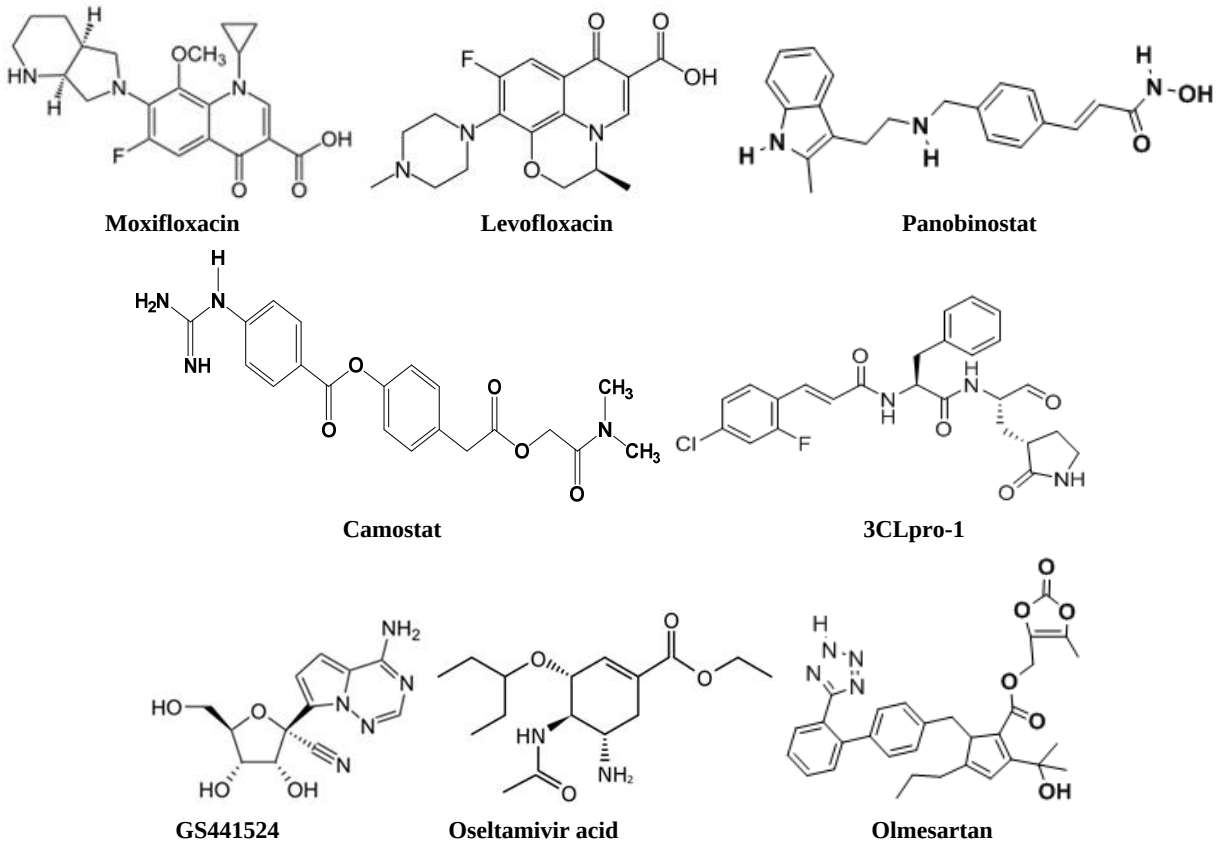


Fig. 1 Chemical structures of the investigated drugs.

5. Results and Discussion

5.1 Molecular Docking

This protocol is based on molecular docking of Levofloxacin and Moxifloxacin in comparison with FDA-Approved antiviral agents, HDACs and ACE2 receptor site, using GOLD suite V.5.6.2. The chemical structures of these drugs and their smiles notation were obtained from chemoffice draw version 12 (Figure 1). The structures of the receptors were retrieved from

Protein Data Bank (PDB). These types are; RNA dependent RNA polymerase, RdRp; PDB (1YVF), Angiotensin converting enzyme 2, PDB (ID: 1R4I), Main Protease PDB (ID: 1DMP), Trans-Membrane Protease Serine 2 (TMPRSS2, HTRA1 Human, 3NZI), Human coronavirus replicase polyproteins, 1a, 1ab, PDB (ID: 2GZ8), Histone Deacetylase PDB (2VQQ), Neuraminidase, PDB (1A4Q), 3C-Like protease, 2Q6F.

