Drug Design, Development and Therapy

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REVIEW

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

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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, oxazole, quinoline, isoxazole, thiazole, quinoxaline, 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

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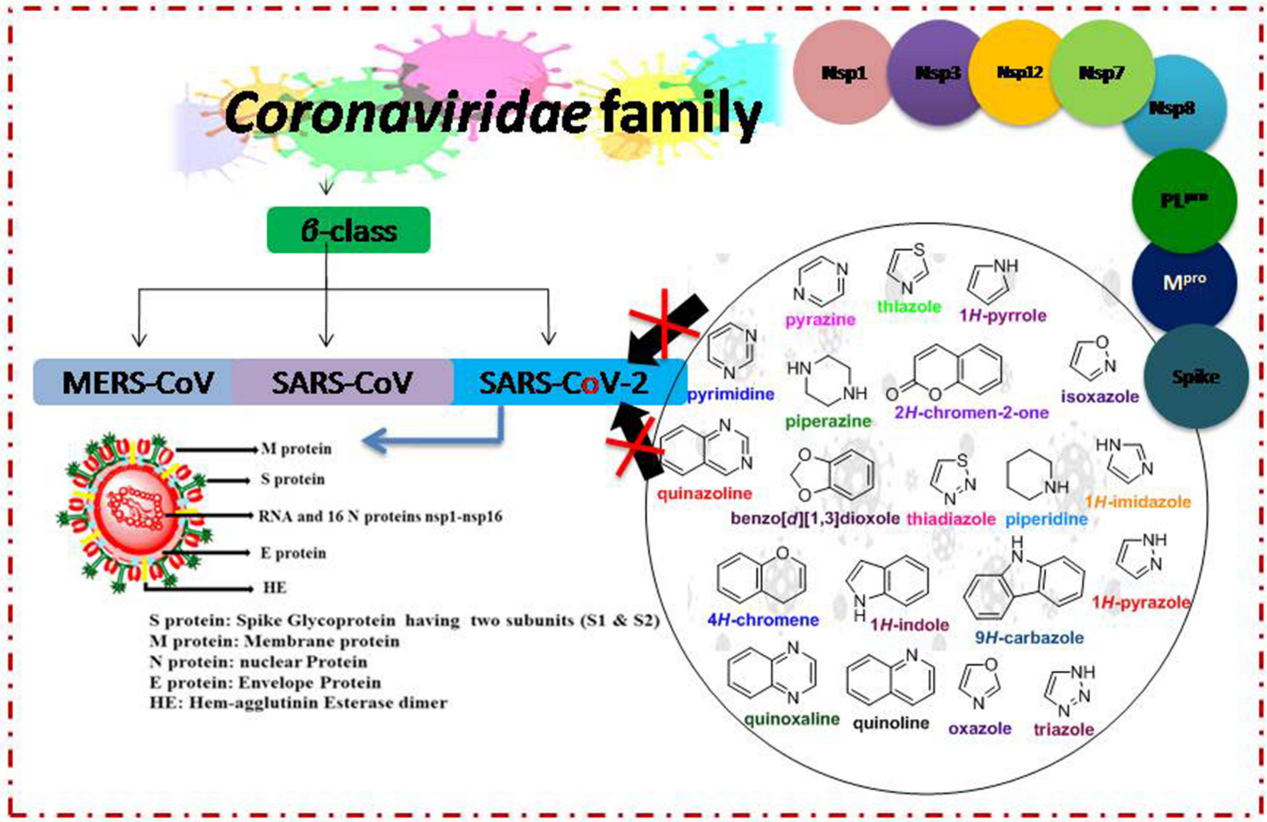
Graphical Abstract



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.[13] The SARS-CoV-2 genome contains approximately 30,000 nucleotides in the sequence of 5′-replicase-S-E-M-N-3′.[14] 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.[15] The nsps1–nsps16 are encoded proteins that are responsible for viral transcription and replication, such as papain-like protease (PL^{pro}; nsp^3), RNA-dependent RNA polymerase (RdRp; nsp^{12}), 3-chymotrypsin-like protease ($3CL^{pro}$; nsp^5), also called the main protease (M^{pro}), and RNA helicase (nsp^{13}).[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.[19] The cells with greater levels of ACE2 expression may be considered as potent SARS-CoV-2 infection sites.[20] The increased ACE2 expression causes a rise in SARS-CoV-2 viral infection.[21] COVID-19 disease may be treated effectively by targeting ACE2 expression.[22] Preventing SARS-CoV-2 entry into the host cell via ACE2 receptor sites may be an effective method of combating COVID-19.[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.[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 adenylyl-transferase function.[25,26]

The chymotrypsin-like cysteine protease ($3CL^{pro}$), also called M^{pro}, 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).[27] The enzyme encodes the translated polyproteins from coronaviruses (CoVs) RNA along with papain-like proteases (PL^{pro}).[28]

In structural identification, $3CL^{pro}$ 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 $3CL^{pro}$ 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.[29] Consequently, two distinct potent $3CL^{pro}$ inhibitors, including both non-peptidic inhibitors and peptidomimetic inhibitors, have been reported to fight a rapid rise in the COVID-19 pandemic.[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.[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.[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^{pro}.[36] Drug repurposing is the quickest option to propose effective treatments for the ongoing COVID-19 pandemic.[37] Several FDA-approved anti-retroviral drugs are already in use, including, oseltamivir **I**,[38] favipiravir **II**/ribavirin **III**,[39] lopinavir **IV**/ritonavir **V**,[40] chloroquine **VI**, and hydroxychloroquine **VII**,[41] ensitrelvir **VIII**,[42] cordycepin **IX**,[43] didanosine **X**,[44] teriflunomide **XI**,[45] riboprine **XII**, and forodesine **XIII**,[46] as well as other similar nucleoside and nucleotide analogs, which showed effective antiviral activities against SARS-CoV-2. Thymohydroquinone, dithymoquinone, ginger, strychnine bush, and pineapple ingredients are used as natural enemies of SARS-CoV-2.[47–49]

