A Comprehensive Update of Anti-COVID-19 Activity of Heterocyclic Compounds

Muhammad Shahid Nazir¹, Matloob Ahmad¹, Sana Aslam², Ayesha Rafiq¹, Sami A Al-Hussain³, Magdi EA Zaki⁴

¹Department of Chemistry, Government College University, Faisalabad, Pakistan; ²Department of Chemistry, Government College Women University, Faisalabad, Pakistan; ³Department of Chemistry, Faculty of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia.

Correspondence: Matloob Ahmad; Magdi EA Zaki, Email Matloob.Ahmad@gcuf.edu.pk; mezaki@imamu.edu.sa

Abstract: The Coronavirus disease 2019 (COVID-19) pandemic is one of the most considerable health problems across the world. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the major causative agent of COVID-19. The severe symptoms of this deadly disease include shortness of breath, fever, cough, loss of smell, and a broad spectrum of other health issues such as diarrhea, pneumonia, bronchitis, septic shock, and multiple organ failure. Currently, there are no medications available for coronavirus patients, except symptom-relieving drugs. Therefore, SARS-CoV-2 requires the development of effective drugs and specific treatments. Heterocycles are important constituents of more than 85% of the physiologically active pharmaceutical drugs on the market now. Several FDA-approved drugs have been reported including molnupiravir, remdesivir, ritonavir, oseltamivir, favipiravir, chloroquine, and hydroxychloroquine for the cure of COVID-19. In this study, we discuss potent anti-SARS-CoV-2 heterocyclic compounds that have been synthesized over the past few years. These compounds included; indole, piperidine, pyrazine, pyrimidine, pyrrole, piperazine, quinazoline, pyrimidine, pyrazole, azafluorene, imidazole, thiadiazole, triazole, coumarin, chromene, and benzodioxole. Both in vitro and in silico studies were performed to determine the potential of these heterocyclic compounds in the fight against various SARS-CoV-2 proteins.

Keywords: COVID-19, SARS-CoV-2, heterocyclic nucleus, in vitro, in silico, molecular docking studies

Introduction

Coronavirus disease 2019 (COVID-19) or 2019-nCoV, a fatal respiratory illness, was first identified in late December 2019 in Wuhan, China, and spread rapidly across 200 countries around the world.¹ On March 11, 2020, the World Health Organization (WHO) declared COVID-19 as a pandemic.² The WHO renamed the virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on February 20, 2020.³ The Beta, Delta, and Omicron variants started to emerge in Africa in May 2020, in India by October 2020, and in various other regions in November 2021, causing many concerns worldwide.⁴ The coronavirus, is highly contagious in the air, can transmit from one person to another through respiratory aerosols and droplets.⁵,⁶ The breathing problems caused by COVID-19 can be fatal in some cases, resulting in a global public health emergency.⁷ Common symptoms of the disease include; shortness of breath (19%), fever (88%), loss of smell (15–30%), and cough (68%), as well as diarrhea, pneumonia, bronchitis, septic shock, multiple organ failure, and acute respiratory distress syndrome.⁸,⁹

The causative agent of COVID-19 is a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).¹⁰ The SARS-CoV-2 is an enveloped, crown-shaped, single-stranded positive RNA virus that belongs to the genus β-coronavirus, subgenus Sarbecovirus, and family Coronaviridae.¹¹ The SARS-CoV-2 is the seventh type of human coronavirus (HCoVs) after SARS-CoV, HCoV-229E, HCoV-HKU1, HCoV-NL63, HCoV-OC43, and middle east respiratory syndrome (MERS-CoV).¹² The SARS-CoV-2, MERS-CoV, and SARS-CoV viruses cause severe acute respiratory infections, whereas the HCoV-HKU1, HCoV-229E, HCoV-OC43, and HCoV-NL63 viruses affect with milder symptoms. The genome sequence of SARS-CoV-2 is...
88% similarity to SARS-like bat coronaviruses (bat-SL-CoVZXC21 and bat-SL-CoVZC45), about 79% similar to SARS-CoV, and has about 50% similarity to MERS-CoV. The SARS-CoV-2 genome contains approximately 30,000 nucleotides in the sequence of 5′-replicase-S-E-M-N-3′. The SARS-CoV-2 genome has two major overlapping open reading frames (ORF1a/b) that encode four structural proteins (sps): spike (S), envelope (E), membrane (M), and nucleocapsid (N), and nonstructural proteins (nsps), as well as accessory factors. The nsps1–nsps16 are encoded proteins that are responsible for viral transcription and replication, such as papain-like protease (PLₚₚₒ; nsp₃), RNA-dependent RNA polymerase (RdRp; nsp₁₂), 3-chymotrypsin-like protease (3CLₚₚₒ; nsp₅), also called the main protease (Mₚₚₒ), and RNA helicase (nsp₁₃).\(^\text{16–18}\)

The spike protein is responsible for SARS-CoV-2 penetration into human ACE2-expressing cells. SARS-CoV-2 spike protein binds to angiotensin-converting enzyme 2 (ACE2) on the surface of human lung epithelial cells, allowing the virus to enter the host cells via the ACE2 receptor.\(^\text{19}\) The cells with greater levels of ACE2 expression may be considered as potent SARS-CoV-2 infection sites.\(^\text{20}\) The increased ACE2 expression causes a rise in SARS-CoV-2 viral infection.\(^\text{21}\) COVID-19 disease may be treated effectively by targeting ACE2 expression.\(^\text{22}\) Preventing SARS-CoV-2 entry into the host cell via ACE2 receptor sites may be an effective method of combating COVID-19.\(^\text{23}\)

RNA-dependent RNA polymerase (RdRp) is a nonstructural protein that catalyzes the synthesis of the viral genome and thus plays a pivotal role in the replication and transcription of SARS-CoV-2.\(^\text{24}\) Viral RNA synthesis also requires the presence of two additional proteins, nsp7 and nsp8, which are thought to have a primase or 3′-terminal adenyl-yl-transferase function.\(^\text{25,26}\)

The chymotrypsin-like cysteine protease (3CLₚₚₒ), also called Mₚₚₒ, belongs to the 16 nonstructural proteins of SARS-CoV-2 and is an essential enzyme that plays a valuable role in viral RNA replication and transcription of coronaviruses (CoVs).\(^\text{27}\) The enzyme encodes the translated polyproteins from coronaviruses (CoVs) RNA along with papain-like proteases (PLₚₚₒ).\(^\text{28}\)
In structural identification, 3CL\textsuperscript{pro} comprises three domains: the chymotrypsin-like domain (domain I, residues 8–101), picornavirus 3C protease-like domain (domain II, residues 102–184), and a globular cluster produced via five helices (domain III, residues 201–303). The 3CL\textsuperscript{pro} active site (substrate-binding site), made up of four subsites (S1, S2, S3, and S4), is situated between domains I and II on β barrels.\textsuperscript{29} Consequently, two distinct potent 3CL\textsuperscript{pro} inhibitors, including both non-peptidic inhibitors and peptidomimetic inhibitors, have been reported to fight a rapid rise in the COVID-19 pandemic.\textsuperscript{30–33}