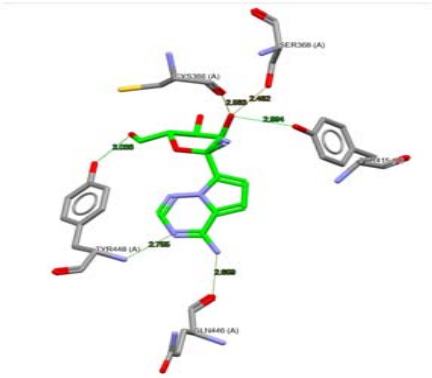
Table 1 Molecular docking of the investigated FDA-Approved Drugs on critically important enzymes and ACE2 receptor site and the amino acids that are involved in interaction.

Target site	Drug	Amino acids involved in interaction	Docking scores (Kcal/Mol)
1DMP *Main Proteases*	3CL pro-1	iLe50 A (2.765 °A), Arg8 A (2.675 °A), Thr80 A (2.984 °A)	77.41
	Levofloxacin	Asp29 A (2.862 °A), Asp30 (3.035 °A), Arg8 B (2.785 °A)	54.37
	Moxifloxacin	Asp29 A (3.017 °A), Asp30 A (2.827 °A)	64.07
2GZ8 *Human coronavirus Replicase polyproteins 1a, 1ab*	3CL pro-1	Gly143 A (2.80 °A), Cys145 A (3.021 °A), Ser144 A (2.80 °A), Ser144 A (2.704 °A)	86.36
	Levofloxacin	Cys44 A (2.68 °A), Cys44 A (3.05 °A), Tyr54 A (2.614 °A), Asp187 A (2.83 °A)	61.10
	Moxifloxacin	His41 A (2.709 °A), Cys44 A (2.588 °A)	60.50
2Q6F 3C-Like protease	3CL pro-1	Gly26 A (2.832 °A), Glu164 A (2.987 °A), Glu187 A (3.062 °A)	82.89
	Levofloxacin	His41 A (2.904 °A), Glu164 A (3.04 °A)	62.38
	Moxifloxacin	His41 A (2.864 °A), Glu164 A (3.021 °A)	64.30
1YVF *RNA dependent RNA polymerase*	GS441524	Cys366 A (2.563 °A), Ser368 A (2.482 °A), Tyr415 A (2.994 °A), Gln446 A (2.659 °A), Tyr448 A (2.755 °A), Tyr 448 A (3.036 °A)	46.68
	Levofloxacin	Ser556 A (2.866 °A)	23.81
	Moxifloxacin	Met414 A (2.387 °A), Tyr448 A (2.741 °A) Gly449 A (2.887 °A), Ser556 A (2.735 °A)	28.72
3NZI *Trans-Membrane Protease Serine 2 (TMPRSS2*)	Camostat	Tyr325 A (3.006 °A), Lys346 A (3.064 °A)	61.54
	Levofloxacin	Ser284 A (2.926 °A), Ser287 A (2.654 °A), Ser287 A (2.946 °A), Tyr325 A (2.931 °A)	47.14
	Moxifloxacin	Ser203 A (2.40 °A), Gly326 A (2.730 °A)	45.84
1A4Q *Neuramidase*	Oseltamivir	Arg149 A (2.979 °A), Arg291 A (2.857 °A), Arg291 A (2.964 °A), Arg373 A (3.067 °A)	55.47
	Levofloxacin	Asn293 A (2.781°A), Arg344 A (2.935°A), Arg344 A (2.994 °A)	36.78
	Moxifloxacin	Arg291 A (2.950 °A), Arg291 A (2.780 °A), Asn293 A (2.794 °A), Asn293 A (2.968 °A)	46.25
2VQQ *HDACs*	Panobinostat	His159 A (2.584°A), His158 A (2.782 °A), His158 A (2.573 °A), Asp196 A (2.919 °A)	82.82
	Levofloxacin	Thr79 B (2.979 °A)	64.60
	Moxifloxacin	Try170 B (2.849 °A)	77.92
1R4L *ACE2*	Olmesartan	Cys361 A (3.022°A), (2.597 °A), Lys363 A (2.720 °A), (2.951 °A), Thr371 A (2.888°A), (2.708 °A)	81.97
	Levofloxacin	Glu406 A (2.727 °A), Thr445 A (3.001°A)	54.15
	Moxifloxacin	Arg518 A (2.597 °A), (2.998 °A)	64.04