Remdesivir **XIV** is an adenosine analogue RNA-dependent RNA polymerase (RdRp) inhibitor used for the treatment of SARS-CoV-2-infected patients.[50] Remdesivir, which has been recommended by the FDA for emergency use, can be injected intravenously into hospitalized patients. It reduces recovery time but not mortality. It is highly efficient with rotavirus polymerases, therefore exhibiting a low potential to cause human toxicity.[51] Molnupiravir (EIDD-2801) **XV**, a prodrug of the nucleoside hybrid N4-hydroxycytidine (NHC), was approved by the FDA for the cure of COVID-19 in specific adults. It exhibits remarkable toxic effects in certain cell-based processes, such as induced mutagenesis in mammalian cells.[52–54] Molnupiravir has some limitations, including its use in patients over the age of 18 because of its side effects on the growth of cartilage and bone and in non-pregnant women because of the potential risk of fatal harm. The most widely used $3CL^{pro}$ inhibitors are peptidomimetic inhibitors, such as YH-53 with benzothiazolyl ketone **XVI**,[55] and nirmatrelvir with nitrile **XVII**.[56] Nonpeptidic inhibitors include covalent and noncovalent inhibitors. Covalent inhibitors include shikonin **XVIII** and carmofur **XIX**.[57] Non-covalent inhibitors of flavonoid derivatives include baicalin **XX** and baicalein **XXI**[58] (Figure 1).

Some repurposing drugs are used for the treatment of COVID-19, however, their effectiveness and side effects are still unknown. Therefore, there is a tremendous need to synthesize novel heterocyclic moieties with improved potency and optimized pharmacological properties. The review aims to report in vitro and in silico studies of chemically synthesized heterocyclic compounds against the structural and non-structural proteins of SARS-CoV-2.

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,[59] 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_{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_{50} value of 6.721μM. Tanaka et al,[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).

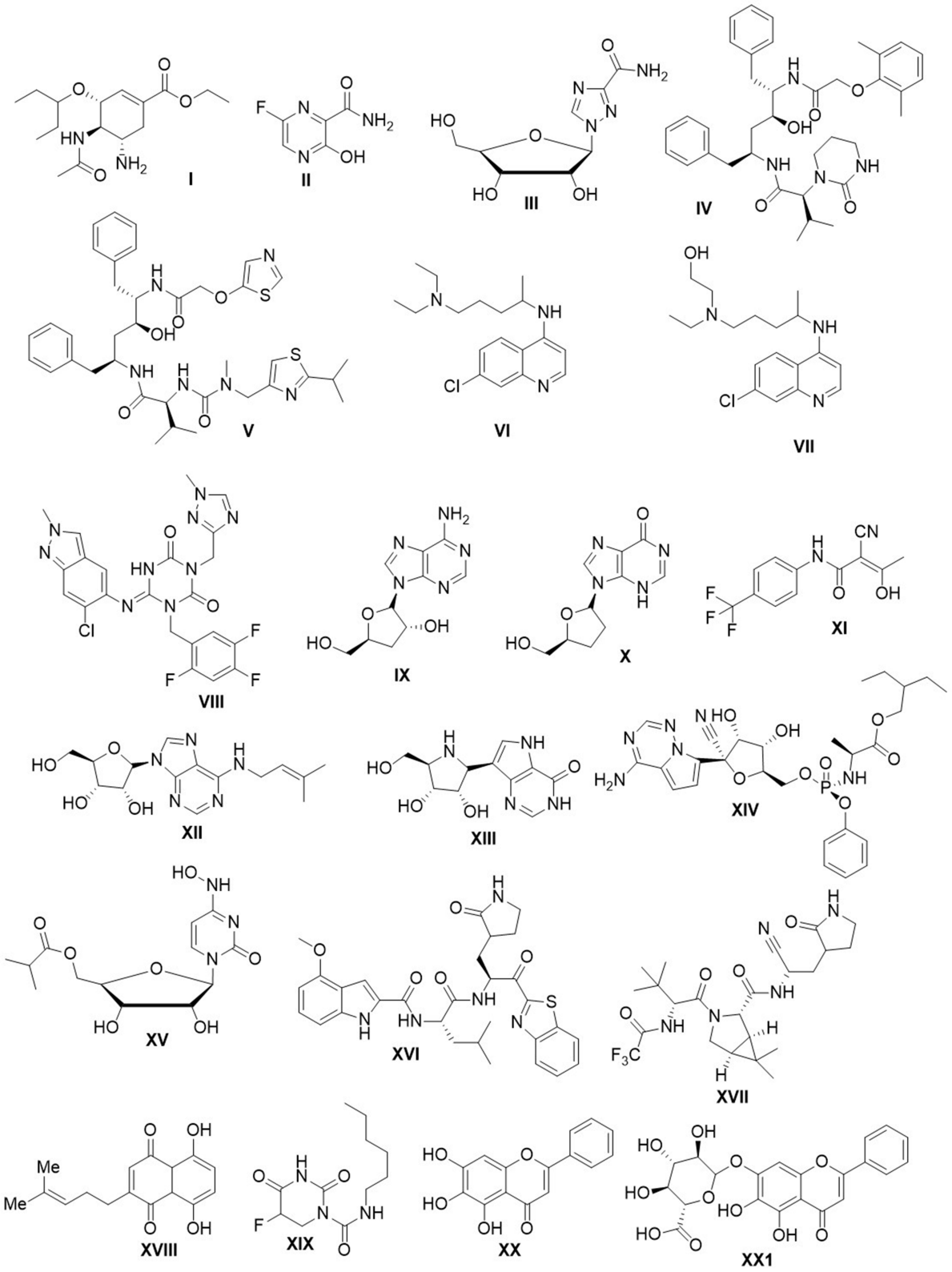


Figure 1 Commercially available drugs as COVID-19 Inhibitors.