The effects of the SARS-CoV-2 epidemic were profound regarding the world’s economy as well as on public health. Consequently, the World Health Organization (WHO) declared a worldwide health emergency in order to coordinate scientific and medical efforts to develop a treatment for patients as soon as possible.\textsuperscript{34} There is a dire need for the discovery of new anti-SARS-CoV-2 inhibitors to reduce the severity of illness in COVID-19 affectees and to target the various types of SARS-CoV-2 proteins because there are no effective medicines available for the treatment of the coronavirus disease. Several anti-malarial, and herbal medicines have been used as alternative drugs to cure COVID-19.\textsuperscript{35} Computer-aided drug design methods have been successfully implemented in anti-COVID-19 analyses to find potential inhibitors rapidly, in repurposing drugs, and in explaining the action mechanism of the therapeutic agents against SARS-CoV-2 based on the crystal structures of SARS-CoV or SARS-CoV2 M\textsuperscript{pro}.\textsuperscript{36} Drug repurposing is the quickest option to propose effective treatments for the ongoing COVID-19 pandemic.\textsuperscript{37} Several FDA-approved anti-retroviral drugs are already in use, including, oseltamivir,\textsuperscript{38} favipiravir,\textsuperscript{39} ribavirin,\textsuperscript{40} lopinavir,\textsuperscript{41} chloroquine,\textsuperscript{42} and hydroxychloroquine,\textsuperscript{43} ensitrelvir,\textsuperscript{44} cordycepin,\textsuperscript{45} didanosine,\textsuperscript{46} teriflu- 

In vitro Studies Against COVID-19

Current Advances in Heterocycles as Anti-COVID-19 Agents

Indole Derivatives as Anti-SARS-CoV-2 Agents

Barakat et al.\textsuperscript{50} synthesized spirooxindole-based phenylsulfonyl hybrids as potent SARS-CoV-2 inhibitors and screened their anti-COVID-19 activity using a colorimetric crystal violet assay. Among them, the combination of compound 1 with compound 3 and the combination of compound 2 with compound 3 was found to be more potent and active. These compounds showed the most potent activity against SARS-CoV-2 in Vero E6 cells, with half-maximal inhibitory concentration (IC\textsubscript{50}) values of 3.657 and 3.275µM and with a selectivity index of 698.4 and 3612.8, respectively. Remdesivir was used as the standard drug with an IC\textsubscript{50} value of 6.721µM. Tanaka et al.\textsuperscript{60} prepared umifenovir analogs and tested their anti-SARS-CoV-2 activities using biological assays. From all the derivatives that were examined, compound 4 exhibited the maximum 20% inhibition of the SARS-CoV-2 spike protein and ACE2 receptor expression (Figure 2).
Figure 1 Commercially available drugs as COVID-19 Inhibitors.
Piperidine Derivatives as Anti-SARS-CoV-2 Agents
A novel series of aspirin-curcumin mimic conjugates was synthesized by Srour et al., and their anti-SARS-CoV-2 properties were evaluated using the MTT-bio assay. The compounds 5 and 6 bearing a thiénylidene ring system showed excellent inhibitory activity against SARS-CoV-2 with IC$_{50}$ values of 8.828 and 3.316µM, CC$_{50}$ values of 206.2 and 416.5µM, and selective index values of 233.6 and 125.6, respectively, as compared to the standard drugs hydroxychloroquine and chloroquine (IC$_{50}$ = 36.92 and 24.98µM, CC$_{50}$ = 356.4 and 377.7µM and SI= 9.7 and 15.1). Yang et al., reported a number of non-peptide inhibitors with anti-SARS-CoV-2 potential. The results of the surface plasmon resonance experiment showed that, among the 49 tested molecules, compound 6 was bound to SARS-CoV-2 M$_{pro}$. Furthermore, fluorescence resonance energy transfer analysis was carried out and obtained IC$_{50}$ values ranging from 0.68 to 2.05µM. Among them, compounds 7 and 8 showed the best potent activity against SARS-CoV-2 M$_{pro}$ with IC$_{50}$ values of 0.73 and 0.69µM, respectively. These compounds reduced viral replication in Vero E6 cells with half maximal effective concentration (EC$_{50}$) values of 4.98 and 8.52µM, respectively (Figure 3).

Pyrazine Derivatives as Anti-SARS-CoV-2 Agents
Pyrazine conjugates were synthesized and evaluated for their anti-SARS-CoV-2 activity using the MTT assay by Seliem et al.. Among these prepared derivatives, compound 9 exhibited significant activity towards coronavirus (IC$_{50}$= 0.120mM, CC$_{50}$= 0.378 m, SI= 3.150) in comparison to the control drug favipiravir (IC$_{50}$= 0.1382mM, CC$_{50}$= 5.262 mM, SI= 3.808). Rabie, synthesized a novel favipiravir analog (cyanorona-20) and found it was the most potent anti-SARS-CoV-2 agent. This derivative showed excellent inhibitory activity against RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2, with an EC$_{50}$ value of 450nM (Figure 4).

Pyridine Derivatives as Anti-SARS-CoV-2 Agents
Ghosh et al., synthesized 5-chloropyridinyl indole carboxylate hybrids and found that compound 11 was the most effective anti-COVID-19 agent targeting SARS-CoV-2 3CL$_{pro}$ with an IC$_{50}$ value of 250nM. The MTT assay was performed for the in vitro analysis of the synthesized compounds. Compound 11 significantly reduced the cytopathic effect and replication of SARS-CoV-2 in Vero E6 cells (EC$_{50}$= 15μM) as compared with the standard drug (EC$_{50}$=...
This compound also showed a half-maximal cytotoxic concentration (CC_{50} > 100\mu M) against coronavirus 2. A derivative of glycyrrhizin nicotinate, “glycyvir”, was prepared by Fomenko et al., and, its anti-SARS-CoV-2 activity was determined through in vitro analysis. Glycyvir 12 exhibited significant antiviral activity to inhibit the replication of SARS-CoV-2 in Vero E6 cells, with an IC_{50} value in the range of 2–8\mu M, as compared to the reference drug remdesivir (IC_{50} = 1.5–3.3\mu M) (Figure 5).

**Pyrimidine Derivatives as Anti-SARS-CoV-2 Agents**

Abu-Zaied et al., prepared pyrimidine derivatives and their corresponding phosphoramidates, and tested their anti-SARS-CoV-2 activity using MTT and plaque reduction assays. The most active derivatives reported in this study were 13 and 14 against SARS-CoV-2, with IC_{50} values of 14.91 and 12.16\mu M and CC_{50} values of 420.9 and 327.1\mu M, respectively. Cytosine thioglycoside phosphoramidates 13 and 14 was proven the most effective profiles in Vero E6 cells, with 83% and 86% inhibition at 0.25\mu M concentrations respectively. The selective index values of these compounds were 28.2 and 26.9. Pyrimidine thioglycoside derivatives were prepared and evaluated for their anti-SARS-CoV-2 properties through in vitro analysis using MTT assay by the same authors. Among all the derivatives, compounds 15 and 16 showed the best potent activity against SARS-CoV-2, with IC_{50} values of 18.47 and 15.41\mu mol and selectivity index (SI) values of 25.33 and 23.40, respectively. These compounds exhibited cytotoxicity in Vero E6 cells with half maximal cytotoxic concentrations (CC_{50}) values of 467.9 and 360.9\mu M. Harbi et al., prepared novel nanocrystallites of violurate-based Mn(II) and Cu(II) complexes and screened their anti-SARS-CoV-2 activities using plaque reduction and MTT assays. Violuric acid and its Mn(II) and Cu(II) complexes displayed good anti-SARS-CoV-2 properties with CC_{50} values of 43.87, 93.45 and 88.38\mu M, respectively. These compounds inhibited the replication of SARS-CoV-2 in Vero-E6 cells with IC_{50} values of 84.01, 39.58, and 44.86\mu M, respectively. Of these, Mn(II) complex 17 was the most effective against SARS-CoV-2, with an IC_{50} value of 39.58\mu M, whereas the control drug remdesivir had an IC_{50} value of 0.2\mu M (Figure 6).