The best docking scores for all cases has been recorded. RdRp (1YVF) = RNA dependent RNA polymerase, 2VQQ = Histone Deacetylase; ACE2 (1R4L) = Angiotensin Converting Enzyme Type 2, Trans-membrane protease serine 2 (TMPRSS2), 2GZ8 = Human SARs coronavirus replicase polyproteins, 1DMP = Protease of human immunodeficiency virus type 1 group M Subtype B. 3C-Like Protease of coronavirus, 3CL pro-1 inhibitor of 3C-Like protease. 1A4Q = Neuramidase.

All the investigated drugs were docked on several critically important viral enzymes, HDACs and ACE2 receptor, and the docking scores are listed on (Table 1). To the best of the author's knowledge, neither Levofloxacin nor Moxifloxacin proved to inhibit any of the viral enzymes listed in this study. The docking scores showed very interesting results that may indicate binding affinity of Levofloxacin and Moxifloxacin to ACE2 and possibly interact to this receptor on a certain binding site (Table 1), in comparison with Olmesartan, which is an ACE2 blocker [49]. Levofloxacin and Moxifloxacin interaction to ACE2 (1R4L) were shown to be on different amino acid residues (Table 1). Levofloxacin binding was as follows; C3-OH of COOH to Glu406-carbonyl (2.727 ^{o}A) and C4-carbonyl to Thr445-OH (3.001 ^{o}A). While binding of Moxifloxacin was as follows: C3-carbonyl to Arg518-NH2 (2.597 ^{o}A) and C4-carbonyl to Arg518-NH2 (2.998 ^{o}A). This interaction may refer to the possibility of blocking this receptor and protect the lung from Coronavirus, as there are obvious interactions with this receptor with reasonable docking scores on certain amino acids (Table 1).

Docking of GS441524 on RdRp, 1YVF showed the binding affinity on the active site of the enzyme with a binding score of 46.68, as it is a known inhibitor (Figure 2a). The interaction of GS441524 to 1YVF was as follows; Tyr448-NH to N = CH (2.755 ^{o}A), Tyr448-OH to OH (3.036 ^{o}A), Tyr415-OH to OH (2.994 ^{o}A), Gln446-COOH to NH2 (2.659 ^{o}A), Ser368-OH to OH (2.482 ^{o}A), Cys366-carbonyl to OH (2.563 ^{o}A) (Figure 2a). Levofloxacin and Moxifloxacin recorded higher docking scores (23.81 and 25.72 respectively) than that of GS441524 (46.68), which may indicate that they are less active than GS441524 (Table 1). Interaction of Levofloxacin on RdRp (1YVF) was as follows; Ser556-OH to C3-carbonyl (2.866 ^{o}A). While, Moxifloxacin binding was as follows: Ser556-OH to C3 carbonyl) (2.735 ^{o}A), Gly449-NH to C4-carbonyl (2.887 ^{o}A), Tyr448-NH to C4-carbonyl (2.741 ^{o}A), Met414-S-CH3 to F (2.387 ^{o}A) (Figure 2b).



(a)

Fig. 2a Interaction of GS441524 to 1YVF (RdRp).



(b)

Fig. 2b: Interaction of Moxifloxacin to 1YVF.

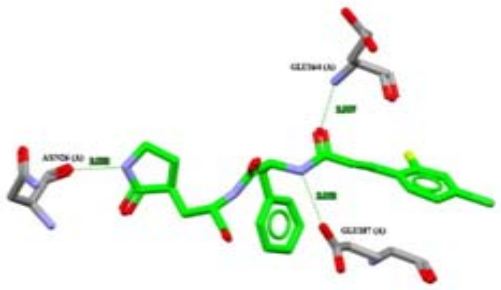
Moxifloxacin and GS441524 interacted on different amino acids on the surface of the enzyme, except that they both interacted on Tyr448. There is an obvious binding of Moxifloxacin with 1YVF, which may refer to the possibility of inhibiting or blocking this enzyme by interacting with a binding site very close to the active site. Levofloxacin binds to Ser556 only, which is also the site of interaction of Moxifloxacin (Table 1). Such interactions may refer to a situation of an umbrella-like binding to the surface of the enzyme very close to the active site of RdRp, 1YVF [62].