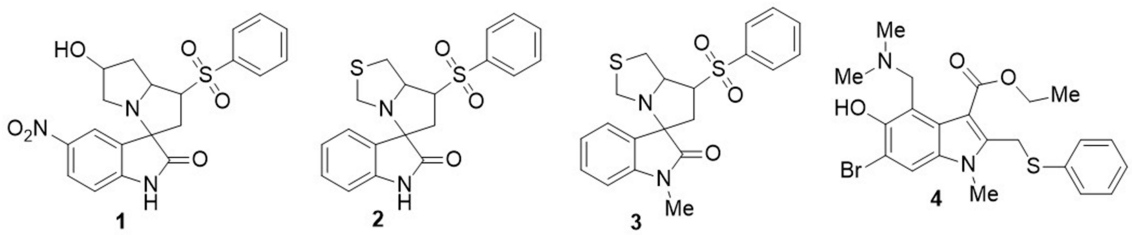


Figure 2 Indole derivatives (**1–4**) as anti-SARS-CoV-2 agents.

Piperidine Derivatives as Anti-SARS-CoV-2 Agents

A novel series of aspirin-curcumin mimic conjugates was synthesized by Srour et al,[61] and their anti-SARS-CoV-2 properties were evaluated using the MTT-bio assay. The compounds **5** and **6** bearing a thienylidene 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,[36] 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.[62] 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,[63] synthesized a novel favipiravir analog (cyanorona-20) **10** 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,[64] 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}=

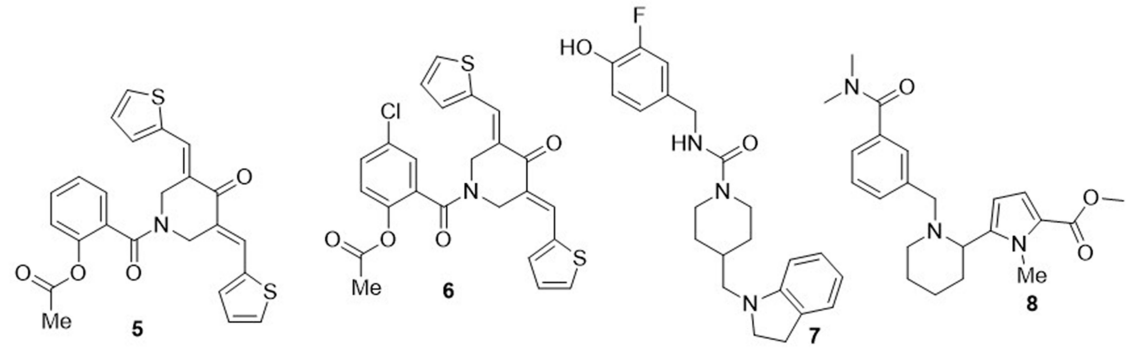


Figure 3 Piperidine derivatives (**5–8**) as anti-SARS-CoV-2 agents.

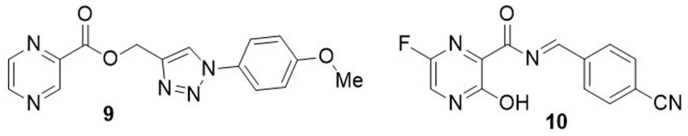


Figure 4 Pyrazine derivatives (**9** and **10**) as anti-SARS-CoV-2 agents.

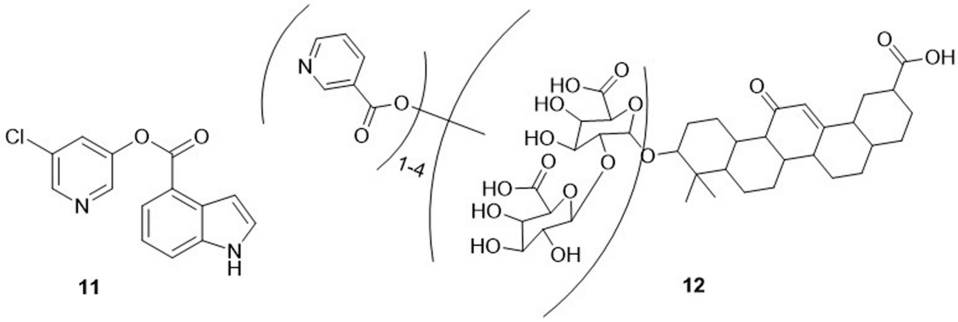


Figure 5 Pyridine derivatives (**11** and **12**) as anti-SARS-CoV-2 agents.

1.2μM,). This compound also showed a half-maximal cytotoxic concentration (CC_{50}> 100μM) against coronavirus 2. A derivative of glycyrrhizin nicotinate, "glycyvir", was prepared by Fomenko et al,[65] 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 2–8μM, as compared to the reference drug remdesivir (IC_{50}= 1.5–3.3μM) (Figure 5).

Pyrimidine Derivatives as Anti-SARS-CoV-2 Agents

Abu-Zaied et al,[12] 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 12.16μM and CC_{50} values of 420.9 and 327.1μM, respectively. Cytosine thioglycoside phosphoramidates **13** and **14** was proven the most effective profiles in Vero E6 cells, with 83% and 86% inhibition at 0.25μM 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.[66] 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 15.41μmol 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 360.9μmol. Harbi et al,[67] 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 88.38μM, respectively. These compounds inhibited the replication of SARS-CoV-2 in Vero-E6 cells with IC_{50} values of 84.01, 39.58, and 44.86μM, respectively. These compounds suppressed SARS-CoV-2 replication by 20%, 49%, and 72%, respectively. Of these, Mn(II) complex **17** was the most effective against SARS-CoV-2, with an IC_{50} value of 39.58μM, whereas the control drug remdesivir had an IC_{50} value of 0.2μM (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.[27] Among them, compound **18** displayed significant inhibitory activity towards the main protease ($3CL^{pro}$) of SARS-CoV-2, with an IC_{50} value of 0.034μM. This compound reduced the replication of SARS-CoV-2 in Vero E6 cells with an EC_{50} value of 0.29μM. The half maximal cytotoxic concentration (CC_{50}), and selectivity index (SI) values of these compounds were 808.7, and 2786 respectively (Figure 7).