**Pyrrole Derivative as Anti-SARS-CoV-2 Agent**

A series of peptidomimetic aldehydes was designed and tested through in vitro analysis for their anti-SARS-CoV-2 activity using a fluorescence resonance energy transfer (FRET) protease assay. Among them, compound 18 displayed significant inhibitory activity towards the main protease (3CL\_pro) of SARS-CoV-2, with an IC_{50} value of 0.034\mu M. This compound reduced the replication of SARS-CoV-2 in Vero E6 cells with an EC_{50} value of 0.29\mu M. The half maximal cytotoxic concentration (CC_{50}), and selectivity index (SI) values of these compounds were 808.7, and 2786 respectively (Figure 7).
Piperazine Derivatives as Anti-SARS-CoV-2 Agent
Nishiuchi et al, synthesized neoechinulin B and its derivatives and evaluated their anti-SARS-CoV-2 activities in vitro. Most of the synthesized derivatives exhibited the best anti-SARS-CoV-2 properties. Among the studied analogs, compound 19 demonstrated the highest activity against SARS-CoV-2, with an IC$_{50}$ value of 9.3μM. This compound exhibited low cytotoxicity against Vero E6 cells (CC$_{50}$ >80 μM) (Figure 8).

Oxazole Derivatives as Anti-SARS-CoV-2 Agents
Guo et al, prepared honokiol hybrids containing 3-(5-phenyl-1,3,4-oxadiazol-2-yl)methyl)oxazol-2(3H)-ones and tested their anti-SARS-CoV-2 activity using an MTT bioassay. Of these, compounds 20 and 21 showed remarkable inhibition of SARS-CoV-2 pseudovirus entry into HEK-293T-ACE2$_h$ cells, with IC$_{50}$ values of 29.23 and 9.82μM, respectively, compared to the parental honokiol with IC$_{50}$ values of >50μM. The positive control inhibitor used in this assay suppressed pseudovirus entry into host cells, with an IC$_{50}$ value of 0.035μM. Compound 21 with a TC$_{50}$> 100 μM showed a biologically safe profile for HEK-293T-ACE2$_h$ cells compared to parental honokiol (TC$_{50}$> 48.23μM). From all honokiol derivatives, compound 21 was found to be the most potent against a pseudovirus, with a selective index (SI)
value exceeding 10.18. A competitive enzyme-linked immunosorbent assay (ELISA) was employed to investigate the interactions of the SARS-CoV-2 spike RBD with the ACE2 protein. A SARS-CoV-2 inhibitor with an IC₅₀ value of 2.59nM was utilized as the positive control in the ELISA assay. Compound 21 suppressed the binding of the SARS-CoV-2 spike RBD and ACE2 by 26.08%, 37.16%, and 50.41% at 12.5, 25, and 50µM concentrations. The inhibition rates of parental honokiol were 13.81% and 20.94% at 25 and 50µM concentrations (Figure 9).

Quinazoline Derivatives as Anti-SARS-CoV-2 Agents

A series of 2-benzylaminoquinazolin-4(3H)-one analogues were prepared and evaluated for their anti-SARS-CoV-2 activity using human ether-a-go-go-related gene (hERG), cytotoxicity, cytochrome P450 (CYP) inhibition, microsomal stability, and the plasma protein binding (PPB) assay. Among them, compound 22 demonstrated the most potent activity against SARS-CoV-2 with an IC₅₀ value of 4.2µM, whereas the IC₅₀ values of the standard drugs remdesivir, chloroquine, and lopinavir were 7.6, 9.4, and 16.6µM, respectively. This compound showed lower cytotoxicity in Vero E6 cells with CC₅₀ and selective index (SI) values of 14.3µM and 3.4M, respectively, as compared to the reference drugs (CC₅₀>25µM and SI >3.2, >2.6, and >1.5µM). A novel series of 2-aminoquinazolin-4-(3H)-one hybrids was prepared by Shin et al, and screened for anti-SARS-CoV-2 activity through in vitro studies. An immunofluorescence assay was used to determine the half-maximal inhibitory concentration (IC₅₀) of these derivatives in Vero E6 cells at a 10µM concentration. The cytotoxicity (CC₅₀) was also examined in uninfected Vero E6 cells. Among the reported derivatives, acetylated compounds 23, 24, and 25 showed the best inhibitory activity against SARS-CoV-2 with IC₅₀ values of 0.29, 0.11, and 0.33µM, respectively, as compared to the reference drug remdesivir (IC₅₀ = 3.47µM) (Figure 10).

Quinoline Derivatives as Anti-SARS-CoV-2 Agents

Guevara-Pulido et al designed and synthesized a series of chloroquine analogs and evaluated their anti-SARS-CoV-2 activity using in vitro drug-release assay. From these, 4-(7-chloroquinolin-4-yl)amino)phenol hybrid 26 was less toxic and exhibited a significant therapeutic effect against SARS-CoV-2 main protease (Mₚro) with an IC₅₀ value of 12nM, whereas the parent compound chloroquine had an IC₅₀ value of 27nM. Nizi et al reported two phenylquinoline derivatives as potential inhibitors of SARS-CoV-2 replication, using a colorimetric formazan-based MTS assay. Amongst

Figure 9 Oxazole Derivatives (20 and 21) as anti-SARS-CoV-2 agents.

Figure 10 Quinazoline derivatives (22–25) as anti-SARS-CoV-2 agents.
the studied compounds, Compound 27 bearing two methoxy groups at the C-4 position of the 2-phenylquinoline core, showed significant inhibitory activity against SARS-CoV-2 helicase (nsp13) with an IC50 value of 0.42μM. This compound reduced viral replication in Vero E6 cells with an EC50 value of 8.8μM as compared with the control drug chloroquine, with an EC50 value of 10.9μM. This compound demonstrated cytotoxicity in Vero E6 cells, with a CC50 value greater than 100μM. Zhao et al.,73 tested 101 quinoline hybrids against SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) using cell-based assays. Compounds 28, 29, and 30 inhibited RNA synthesis by the RdRp of SARS-CoV-2, with EC50 values of 1.08, 2.08, and 3.92μM, respectively. Remdesivir was used as the standard drug with an EC50 value of 1.39μM. These compounds showed cytotoxic effects on Vero E6 cells, with CC50 values of 70.79, 72.44, and 79.43μM, respectively. The therapeutic indexes (TI) for these compounds were 65.55, 34.83, and 20.26μM, respectively. Chen et al.,74 investigated chloroxine and hybrids of tanshinone IIA sulfonate sodium (TSS) as potential inhibitors of SARS-CoV-2 PLpro using a fluorescence polarization assay (FPA) and cell-based assay. Amongst them, chloroxine 31 inhibited the binding of SARS-CoV-2 PLpro to ISG15 (which plays an important role in viral replication) with an IC50 value of 6.0μM. Compound 31 also demonstrated excellent potent activity against omicron, WT, and delta variants of SARS-CoV-2, with EC50 values of 2.98, 4.59, and 2.70μM, respectively. The cytotoxicity of this compound in Vero E6 cells was determined using the cell counting kit-8 (CCK-8) assay, and the results showed a CC50 value of more than 100μM. A series of artesunic acid and peroxide-based derivatives were synthesized and screened for their anti-SARS-CoV-2 activity using a cell viability assay by Herrmann et al.,75 The most potent derivatives reported in this series were artesunic acid-quinoline derivatives 32, 33, and 34 with EC50 values in the range of 13–19μM against SARS-CoV-2, as compared to the parent compound artesunic acid (EC50>50μM). These derivatives showed no cytotoxic effects on Vero E6 cells, with CC50 values in the range of 100–110μM. Isoquinoline derivative with strong anti-SARS-CoV-2 activity was discovered by Rabie et al.,76 Compound 35 demonstrated the best inhibitory activities against anti-SARS-CoV-1 (EC50= 0.39μM), anti-SARS-CoV-2 (EC50= 0.11μM), anti-MERS-CoV (EC50= 0.20μM), anti-ExoN (EC50= 0.27μM), anti-RdRp (EC50= 0.16μM), and anti-Mpro (EC50= 0.077μM). It has remarkable potency towards anti-SARS-CoV-2 activities in vitro (EC50> 0.11μM, reaching as low as 0.077μM) in comparison to ensitrelvir (EC50< 0.29μM, reaching as high as 0.50μM). Wang et al.,77 synthesized a novel series of tetrahydroisoquinoline derivatives and tested them as SARS-CoV-2 inhibitors through in vitro studies. An immunofluorescence assay was used to determine the half-maximum effective concentration (EC50) in Vero E6 cells. Among the studied derivatives, compound 36 exhibited remarkable inhibition of SARS-CoV-2 replication in Vero E6 cells (EC50= 3.15 μM, SI> 63.49). The cytotoxicity of this compound in Vero E6 cells was determined using the CCK-8 assay, and the results showed a CC50 value of more than 200μM. Compound 36 also significantly inhibited the viral replication of SARS-CoV-2 in human lung cells (EC50= 2.78μM, SI> 71.94) as compared to the standard drug chloroquine (EC50= 44.90μM SI= 2.94). (Figure 11).