3CLpro-1 is an inhibitor of 3C-Like protease, 2Q6F with a binding score of 65.56. 3CL-pro-1 NH interacts with Gly26 (2.832 ^{o}A), carbonyl with Glu164 (2.987 ^{o}A) and NH with Glu187 (3.062 ^{o}A) (Figure 3a). Levofloxacin and Moxifloxacin showed binding

scores of 53.42 and 55.63 respectively (Table 1), which are very close to that of the inhibitor. Levofloxacin C3-OH binds to His41-NH (2.904 ^{o}A) and Glu164 (3.04 ^{o}A). Moreover, Moxifloxacin binds to His41 A (2.864 ^{o}A) and Glu164 A (3.021 ^{o}A).

This shows that Levofloxacin, Moxifloxacin and 3CLpro-1 bind on Glu164, and this may refer to the great possibility of inhibiting or blocking the active site of 3C-Like protease by both Moxifloxacin and Levofloxacin. Moxifloxacin and 3CLpro-1 bind to two similar amino acids, His26 A and Glu164 (Figure 3a and 3b). 3CL-pro-1 and Levofloxacin bind with the same amino acid, Glu164.

3CL-pro-1 showed binding to Replicase polyproteins, 2GZ8, with binding score of 86.36 and interacts with the following amino acids; 3CLpro-1 NH with Ser144-OH (2.704 ^{o}A), carbonyl with Ser144-OH (2.80 ^{o}A), and carbonyl with Cys145 NH (3.021 ^{o}A) and Gly143 A (2.80 ^{o}A) (Figure 4a). Levofloxacin recorded a binding score of 61.10 and interacted with the amino acids; C3-OH with Asp187-carbonyl (2.830 ^{o}A), C3-OH with Tyr54-OH (2.614 ^{o}A), C3-carbonyl with Cys44-SH (2.68 ^{o}A) and C3-carbonyl with Cys44 NH (3.05 ^{o}A) (Figure 4b). Moxifloxacin recorded a binding score of 60.50 and interacted with His41 A (2.709 ^{o}A) and Cys44 A (2.588 ^{o}A). The binding scores of Levofloxacin and Moxifloxacin were very close, which may refer to a similar binding affinity to 2GZ8 (Table 1). Levofloxacin binds to three amino acid residues that may block or disturb the activity of this enzyme, as it also showed reasonable binding score.



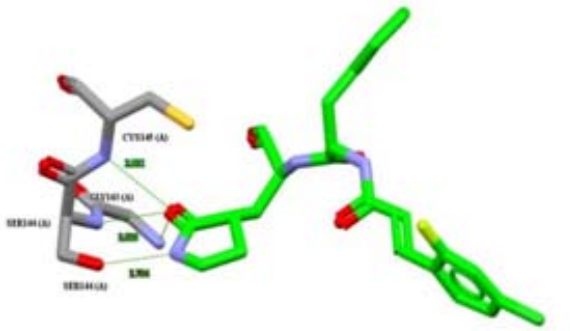
(a)

Fig. 3a Interaction of 3CL pro-1 with 2Q6F.



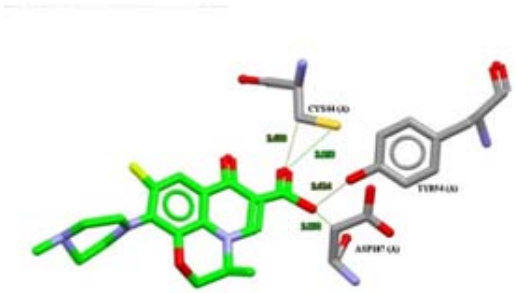
(b)

Fig. 3b Interaction of Moxifloxacin with 2Q6F.



(a)

Fig. 4a Interaction of 3CL pro-1 with 2GZ8.



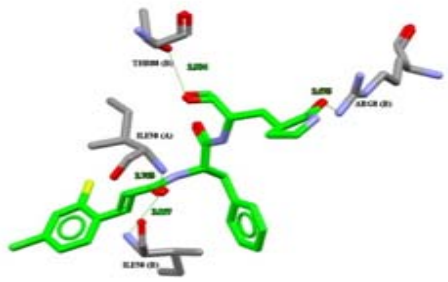
(b)

Fig. 4b Interaction of Levofloxacin with 2GZ8.