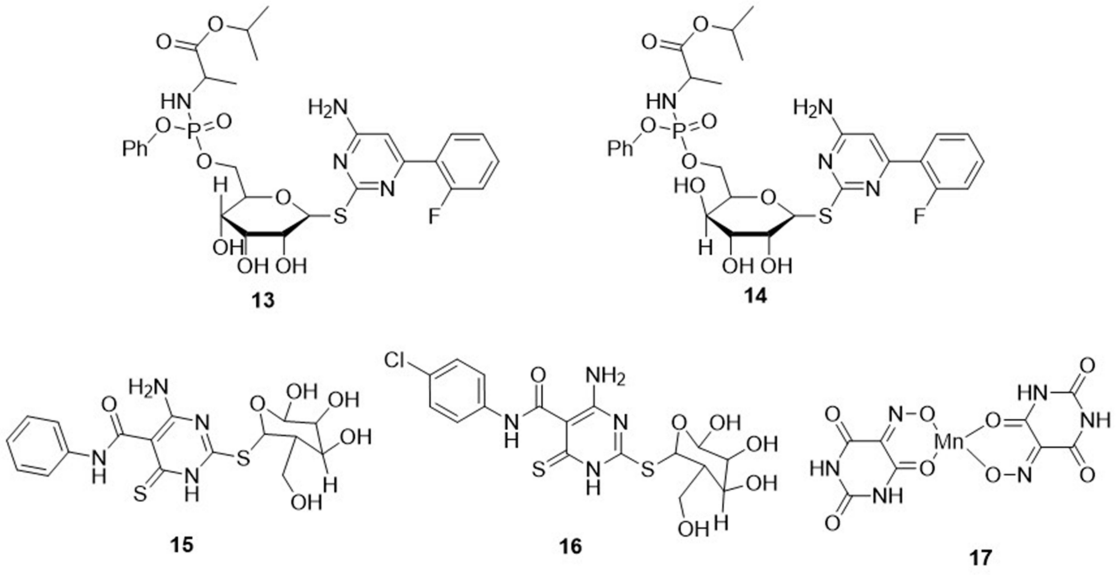


Figure 6 Pyrimidine derivatives (**13–17**) as anti-SARS-CoV-2 agents.

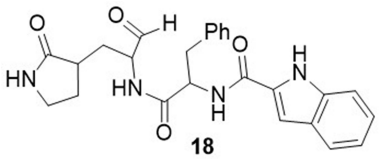


Figure 7 Pyrrole derivatives (**18**) as anti-SARS-CoV-2 agents.

Piperazine Derivatives as Anti-SARS-CoV-2 Agent

Nishiuchi et al,[68] 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,[69] 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)

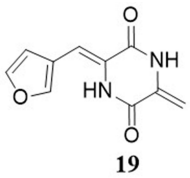


Figure 8 Piperazine derivative (**19**) as an anti-SARS-CoV-2 agent.

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 IC_{50} 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.[70] Among them, compound **22** demonstrated the most potent activity against SARS-CoV-2 with an IC_{50} value of 4.2μM, whereas the IC_{50} 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 CC_{50} and selective index (SI) values of 14.3μM and 3.4M, respectively, as compared to the reference drugs (CC_{50}>25μM and SI >3.2, >2.6, and >1.5μM). A novel series of 2-aminoquinazolin-4-(3*H*)-one hybrids was prepared by Shin et al,[71] and screened for anti-SARS-CoV-2 activity through in vitro studies. An immunofluorescence assay was used to determine the half-maximal inhibitory concentration (IC_{50}) of these derivatives in Vero E6 cells at a 10μM concentration. The cytotoxicity (CC_{50}) 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 IC_{50} values of 0.29, 0.11, and 0.33μM, respectively, as compared to the reference drug remdesivir (IC_{50} = 3.47μM) (Figure 10).

Quinoline Derivatives as Anti-SARS-CoV-2 Agents

Guevara-Pulido et al,[72] 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 (M^{pro}) with an IC_{50} value of 12nM, whereas the parent compound chloroquine had an IC_{50} value of 27nM. Nizi et al,[10] reported two phenylquinoline derivatives as potential inhibitors of SARS-CoV-2 replication, using a colorimetric formazan-based MTS assay. Amongst

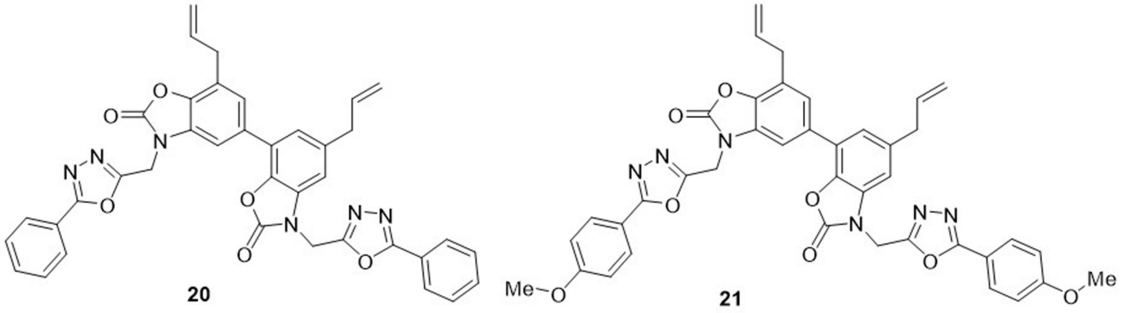


Figure 9 Oxazole Derivatives (**20** and **21**) as anti-SARS-CoV-2 agents.