**Thiazole Derivatives as Anti-SARS-CoV-2 Agents**

Wu et al.,78 synthesized 8H-Indeno[1,2-d]thiazole hybrids and evaluated their anti-SARS-CoV-2 (3CLpro) activities. Amongst them, compound 37 demonstrated the best inhibitory activity against SARS-CoV-2 3CLpro with an IC50 value of 1.28μM as compared to the standard drug nirmatrelvir, with an IC50 value of 0.012μM. This compound showed the highest potency with a 72.5–6.1% inhibition rate at a 20μM concentration. Pohl et al.,79 2022 tested apratoxin S4 (a Sec61 inhibitor) as an anti-SARS-CoV-2 agent using an MTT assay. Apratoxin S4 38 was the most potent activity against SARS-CoV-2. It reduced viral replication in HeLa-hACE2 cells (IC50= 0.71nM) and Vero E6 cells (IC50=0.17μM). Remdesivir was used as the reference drug with an IC50 value of 35nM (Figure 12).

**Quinoxaline Derivative as Anti-SARS-CoV-2 Agent**

Chemboli et al.,80 synthesized pyrrolo[2,3-b]quinoxaline hybrids and tested their ability to inhibit cytokine storms in SARS-CoV-2 infection. All synthesized compounds were tested by in vitro studies for tumor necrosis factor-alpha (TNF-α) inhibition. Among them, Compound 39 demonstrated good inhibitory activity against TNF-α with an IC50 value of 5.14μM. This compound was not found to be better than the reference drug rolipram IC50= 0.91μM, but it was better than another known inhibitor: thalidomide(IC50=198.91μM) (Figure 13).
Pyrazole Derivative as Anti-SARS-CoV-2 Agent

Shcherbakov et al. investigated pyrazole derivatives as potential inhibitors of SARS-CoV-2 3CL^{pro} using an enzymatic assay. The most potent compound reported in this study was compound 40 which showed an IC_{50} value of 0.75μM against the non-structural protein (3CL^{pro}) of SARS-CoV-2, whereas control drugs disulfiram and ebselen had IC_{50} values of 6.1μM and 1.7μM, respectively (Figure 14).
Benzodioxole Derivatives as Anti-SARS-CoV-2 Agents
A novel series of N-aryl amide derivatives were designed and tested for their anti-COVID-19 activity by Wansri et al. All derivatives were evaluated through in vitro analysis at a 100µM concentration. Compounds 41 and 42 reduced the activity of SARS-CoV-2 (3CLpro) by nearly 70% at 100µM concentration. From these, compound 42 demonstrated remarkable activity towards SARS-CoV-2 3CLpro with an IC\textsubscript{50} value of 106.9µM as compared to the control drug rutin, with an IC\textsubscript{50} value of 325.6µM (Figure 15).

Coumarin Derivatives as Anti-SARS-CoV-2 Agents
Mohamed and Eltelbany, prepared 16 coumarin hybrids and evaluated them as potential inhibitors of the SARS-CoV-2 M\textsuperscript{pro}. At 100µM concentration, seven compounds showed approximately 50% inhibition of the main protease (M\textsuperscript{pro}) of SARS-CoV-2. Of these, cyclic amide 43 showed excellent potent activity against SARS-CoV-2 M\textsuperscript{pro} with an IC\textsubscript{50} value of 15.0µg/mL, whereas the reference drug chloroquine had an IC\textsubscript{50} of 13.1µg/mL. Compound 44 inhibited viral replication by 63.9%, with an IC\textsubscript{50} value of 25.8µg/mL at 100µM concentration (Figure 16).
Thiadiazole Derivative as Anti-SARS-CoV-2 Agents

Rabie and Eltayb. screened a novel series of aminothiadiazoles as inhibitors of COVID-19 employing in vitro anti-SARS-CoV-2 and anti-RdRp/ExoN bioassays. In Vero E6 cells, it was found that all the derivatives effectively inhibited and impaired SARS-CoV-2 replication and transcription. Amongst the studied derivatives, the compound 45 showed significant activity against the SARS-CoV-2 Omicron variant (EC$_{50}$ = 0.41µM), which was found to be 6.4 times more potent than the standard drug molnupiravir (EC$_{50}$ = 2.61µM). Compound 45 also displayed excellent anti-RdRp with an EC$_{50}$ value of 0.17 µM as compared to the reference drug molnupiravir (EC50= 0.24 µM). The cytotoxicity assay showed that the CC$_{50}$ value of compound 45 is remarkably greater than 100µM, and is expected to have a higher selectivity index (SI> 243.9) in comparison to the reference molnupiravir (SI> 38.3) (Figure 17).

In silico Studies of COVID-19

Current Advances in Heterocycles as Anti-COVID-19 Agents

Azafluorene Derivative as Anti-SARS-CoV-2 Agents

Azafluorene hybrids were synthesized and screened as potential inhibitors of SARS CoV-2 RdRp by Venkateshan et al. The pharmacological model of the synthesized compounds was predicted using ADMET analysis. AutoDock Vina and MM-PB(GB)SA analyses were used to determine the binding and binding free energies. The compounds 46 and 47 showed good binding affinity against non-structural protein RdRp (nsp12) of SARS CoV-2 with binding energy values (−8.3 and −7.8kcal/mol) and binding free energy values (−11.04 and −39.42 kcal/mol), whereas, control drugs favipiravir, hydroxychloroquine, and Galidesivir had binding energy values of −5.8, −6.3, and −7.6kcal/mol. Compound 46 exhibited H-bonding interactions with amino acid residues ASP-502, LYS-682, and TYR-503, and compound 47 displayed H-bonding interactions with amino acid residue ASP-507 (Figure 18).
Imidazole Derivatives as Anti-SARS-CoV-2 Agents