Moreover, 3CL pro-1 interacted with 1DMP on the following amino acids; carbonyl with iLe50-NH (A) (2.765 ^{o}A), OH with Thr80 A-OH (2.934 ^{o}A), and carbonyl with Arg8-NH A (2.675 ^{o}A), with binding score of 77.41 (Figure 5a). Levofloxacin binding with 1DMP on three different amino acids: C3-OH with Asp29 A-carbonyl (2.862 ^{o}A), C3-OH with Asp30 A-OH (3.035 ^{o}A) and C4-carbonyl with Arg8 A-N (2.785 ^{o}A), with docking score of 54.37 (Figure 5b).

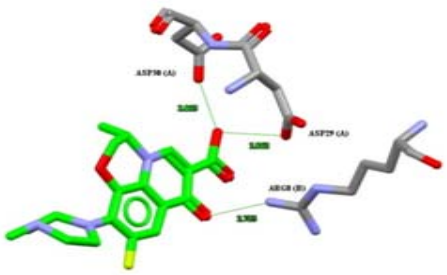
Levofloxacin and Moxifloxacin recorded slightly higher docking scores on Neuraminidase, 1A4Q (36.78 and 46.25 respectively) than Oseltamivir acid (55.47) (Table 1). Carbonyl group of Oseltamivir acid binds to Arg149-NH (A) (2.979 ^{o}A), carbonyl of COOH with Arg291-NH(A), (2.857 ^{o}A) and carbonyl of COOH with Arg373-NH (A) (3.067 ^{o}A) (Figure 6a). While, Moxifloxacin showed the following interactions: C3-OH with Asn293-NH (A) (2.794 ^{o}A), C3-OH with Asn293-carbonyl (A) (2.968 ^{o}A) C3-OH with Arg291-NH (A) (2.780 ^{o}A) and C4-carbonyl with Arg291-NH (A) (2.950 ^{o}A). Levofloxacin binding was on; C3-OH with Arg344-NH (A) (2.935 ^{o}A), C3-carbonyl with Arg344-NH (A) (2.994 ^{o}A) and C4-carbonyl with Asn203-NH (A), (2.781 ^{o}A). A similar possibility of inhibiting or blocking the catalytic activity of Neuraminidase by Moxifloxacin was observed, as it binds with Arg291, which is also the binding site of Oseltamivir acid on 1A4Q (Figure 6a and 6b).

Camostat is a serine protease inhibitor (Trans-Membrane Serine 2, TMPRSS2) and is used as a standard inhibitor of the enzyme, 3NZI, with a docking score of 61.54. Camostat-ester interacts to Tyr325-OH (A) (3.006 ^{o}A) and Lys346-NH (A) (3.064 ^{o}A) (Figure 7a). Levofloxacin and Moxifloxacin were also docked on 3NZI and recorded docking scores of 47.14 and 45.84, respectively. Levofloxacin-C3-OH interacts with Ser284-carbonyl (2.926 ^{o}A) and C3-OH with Tyr325-carbonyl (A), (2.931 ^{o}A). C3-carbonyl of COOH binds to Ser287-OH (A) (2.654 ^{o}A) and C3-carbonyl with Ser287-NH (A) (2.946 ^{o}A) (Figure 7b). While, Moxifloxacin interaction with 3NZI was as follows; C3-OH of COOH to Ser203-carbonyl (2.40 ^{o}A). C4-carbonyl interacts to Gly326-NH (2.730 ^{o}A). There is obvious evidence that Levofloxacin may inhibit or block the catalytic activity of this enzyme, as it interacts with the same amino acid residue (Tyr325) as Camostat (Figure 7b).



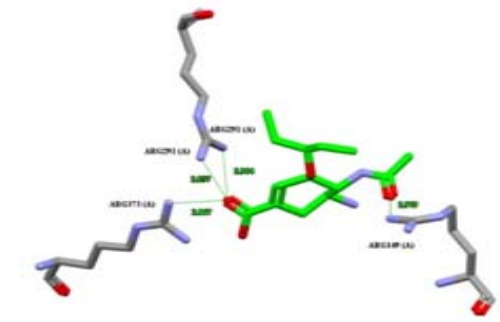
(a)

Fig. 5a Interaction of 3CL pro-1 with 1DMP.