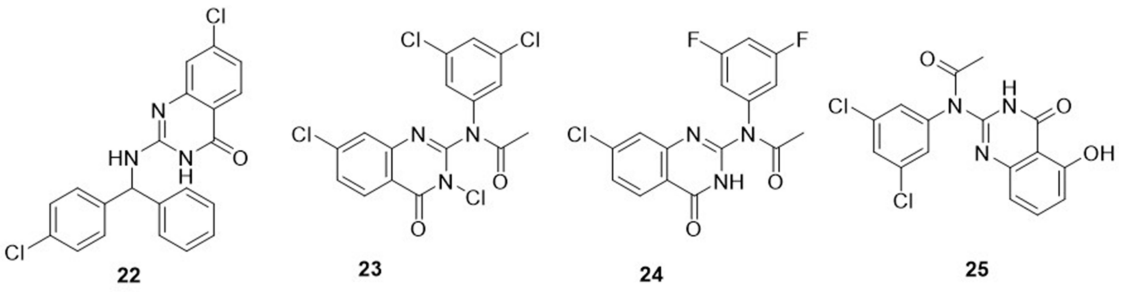


Figure 10 Quinazoline derivatives (**22–25**) as anti-SARS-CoV-2 agents.

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 IC_{50} value of 0.42μM. This compound reduced viral replication in Vero E6 cells with an EC_{50} value of 8.8μM as compared with the control drug chloroquine, with an EC_{50} value of 10.9μM. This compound demonstrated cytotoxicity in Vero E6 cells, with a CC_{50} 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 EC_{50} values of 1.08, 2.08, and 3.92μM, respectively. Remdesivir was used as the standard drug with an EC_{50} value of 1.39μM. These compounds showed cytotoxic effects on Vero E6 cells, with CC_{50} 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 PL^{pro} using a fluorescence polarization assay (FPA) and cell-based assay. Amongst them, chloroxine **31** inhibited the binding of SARS-CoV-2 PL^{pro} to ISG15 (which plays an important role in viral replication) with an IC_{50} value of 6.0μM. Compound **31** also demonstrated excellent potent activity against omicron, WT, and delta variants of SARS-CoV-2, with EC_{50} 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 CC_{50} 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 EC_{50} values in the range of 13–19μM against SARS-CoV -2, as compared to the parent compound artesunic acid (EC_{50}>50μM). These derivatives showed no cytotoxic effects on Vero E6 cells, with CC_{50} 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 (EC_{50}= 0.39μM), anti-SARS-CoV-2 (EC_{50}= 0.11μM), anti-MERS-CoV (EC_{50}= 0.20μM), anti-ExoN (EC_{50}= 0.27μM), anti-RdRp (EC_{50}= 0.16μM), and anti-M^{pro} (EC_{50}= 0. 077μM). It has remarkable potency towards anti-SARS-CoV-2 activities in vitro (EC_{50}> 0.11μM, reaching as low as 0.077μM) in comparison to ensitrelvir (EC_{50}< 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 (EC_{50}) in Vero E6 cells. Among the studied derivatives, compound **36** exhibited remarkable inhibition of SARS-CoV-2 replication in Vero E6 cells (EC_{50}= 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 CC_{50} value of more than 200μM. Compound **36** also significantly inhibited the viral replication of SARS-CoV-2 in human lung cells (EC_{50}= 2.78μM, SI> 71.94) as compared to the standard drug chloroquine (EC_{50}= 44.90μM SI= 2.94). (Figure 11).

Thiazole Derivatives as Anti-SARS-CoV-2 Agents

Wu et al,[78] synthesized 8*H*-Indeno[1,2-*d*]thiazole hybrids and evaluated their anti-SARS-CoV-2 ($3CL^{pro}$) activities. Amongst them, compound **37** demonstrated the best inhibitory activity against SARS-CoV-2 $3CL^{pro}$ with an IC_{50} value of 1.28μM as compared to the standard drug nirmatrelvir, with an IC_{50} 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 ($IC5_0$= 0.71nM) and Vero E6 cells (IC_{50}=0.17μM). Remdesivir was used as the reference drug with an IC_{50} 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 IC_{50} value of 5.14μM. This compound was not found to be better than the reference drug rolipram IC_{50}= 0.91μM, but it was better than another known inhibitor: thalidomide(IC_{50}=198.91μM) (Figure 13).