Satheesh et al., investigated imidazolium salts as inhibitors of the non-structural RNA-binding protein (nsp9) of SARS CoV-2 (PDB code: 6W4B) through molecular docking analysis. Among the docked derivatives, compounds 48, 49, and 50 exhibited strong binding affinities against the non-structural RNA-binding protein (nsp9) of SARS CoV-2 with docking scores of −4.2, −4.0, and −3.5kcal/mol, respectively. Hydroxychloroquine and chloroquine were used as standard drugs with binding affinities of −3.6kcal/mol. Compounds 48 and 49 showed hydrogen bonding interactions with the amino acid residues SER-6A and LYS-37A and hydrophobic interactions with the amino acid residues THR-36 and PHE-41. Compound 50 interacted with amino residues PRO-7A and VAL-77B via hydrophobic interactions. Belhassan et al., evaluated imidazole hybrids for the anti-SARS CoV-2 activity of imidazole hybrids using a computational approach. Among them, compounds 51, 52, and 53 showed good binding affinities against the main protease (M\textsuperscript{pro}) (Code PDB: 6LU7) of SARS CoV-2 with values of −8.5, −8.3, and −8.3kcal/mol, respectively, as compared to the reference drugs chloroquine (−5.9kcal/mol) and hydroxychloroquine (−6.6kcal/mol). Compound 51 exhibited H-bonding interactions with amino acid residues ARG-188A, HIS-164A, LEU-141A, and SER-144A and hydrophobic interactions with residues HIS-41A and MET-165A. Compound 52 displayed H-bonding interactions with the amino acid residue GLN-189A and hydrophobic interactions with the residues MET-165A and MET-49. Compound 53 demonstrated H-bonding interactions with the amino acid residues GLY-143A and hydrophobic interactions with MET-165A and CYS-145A. A series of substituted benzimidazole derivatives was synthesized by Mudi et al., and their anti-SARS CoV-2 activities were tested using a molecular docking approach. Of these, thiophene-substituted compound 54 displayed the highest binding affinity for M\textsuperscript{pro} (PDB code: 6LU7), nsp2 (PDB code: 7JLT), and nsp7 (PDB code: 7EXM) of SARS CoV-2 with docking scores of −10.52, −5.07, and −6.63 kcal/mol. It showed H-bonding interactions with amino acid residues LYS-113 and GLU-110 of nsp\textsuperscript{2} and ASP-67 of nsp7. It displayed one H-bonding interaction with amino acid residue GLY-143 and hydrophobic interactions with amino acid residue HIS-41 of the CoV-2 main protease (M\textsuperscript{pro}). Compound 54 showed the lowest docking score (−10.52 kcal/mol) towards the main protease (M\textsuperscript{pro}) of SARS CoV-2 rather than the non-structural proteins nsp2 and nsp7 (Figure 19).

Oxazole Derivative as Anti-SARS-CoV-2 Agent

Algethami et al., prepared isoxazole linked pyranopyrimidinone derivatives and screened them for inhibitors of the SARS CoV-2 main protease (M\textsuperscript{pro}) via molecular docking analysis. The most potent derivative in this study was compound 55 with the lowest binding energy score of −8.9 kcal/mol against the main protease (M\textsuperscript{pro}) of SARS CoV-2 as compared to the reference N3 inhibitor (−7.0kcal/mol). Compound 55 showed H-bonding interactions with the amino acid residue THR-26 and hydrophobic interactions with the amino acid residues PRO-168, MET-49, MET-165, and HIS-41 (Figure 20).

![Figure 19 Imidazole derivatives (48–54) as anti-SARS-CoV-2 agents.](https://doi.org/10.2147/DDDT.S450499)
Piperazine Derivative as Anti-SARS-CoV-2 Agent
Gatfaoui et al. synthesized 1-ethylpiperazine-1,4-diium bis(nitrate) and tested their anti-SARS-CoV-2 (PDB code: 6W4B) activity using docking studies. Compound 56 showed the highest binding affinity for SARS-CoV-2, with a binding energy of $-68.15$ kcal/mol. It displayed H-bonding interactions with the amino acid residue SER-47B (Figure 21).

Piperidine Derivative as Anti-SARS-CoV-2 Agent
Anthony et al. prepared a piperidine derivative and screened it for its inhibitory potential against COVID-19’s main protease (M\textsubscript{pro}) through molecular docking and molecular dynamics simulation. Compound 57 demonstrated the highest binding affinity against COVID-19 (6WCF-6Y84) receptors with docking scores of $-11.10$ and $-11.25$ kcal/mol, whereas the control drug hydroxychloroquine had docking score values of $-8.96$ and $-8.68$ kcal/mol. It formed H-bonds with the amino acid residues VAL-41 of the COVID-19 (6WCF) receptors and PRO-9 of the COVID-19 (6Y84) receptors (Figure 22).

Pyrazole Derivatives as Anti-SARS-CoV-2 Agents
Alotaibi et al. synthesized a series of novel pyrazole derivatives and examined their inhibitory potential against SARS-CoV-2 main protease (M\textsubscript{pro}) (PDB ID: 6LU7) through molecular docking analysis. From the examined derivatives, compound 58 demonstrated an excellent binding affinity for the main protease (M\textsubscript{pro}) of SARS-CoV-2 with a binding energy value of $-8.7$ kcal/mol. Azithromycin, lopinavir, hydroxychloroquine, chloroquine, remdesivir, favipiravir, and baloxvir were used as standard drugs with binding energy values of $-8.55$, $-9.26$, $-9.23$, $-8.95$, $-9.24$, $-9.23$, and $-9.18$ kcal/mol. It exhibited interactions with the amino acid residue of main protease (M\textsubscript{pro}) through a variety of interactions, such as van der Waals, hydrogen bonding, π-alkyl, and π-sigma interactions. Gupta et al. prepared a series of novel 4,4’-(arylmethylene)bis(1H-pyrazol-5-ols) compounds and tested them as potent inhibitors of COVID-19 main protease (3CL\textsubscript{pro}) (PDB ID: 6LU7) using molecular docking. The compounds 59 and 60 were found to exhibit significant binding affinity against COVID-19 M\textsubscript{pro} with binding energy scores of $-8.3$ and $-8.8$ kcal/mol, respectively, as compared to the reference drugs oseltamivir ($-4.7$ kcal/mol), remdesivir ($-6.5$ kcal/mol), hydroxychloroquine ($-5.3$ kcal/mol), chloroquine ($-5.1$ kcal/mol), ribavirin ($-5.6$ kcal/mol), ritonavir ($-7.3$ kcal/mol) and favipiravir ($-5.4$ kcal/mol). Compound 59 showed H-bonding interactions with the amino acid residue GLU-288; hydrophobic interactions with residues LEU-286, LYS-137, andALA-285; and pi-anion and pi-cation interactions with residues LYS-

![Figure 20 Oxazole derivative (55) as an anti-SARS-CoV-2 agent.](https://doi.org/10.2147/DDDT.S450499)