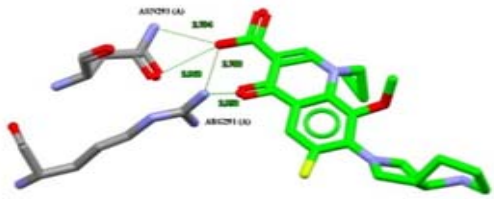
(b)

Fig. 5b Interaction of Levofloxacin with 1DMP.



(a)

Fig. 6a Interaction of Oseltamivir acid with 1A4Q.



(b)

Fig. 6b Interaction of Moxifloxacin with 1A4Q.



(a)

Fig. 7a Interaction of Camostat with 3NZI.



(b)

Fig. 7b Interaction of Levofloxacin with 3NZI.

Panobinostat is a histone deacetylase inhibitor recorded docking score of 82.82 on HDAC, 2VQQ, while, Levofloxacin and Moxifloxacin recorded docking scores of 64.60 and 77.92 respectively. Panobinostat interacted with His158 A (2.782 ^{o}A), His158 A (2.573 ^{o}A), His159 A (2.584 ^{o}A), and Asp196 A (2.919 ^{o}A). Levofloxacin and Moxifloxacin interacted only on Tyr79 A (2.979 ^{o}A) and Tyr170 A (2.849 ^{o}A), respectively (Table 1). Levofloxacin recorded reasonable binding affinities and docking scores on 1DMP, 3NZI and 2GZ8. Moxifloxacin showed interaction and comparable docking scores on 1YVF, 2Q6F and 1A4Q. Based on those binding affinities and interaction to similar amino acids of certain viral enzymes, the results revealed that Levofloxacin and Moxifloxacin may inhibit or disturb the activity of one or more of those viral enzymes, and consequently has a pronounced antiviral activity, particularly against Coronavirus. However, Levofloxacin is superior in this protocol over Moxifloxacin, due to its high excretion, as unchanged (approximately 83% of the initial dose) through the kidneys [30], while Moxifloxacin is only 20% of the initial dose is excreted unchanged through the kidneys [63]. It is important to know that Coronavirus attacks the lungs, kidneys and heart muscles and arteries. So, it is an extra advantage to manage coronavirus in the kidneys too, and that is why Levofloxacin is superior and highly recommended to be used to treat Covid-19. Levofloxacin has previously shown to have antiviral activities on certain viruses [57-60].

5.2 Validity of the Docking Study

Validation is the essential part of docking studies and for the validation purposes a 3D model of the ligand was prepared and energy minimized. Then, the ligand was docked and the docking results were compared with those of the FDA-Approved drugs. These steps were performed to determine whether the docked ligands bound with the same amino-acid

residues, as they bound in the crystal structure of the enzyme, or bound differently to the enzyme. The application of this molecular docking approach on known molecules, such as, the antiviral agents, histone deacetylase inhibitors and angiotensin converting enzyme blockers was validated for its reliability as an important source of database screening, prediction and selection of the most potent compound in comparison with their docking scores. It is a relative comparison for all drugs and candidates recorded on the same bases.

6. Conclusion and Recommendation

In conclusion, the expected results of using such protocol are the followings: Reduce the Cytokine storm, shortening the period for hospitalization. Levofloxacin is potentially managing Covid-19, in terms of all required activities, including treating infection, reducing the Cytokine storm and possibly inhibiting one of the critical viral enzymes, and act as an antiviral agent, as previously proved with few types of viruses, decrease and control of body temperature, less frequent use of ventilators and intensive care unit (ICU), supplemental Oxygen will not be needed or may be used for a short period of time, cost-effective protocol. The use of this A-TP is highly recommended, as it serves the full requirements for excellent and potential therapy for the severe infections associated with Covid-19. Vitamin D3 and Zinc have already known for their antiviral activities and great role in Covid-19. Levofloxacin and Moxifloxacin are already considered as Respiratory Fluoroquinolone, based on their activity on severe infections of the respiratory system. Levofloxacin and Moxifloxacin may interact with one or more of the investigated viral enzymes and inhibit their action or bind to certain binding sites on the surface of those enzymes, and in either case block or disturb their catalytic activities. Levofloxacin is superior in this protocol over Moxifloxacin, as previously explained. This protocol will definitely treat and manage Covid-19 and reduce the mortality rate and save lives. The use of this A-TP is highly recommended, as it serves the full requirements for excellent and potential therapy for the severe infections associated with Covid-19.

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