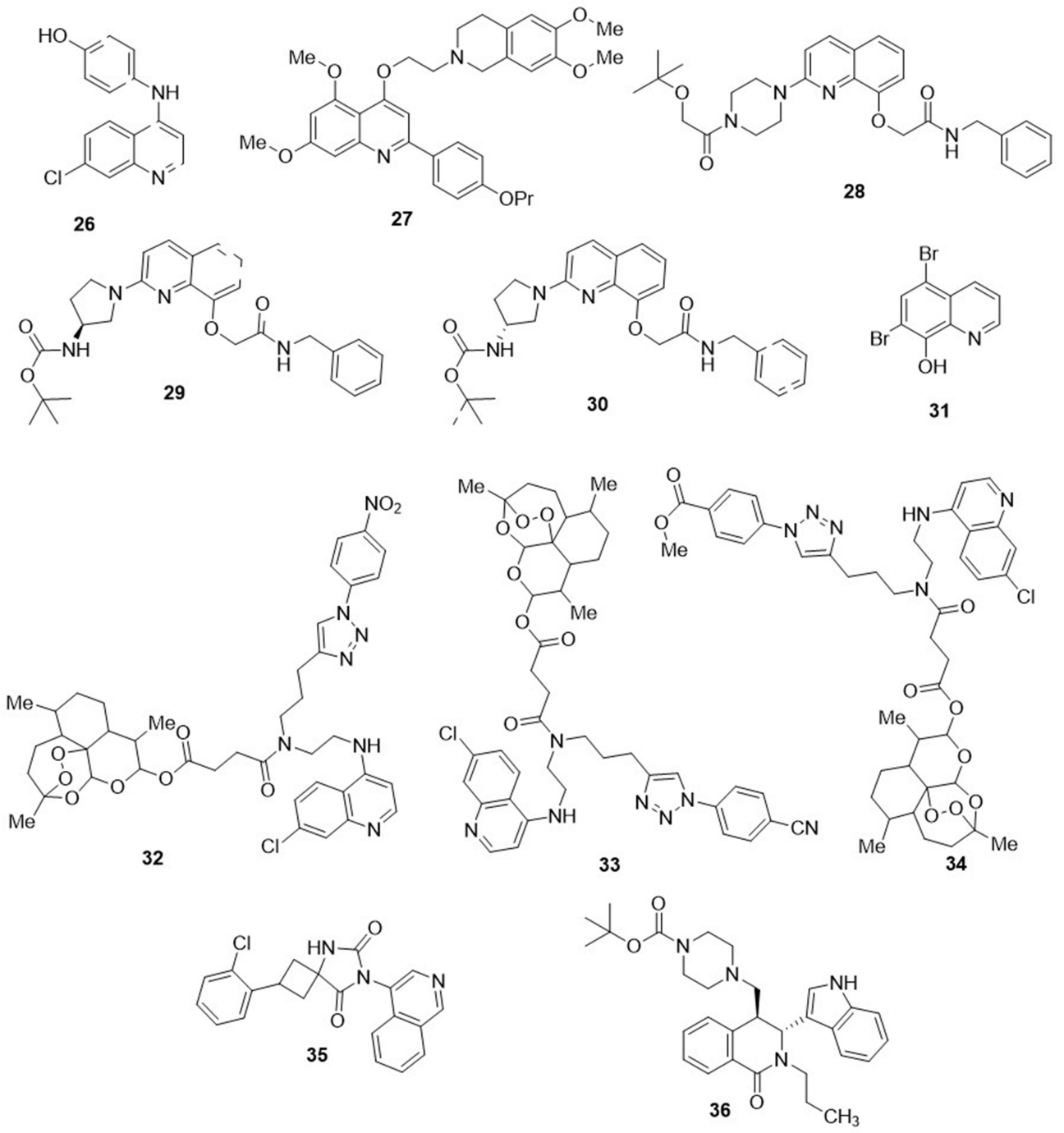


Figure 11 Quinoline derivatives (**26–36**) as anti-SARS-CoV-2 agents.

Pyrazole Derivative as Anti-SARS-CoV-2 Agent

Shcherbakov et al,[81] 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).

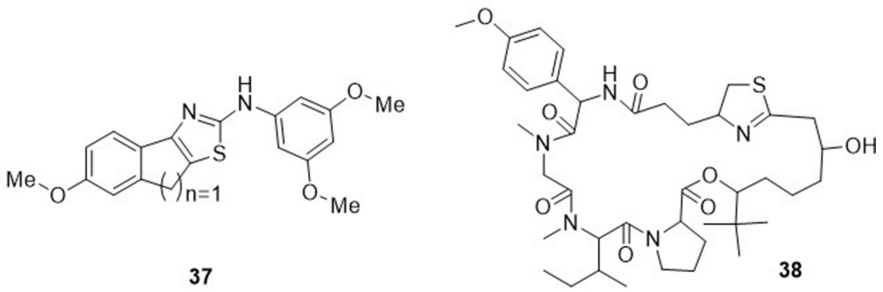


Figure 12 Thiazole derivatives (**37** and **38**) as anti-SARS-CoV-2 agents.

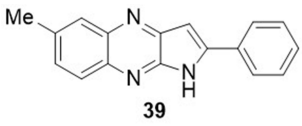


Figure 13 Quinoxaline derivative (**39**) as an anti-SARS-CoV-2 agent.

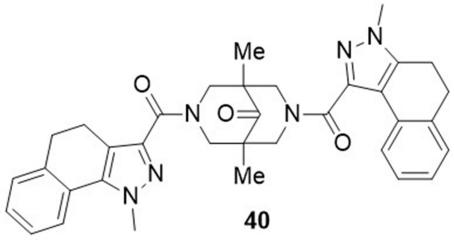


Figure 14 Pyrazole derivative (**40**) as an anti-SARS-CoV-2 agent.

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.[82] All derivatives were evaluated through in vitro analysis at a 100μM concentration. Compounds **41** and **42** reduced the activity of SARS-CoV-2 ($3CL^{pro}$) by nearly 70% at 100μM concentration. From these, compound **42** demonstrated remarkable activity towards SARS-CoV-2 $3CL^{pro}$ with an IC_{50} value of 106.9μM as compared to the control drug rutin, with an IC_{50} value of 325.6μM (Figure 15).

Coumarin Derivatives as Anti-SARS-CoV-2 Agents

Mohamed and Eltelbany,[83] prepared 16 coumarin hybrids and evaluated them as potential inhibitors of the SARS-CoV-2 M^{pro}. At 100μM concentration, seven compounds showed approximately 50% inhibition of the main protease (M^{pro}) of SARS-CoV-2. Of these, cyclic amide **43** showed excellent potent activity against SARS-CoV-2 M^{pro} with an IC_{50} value of 15.0μg/mL, whereas the reference drug chloroquine had an IC_{50} of 13.1μg/mL. Compound **44** inhibited viral replication by 63.9%, with an IC_{50} value of 25.8μg/mL at 100μM concentration (Figure 16).

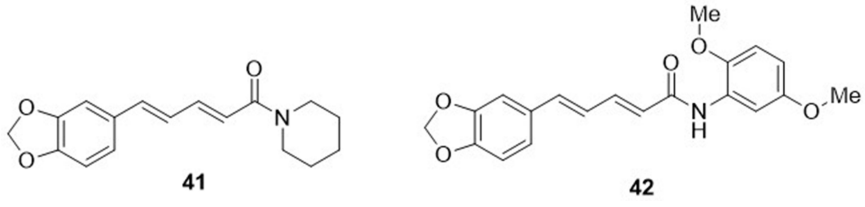


Figure 15 Benzodioxole derivatives (**41** and **42**) as anti-SARS-CoV-2 agents.