![Figure 21 Piperazine derivative as a (56) anti-SARS-CoV-2 agent.](https://doi.org/10.2147/DDDT.S450499)
5 and GLU-288. Compound 60 displayed hydrophobic interactions with amino acid residues TRP-218 Immunofluorescence assay and LEU-220. Addoum et al,\textsuperscript{92} prepared pyrano[2,3-c]pyrazole derivatives and investigated them as inhibitors of SARS-CoV-2 (3CL\textsuperscript{pro}) (PDB ID: 6LU7) using molecular docking analysis. Among them, compound 61 exhibited a remarkable binding affinity against the main protease (3CL\textsuperscript{pro}) of SARS-CoV-2 with a binding energy score of $-6.2 \text{kcal/mol}$, which was similar to the binding energy score of the reference drug chloroquine ($-6.2\text{Kcal/mol}$) and higher than both favipiravir ($-4.2 \text{kcal/mol}$) and hydroxychloroquine ($-5.5\text{Kcal/mol}$). It showed H-bonding interactions with amino acid residues CYST-145, SER-144, and GLYL-143. Masaret,\textsuperscript{93} designed a series of new spiropyrazole hybrids and evaluated their anti-COVID-19 activity using a molecular docking approach. Amongst them, compound 62 showed the best binding affinity for the main protease (PDB code: 6LU7) of COVID-19, with the lowest docking score of $-7.764\text{kcal/mol}$. Compound 62 displayed H-bonding interactions with amino acid residues THR-26A and GLY-43A. Mohamed et al,\textsuperscript{94} synthesized a tridentate Schiff base along with its complexes and screened them as inhibitors of the SARS-CoV-2 main protease UAW247 (6XBH) through in silico studies. Amongst all the complexes studied, Cr(III) complex 63 had the lowest binding energy for SARS-CoV-2 ($-28.6\text{kcal/mol}$). It demonstrated H-bonding interactions with amino acid residues MET-165 and GLN-166 and Pi-H interaction with residue GLU-189 (Figure 23).

**Pyridine Derivatives as Anti-SARS-CoV-2 Agents**

A series of 3-substituted indole-based 1.2-dihydropyridine hybrids were synthesized by Jayabal et al,\textsuperscript{95} and their inhibitory potential against SARS-CoV-2 was tested using a molecular docking approach. Out of the 20 prepared derivatives, compound

![Pyrazole derivatives (58–63) as Anti-SARS-CoV-2 agents.](https://doi.org/10.2147/DDDT.S450499)

Figure 23 Pyrazole derivatives (58–63) as Anti-SARS-CoV-2 agents.
64 showed good binding affinity against the main protease (M\text{pro}) (PDB code: 6LU7) of SARS-CoV-2 with a binding energy score of −8.6 kcal/mol, as compared to the control drug remdesivir with a binding energy value of −7.7 kcal/mol. In addition, compound 65 exhibited the highest binding affinity towards spike glycoprotein (PDB code: 7NX7) of COVID-19 with a binding energy value of −7.3 kcal/mol, whereby the standard drug remdesivir had the same binding energy value of −7.3 kcal/mol. Compound 64 displayed H-bonding interactions with 6LU7 M\text{pro} amino acid residues HIS-163 and GLY-143, and hydrophobic interactions with residues GLY-143 and GLU-166, while compound 65 showed H-bonding interactions with 7NX7 spike glycoprotein amino acid residues HIS-163 and PHE-140, and hydrophobic interactions with residues GLU-166, CYS-145, HIS-41, and MET-165. Tarika et al.\textsuperscript{66} investigated 4-dimethylamino pyridinium 3,5-dichlorosalicylate (DADS) as a potential SARS-CoV-2 inhibitor in silico studies. DADS\textsuperscript{66} demonstrated the best binding affinity against 6M2N, 7CHF, and 6M03 of the SARS-CoV-2 proteins, with binding energy of −2.66, −2.4, and −2.54 kcal/mol, respectively. It formed H-bonding interactions with 6M2N, 7CHF, and 6M03 proteins. Topal et al.\textsuperscript{97} prepared 3-chloro-2-{(2\text{E})-2-[1-(4-chlorophenyl)ethylidene]hydrazinyl}pyridine 67 and screened it for anti-COVID-19 activity using a molecular docking analysis. Compound 67 has been found to exhibit significant binding affinity (−6.4 kcal/mol) against 3CL\text{pro} (PDB code: 6LU7) of COVID-19. Chloroquine and hydroxychloroquine were used as control drugs, with binding energy values of −4.7 and −5.0 kcal/mol. It showed H-bonding interactions with amino acid residues HIS-164A and HIS-41A, and hydrophobic interactions with residues HIS-164A and MET-165A (Figure 24).

Pyrimidine Derivatives as Anti-SARS-CoV-2 Agents

Nesaragi et al.\textsuperscript{98} synthesized a series of pyrano[2,3-d]pyrimidine and tetrahydrobenzo[b]pyran derivatives and tested them as SARS-CoV-2 inhibitors using a molecular docking approach. From the synthesized derivatives, compounds 68, 69, and 70 exhibited the highest binding affinities against the nucleoplasmid CoV-N-protein (PDB code: 6M3M) of SARS-CoV-2 with docking scores of −87.722, −87.274, and −75.076 kcal/mol, respectively, whereas (6-chloro-7-(2-morpholinoethyl)amino)quinoline-5,8-dione was used as a reference compound with a docking score of −77.045 kcal/mol. These compounds showed different types of interactions with the amino acid residue ARG-89 and preferentially bound to the nucleoplasmid CoV-N-protein (PDB code: 6M3M) compared to the reference compound. Muhammad et al.\textsuperscript{99} prepared azoloazines and tested them as inhibitors of the SARS-CoV-2 3CL\text{pro}. Among these derivatives, compounds 71 and 72 showed the highest docking scores against SARS-CoV-2 3C-Like proteinase (3CL\text{pro}) (PDB code: LU7), with values of −7.529 and −7.517 kcal/mol, respectively. Compound 71 exhibited H-bonding interactions with the amino acid residues ASN-142, CYS-145, and HIS-164, while compound 72 displayed H-bonding interactions with the residues CYS-145, HIS-163, and HIS-164. A novel series of 2-amino-4-chloropyrimidine hybrids was synthesized by Qureshi et al.\textsuperscript{100} and screened for their anti-SARS-CoV-2 activity using a computational approach. Out of these, compound 73 demonstrated good affinity against 3CL\text{pro} (PDB ID: 6LU7) of SARS-CoV-2 with a binding free energy score of −8.12 kcal/mol as compared to the standard drug remdesivir with a binding free energy score of −6.41 kcal/mol. It exhibited H-bonding interactions with amino acid residues ASN-142 and PHE-140, and hydrophobic interactions with amino acid residues HIS-41, HIS-172, MET-165, MET-49, LEU-141, TYR-54, HIS-163, HIS-164, ASP-187, and ARG-188 (Figure 25).

![Figure 24 Pyridine derivatives (64–67) as anti-SARS-CoV-2 agents.](https://doi.org/10.2147/DDDT.S450499)
Quinoline Derivatives as Anti-SARS-CoV-2 Agents

Nepolraj et al. synthesized 3-(hydroxymethyl)-2-phenyl-2,3-dihydroquinolin-4(1H)-one and tested it as an inhibitor of SARS-CoV-2 main protease (M\text{pro}) (PDB code: 6LU7) through in silico studies. Compound 74 showed the highest binding affinity against SARS-CoV-2 main protease (−6.70 kcal/mol by AutoDock) and (−7.52 kcal/mol by AutoDock). It formed H-bonding interactions with amino acid residues GLY-143, SER-144, CYS-145, and LEU-141, and hydrophilic interactions with CYS-145. Drug scoring, drug likeness, and toxicity were determined through in silico studies using ADME analysis. Alshammari et al. designed a series of N-substituted-2-quinolonylacetohydrazides and evaluated their anti-COVID-19 activity using in silico analysis. Among them, compound 75 showed the strongest binding affinity against main protease (M\text{pro}) (PDB code: 6LU7) and RNA-dependent RNA polymerase (RdRp) (PDB code: 6M71) of SARS-CoV-2 with docking scores of −9.7, and −7.7 kcal/mol, respectively. Remdesivir was used as a standard drug and exhibited binding affinity towards RdRP (−5.6 kcal/mol) and M\text{pro} (−8.5 kcal/mol). Compound 75 preferentially binds M\text{pro} to RdRp. It displayed H-bonding interactions with amino acid residues SER-144, GLU-166, LEU-141, and HIS-163 of M\text{pro} and ASP-623, TYR-619, and GLU-811 of RdRp (Figure 26).