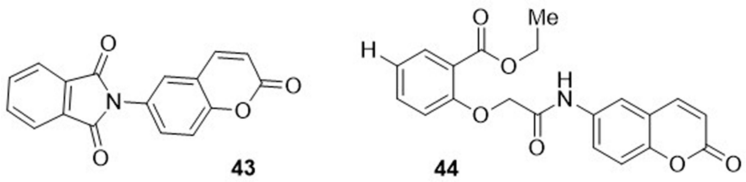


Figure 16 Coumarin derivatives (**43** and **44**) as anti-SARS-CoV-2 agents.

Thiadiazole Derivative as Anti-SARS-CoV-2 Agents

Rabie and Eltayb.[84] 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.[85] 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 dugs 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).

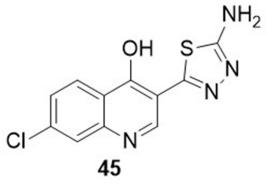


Figure 17 Thiadiazole derivative (**45**) as anti-SARS-CoV-2 agent.

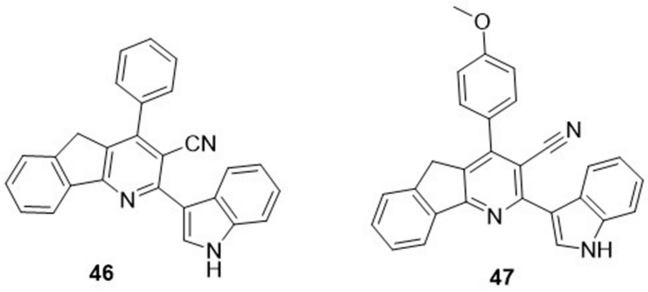


Figure 18 Azafluorenere derivatives (**46** and **47**) as anti-SARS-CoV-2 agents.

Imidazole Derivatives as Anti-SARS-CoV-2 Agents

Satheesh et al,[1] 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,[86] 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^{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,[87] 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^{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[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^{pro}). Compound **54** showed the lowest docking score (−10.52 kcal/mol) towards the main protease (M^{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,[88] prepared isoxazole linked pyranopyrimidinone derivatives and screened them for inhibitors of the SARS CoV-2 main protease (M^{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^{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).

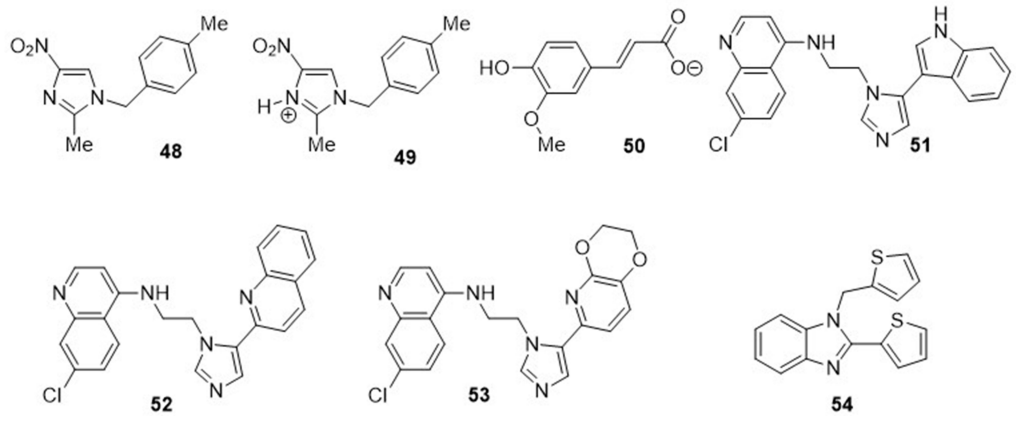


Figure 19 Imidazole derivatives **(48–54)** as anti-SARS-CoV-2 agents.

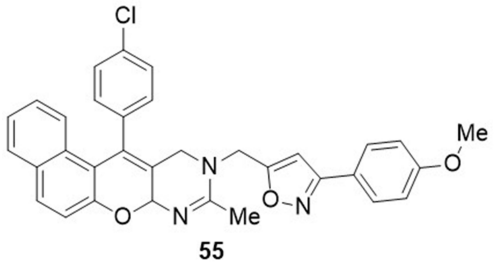


Figure 20 Oxazole derivative (**55**) as an anti-SARS-CoV-2 agent.

Piperazine Derivative as Anti-SARS-CoV-2 Agent

Gatfaoui et al,[89] 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 −68.15kcal/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,[90] prepared a piperidine derivative and screened it for its inhibitory potential against COVID-19's main protease (M^{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 −11.25kcal/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,[11] synthesized a series of novel pyrazole derivatives and examined their inhibitory potential against SARS-CoV-2 main protease (M^{pro}) (PDB ID: 6LU7) through molecular docking analysis. From the examined derivatives, compound **58** demonstrated an excellent binding affinity for the main protease (M^{pro}) of SARS-CoV-2 with a binding energy value of −8.7kcal/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 −9.18kcal/mol. It exhibited interactions with the amino acid residue of main protease (M^{pro}) through a variety of interactions, such as van der Waals, hydrogen bonding, π-alkyl, and π-sigma interactions. Gupta et al,[91] prepared a series of novel 4.4'-(arylmethylene)*bis*(1*H*-pyrazol-5-ols) compounds and tested them as potent inhibitors of COVID-19 main protease ($3CL^{pro}$) (PDB ID: 6LU7) using molecular docking. The compounds **59** and **60** were found to exhibit significant binding affinity against COVID-19 M^{pro} with binding energy scores of −8.3 and −8.8Kcal/mol, respectively, as compared to the reference drugs oseltamivir (−4.7Kcal/mol), remdesivir (−6.5Kcal/mol), hydroxychloroquine (−5.3Kcal/mol), chloroquine (−5.1Kcal/mol), ribavirin (−5.6Kcal/mol), ritonavir (−7.3Kcal/mol) and favipiravir (−5.4Kcal/mol). Compound **59** showed H-bonding interactions with the amino acid residue GLU-288; hydrophobic interactions with residues LEU-286, LYS-137, and ALA-285; and pi-anion and pi-cation interactions with residues LYS-

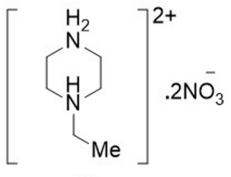


Figure 21 Piperazine derivative as a (**56**) anti-SARS-CoV-2 agent.