Quinoxaline Derivative as Anti-SARS-CoV-2 Agent

The quinoxaline derivative 4-(5-nitro-thiophen-2-yl)-pyrrolo[1,2-a]quinoxaline 5NO\text{2}TAAPP 76 was prepared by Divya et al. and tested its anti-SARS-CoV-2 main protease 3CL\text{pro} (PDB code: 6LU7) activity using molecular docking studies. The compound (5NO\text{2}TAAPP) 76 showed a lower binding energy towards SARS-CoV-2 main protease 3CL\text{pro} (−7.95 Kcal mol\textsuperscript{−1}) as compared to the reference drugs remdesivir (−4.96 Kcal mol\textsuperscript{−1}) and hydroxychloroquine (−6.06 Kcal mol\textsuperscript{−1}). It exhibited H-bonding interactions with the amino acid residues GLY-143A and CYS-145A, and hydrophobic interactions with residues MET-49A and MET-165A (Figure 27).

![Figure 25](image_url) Pyrimidine derivatives (68–73) as anti-SARS-CoV-2 agents.

![Figure 26](image_url) Quinoline derivatives (74 and 75) as anti-SARS-CoV-2 agents.
Thiadiazole Derivatives as Anti-SARS-CoV-2 Agents
A series of novel 1,3,4-thiadiazole hybrids was prepared by Rashdan and Abdel monse, and tested them as anti-Covid-19 agents through in silico studies. Among the docked hybrids, compound 77 showed the highest binding affinity against receptor-binding domain (RBD), main protease (M\textsuperscript{pro}), RNA-dependent RNA polymerase (RdRp), and papain-like protease (PL\textsuperscript{pro}) of SARS-CoV-2 with docking scores of −6.8, −11.4, −8.2, and −9.4 Kcal mol\textsuperscript{−1}, respectively. Compound 77 showed the best bonding interactions with main protease (M\textsuperscript{pro}) (−11.4 Kcal mol\textsuperscript{−1}) as compared to the standard drug darunavir (−7.5 Kcal mol\textsuperscript{−1}). It showed hydrogen bond interactions with amino acid residues THR-24 and SER-144 of the targeted M\textsuperscript{pro}; pi-stacked interactions with residues LYS-218, TYR-251, and PHE-258 of the targeted PL\textsuperscript{pro}; and ARG-116 and PHE-35 of the targeted RdRp. It also displayed H-bonding interactions with amino acid residues TYR-385A and ARG-393A of the targeted RBD. Rashdan and Abdel monse\textsuperscript{104} also designed a thiadiazole-based derivative bearing 1,2,3-triazole ring and evaluated its inhibitory potential towards the main protease SARS-CoV-2 using in silico studies. Of these, compound 78 exhibited the lowest binding energy against COVID-19’s main protease (−8.3 Kcal/mol). It formed H-bonding interactions with the amino acid residues LYS-5 and THR-199, as well as π-cation interactions with residues ARG-131 (Figure 28).

Thiazole Derivatives as Anti-SARS-CoV-2 Agents
Al-janabi et al\textsuperscript{105} synthesized novel thiazole derivatives and screened them for SARS-CoV-2 3CL\textsuperscript{pro} inhibitors using a molecular docking approach. Among these derivatives, N-(6-chlorobenzo[d]thiazol-2-yl)-1-phenyl-1-(pyridin-2-yl)methanimine 79 showed the best binding affinities for SARS-CoV-2 with the same values of −7.6 Kcal/mol. It displayed van der Waals interactions with amino acid residues HIS-41, CYS-145, and GLU-166. Adel Alghamdi et al\textsuperscript{106} synthesized a new series of thiazole-clubbed pyridine hybrids and examined them as potential inhibitors of COVID-19 M\textsuperscript{pro} (PDB code: 6LU7) via in silico studies. From the studied hybrids, compound 80 displayed the highest binding affinity against the main protease of SARS-CoV-2 with a binding energy value of −8.6 kcal/mol as compared to the standard Inhibitor N3 (−8.0 kcal/mol). It displayed H-bonding interactions with the amino acid residues GLY-71A, GLN-19A, and MET-17A and hydrophobic interactions with the amino acid residues GLU-14A, GLY-120A, ALA-70A, LYS-97A, VAL-18A, and TRP-31A. The molecular docking approaches, physical parameters, and toxicity assessment showed that the compound 80 was found to be non-toxic and could bind with the main protease of SARS-CoV-2 via different types of interactions. (Figure 29).

Triazole Derivatives as Anti-SARS-CoV-2 Agents
Murugavel et al\textsuperscript{4} prepared triazole derivatives and tested them as potent inhibitors of COVID-19 using in silico analyses. From these, compound 81 showed a remarkable binding affinity for the spike protein (PDB ID: 7DF4) of SARS-CoV-2 (−8.9 Kcal/mol).
The compounds 81 and 82 exhibited the lowest binding energies towards the ACE2 receptor protein (PDB ID: 7DF4) of SARS-CoV-2, with a similar docking score of −9.1Kcal/mol. BMTPP 81 exhibited H-bonding interactions with amino acid residues ARG-355, GLU-465, and ARG466, as well as hydrophobic interactions with residues GLU-465, PHE-464, and LYS-462 of the spike protein receptors. Compounds 81 and 82 displayed H-bonding interactions with the amino acid residues HIS-401, ASP-350, TRP-349, and ALA-348 and hydrophobic interactions with residues PHE-40, TRP-349, ARG-393, HIS-401, and PHE-390 of the SARS-Cov-2 ACE2 receptor protein. A novel series of 1,2,3-triazole hybrids were synthesized by Aouad et al., and screened for their anti-COVID-19 activity using in silico studies. Amongst them, compound 83 showed the best binding affinity against the main protease (PDB code: 6LU7) of SARS-CoV-2 (−8.8Kcalmol⁻¹). It displayed the highest binding affinity, higher than the standard drugs hederagenin (−6.9Kcalmol⁻¹), cloperastine (−7.0Kcalmol⁻¹), vigabatrin (−4.1Kcalmol⁻¹), methotrexate (−8.2Kcalmol⁻¹), furidiazine (−6.1Kcalmol⁻¹), ursolic acid (−7.1Kcalmol⁻¹), acetazolamide (−5.2Kcalmol⁻¹), doravirine (−7.8Kcalmol⁻¹), desaglybuzole (−6.5Kcalmol⁻¹), and nortriptyline (−7.3Kcal mol⁻¹), and a similar one to maraviroc (−8.8Kcalmol⁻¹) but had a lower one as compared to raltegravir (−9.6Kcalmol⁻¹). It showed either H-bonding or hydrophobic interactions with the amino acid residues GLN-189A, PHE-140A, and SER-144. Singh et al., prepared a bis-triazolyl probe and evaluated its anti-COVID-19 activity using a molecular docking analysis. Compound 84 exhibited the best binding affinity against COVID-19 main protease (PDB code: 6LU7), with a value of −6.43 kcal/mol. It exhibited H-bonding interactions with amino acid residue ARG-298A and hydrophobic interactions with residues PHE-8A, PRO-9A, TYR-154A, and PHE-294A (Figure 30).

**Chloroquine Derivatives as Anti-SARS-CoV-2 Agents**

Hussein and Elkhair synthesized zinc complexes of hydroxychloroquine and chloroquine and evaluated them as potential inhibitors of COVID-19’s Mpro (PDB code: 6LU7) using in silico studies. Among the docked derivatives, complexes 85 and 86 showed the highest binding affinities against the main protease of COVID-19 with values of −7.54 and −7.7Kcal/mol, respectively, whereas parent compounds hydroxychloroquine and chloroquine had docking scores of −6.87Kcal/mol and −7.08Kcal/mol, respectively. Complex 85 formed H-bonding interactions with the main protease...
amino acid residues GLN-189A, HIS-164A, CYS-145A, GLU-166A, MET-165A, and ARG-188A, and complex 86 displayed H-bonding interactions with the main protease amino acid residues GLU-166A and ARG-188A (Figure 31).

**Chromone Derivatives as Anti-SARS-CoV-2 Agents**

A series of dihydrobenzofuro[3,2-$b$]chromene hybrids were prepared by Gorai et al,\textsuperscript{110} and screened as SARS-CoV-2 3CL\textsuperscript{pro} (PDB code: 6lu7) inhibitors through molecular docking analysis. From these, compound 87 demonstrated lower binding energy towards the 3CL\textsuperscript{pro} of SARS-CoV-2 (−7.6kcal/mol). It exhibited H-bonding interactions with amino acid residues CYS-145A; van der Waals interactions with residues TYR-54A, ASN-142A, SER-144A, HIS-164A, GLU-166A, ASP-187A, ARG-188A, GLN-189A, LEU-141A, and GLY-143A; hydrophobic interactions with PRO-52A, MET-49A, and HIS-41; and Pi-sulfur interactions with residue MET-165A. Mary et al,\textsuperscript{111} synthesized N-methyl-2-[(4-oxo-4$H$-chromen-3-yl)methyli-dene]-hydrazinecarbothioamide (MCMH) and evaluated its anti-COVID-19 activity using in silico studies. Compound (MCMH) 88 exhibited the highest binding affinity towards the main protease (M\textsuperscript{pro}) (PDB code: 5r80) of SARS-CoV-2, with a docking score of −8.8kcal/mol. It displayed H-bonding interactions with amino acid residues GLY-143A, ASN-142A, HIS-163A, SER-144A, and THR-26A and hydrophobic interactions with amino acid residue MET-165A (Figure 32).

**Coumarin Derivatives as Anti-SARS-CoV-2 Agents**

Mun et al,\textsuperscript{112} synthesized coumarin derivatives and tested them as anti-COVID-19 agents using a molecular docking analysis. Among them, psoralen 89 showed the highest binding affinity against RdRp (PDB code: 7BV2), PL\textsuperscript{pro} (PDB code: 6W9C), M\textsuperscript{pro} (PDB code: 6W63), and spike glycoprotein (PDB code: 6M0J) of SARS-CoV-2 with values of −6.5, −6.2, −6.6, and −6.4kcal/mol, respectively. Phenprocoumon 90 also exhibited the highest binding potential towards the main protease (M\textsuperscript{pro}) (PDB code: 6W63) of SARS-CoV-2 (−7.2 kcal/mol) in comparison to other target proteins of SARS-CoV-2. Remdesivir was used as a standard drug and had a binding affinity with RdRp (−6.1kcal/mol), PL\textsuperscript{pro} (−7.4kcal/mol), M\textsuperscript{pro} (−7.8kcal/mol), and Spike protein (−6.9kcal/mol). Psoralen 89 displayed H-bonding interactions with the amino acid residues SER-795A and ASP-164A of RdRp; TYR-213A, GLU-214A, TYR-305A, and LYS-217A of PL\textsuperscript{pro}, HIS-164A and GLY-166A of M\textsuperscript{pro}; and HIS-493A, TYR-613A, and ARG-482A of SP. Phenprocoumon 90 formed H-bonding interaction with the amino acid residues GLU-166A and HIS-163A of the main protease (M\textsuperscript{pro}). Chidambaram et al,\textsuperscript{113} designed a series of novel coumarin hybrids and screened them as inhibitors of SARS-CoV-2 main protease (PDB code: 5N5O) through in silico studies. Amongst the studied hybrids, compounds 91 and 92, both showed the highest binding affinities against the main protease of SARS-CoV-2 with a binding energy value of −7.9kcal/mol as compared to the standard drugs alpha-ketoamide (−6.6kcal/mol) and hydroxychloroquine (−5.8kcal/mol). Compound 91 demonstrated hydrophobic interactions with amino acid residues THR-25A, HIS-41A, CYS-145A, and GLY-143A. Compound 92 displayed H-bonding interactions with the main amino acid residues GLN-189A, HIS-164A, CYS-145A, GLU-166A, MET-165A, and ARG-188A, and complex 86 displayed H-bonding interactions with the main protease amino acid residues GLU-166A and ARG-188A (Figure 31).

**Figure 31** Chloroquine derivatives (85 and 86) as anti-SARS-CoV-2 agents.

**Figure 32** Chromone derivatives (87 and 88) as anti-SARS-CoV-2 agents.
residue HIS-163A and hydrophobic interactions with amino acid residues MET-49A, LEU-141A, CYS-145A, ASN-142A, and CYS-143A (Figure 33).

**Conclusion**

Heterocyclic compounds are important in medicinal chemistry owing to their physicochemical properties. The spectrum of heterocycle-containing molecules utilized in medications is growing by the day, and their various derivatives offer a valuable and pivotal pathway for the development of drugs with a broad range of biological applications. This study has revealed that chemically synthesized heterocycles are crucial and versatile compounds with anti-SARS-CoV-2 activities. In vitro studies summarized here state that different heterocycles act against the structural and non-structural proteins of SARS-CoV-2. The half-maximal inhibitory concentration (IC$_{50}$), half-maximal effective concentration (EC$_{50}$), 50% cytotoxic concentration (CC$_{50}$), and selective index of potent compounds were determined. In silico analyses reported herein describe that different heterocycles demonstrate binding affinities towards RdRp (PDB code: 7BV2), PL$^{pro}$ (PDB code: 6W9C), M$^{pro}$ (PDB code: 6W63), and spike glycoprotein (PDB code: 6M0J) of SARS-CoV-2. Various binding interactions with the amino acid residues of the target proteins have also been reported. ADME analysis was performed to predict the pharmacokinetic properties of the prepared molecules.

Nitrogen- and oxygen-containing heterocyclic compounds with significant levels of demonstrated efficient antiviral effects came to the top of the pharmaceutical perspective for COVID-19 therapy. The current detailed in vitro/in silico preclinical research has investigated the anti-COVID-19 potential of various heterocyclic compounds. Interestingly, preclinical research findings reveal that the heterocyclic compounds have excellent and balanced drug-like behavior and could be further processed in vivo/clinical stages of drug assessment and development.

However, knowing the importance of heterocyclic compounds as antiviral agents, particularly against coronaviruses, a greater knowledge of heterocyclic frameworks as anti-SARS CoV, anti-MERS CoV, and anti-SARS CoV-2 agents. This overview focuses on various heterocyclic compounds employed in coronaviruses inhibition via in vitro and in silico techniques, which may serve as leading structures for the design and development of SARS CoV-2 inhibitors.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**


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