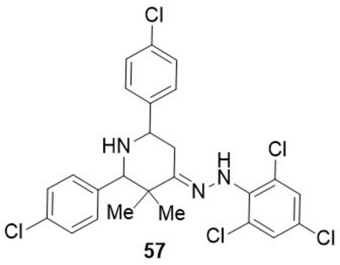


Figure 22 Piperidine derivative (**57**) as anti-SARS-CoV-2 agent.

5 and GLU-288. Compound **60** displayed hydrophobic interactions with amino acid residues TRP-218 Immunofluorescence assay and LEU-220. Addoum et al,[92] prepared pyrano[2,3-*c*]pyrazole derivatives and investigated them as inhibitors of SARS-CoV-2 ($3CL^{pro}$) (PDB ID: 6LU7) using molecular docking analysis. Among them, compound **61** exhibited a remarkable binding affinity against the main protease ($3CL^{pro}$) of SARS-CoV-2 with a binding energy score of −6.2 kcal/mol, which was similar to the binding energy score of the reference drug chloroquine (−6.2Kcal/mol) and higher than both favipiravir (−4.2 kcal/mol) and hydroxychloroquine (−5.5Kcal/mol). It showed H-bonding interactions with amino acid residues CYST-145, SER-144, and GLYL-143. Masaret,[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 −7.764kcal/mol. Compound **62** displayed H-bonding interactions with amino acid residues THR-26A and GLY-43A. Mohamed et al,[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 (−28.6kcal/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,[95] and their inhibitory potential against SARS-CoV-2 was tested using a molecular docking approach. Out of the 20 prepared derivatives, compound

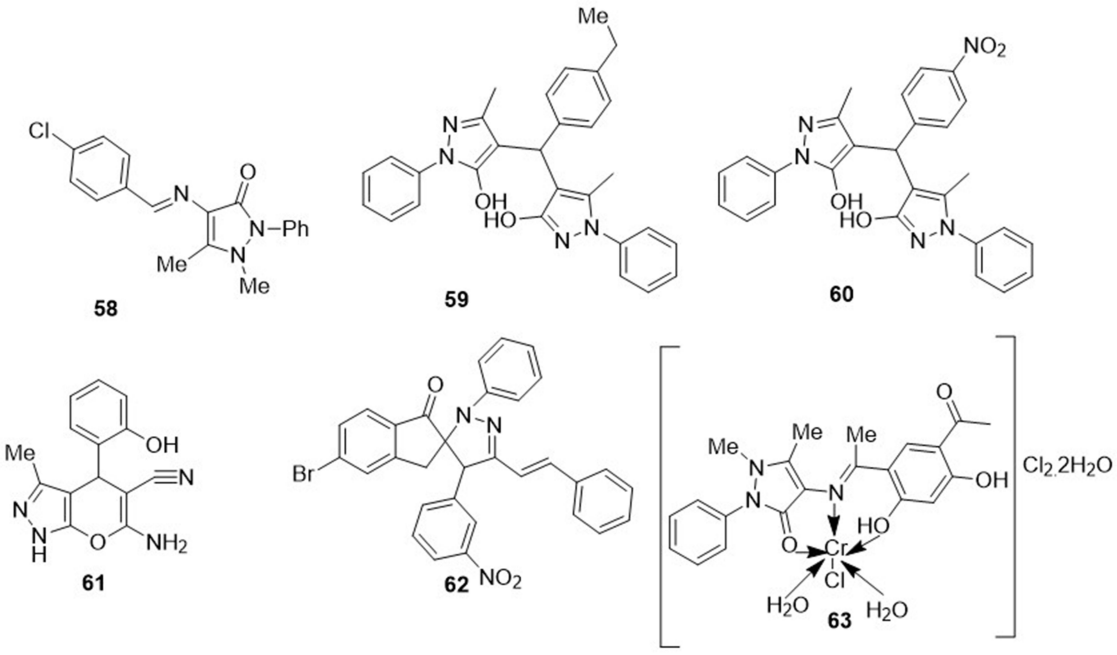


Figure 23 Pyrazole derivatives (**58–63**) as Anti-SARS-CoV-2 agents.

64 showed good binding affinity against the main protease (M^{pro}) (PDB code: 6LU7) of SARS-CoV-2 with a binding energy score of −8.6kcal/mol, as compared to the control drug remdesivir with a binding energy value of −7.7kcal/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 −7.3kcal/mol, whereby the standard drug remdesivir had the same binding energy value of −7.3kcal/mol. Compound **64** displayed H-bonding interactions with 6LU7 M^{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,[96] investigated 4-dimethylamino pyridinium 3.5-dichlorosalicylate (DADS) as a potential SARS-CoV-2 inhibitor in *silico* studies. DADS **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 6 M03 proteins. Topal et al,[97] prepared 3-chloro-2-{(2*E*)-2-[1-(4-chlorophenyl)ethylidene]hydra-zinyl}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 $3CL^{pro}$ (PDB code: 6LU7) of COVID-19. Chloroquine and hydroxychloroquine were used as control drugs, with binding energy values of −4.7 and −5.0kcal/mol. It showed H-bonding interactions with amino acid residues HIS-164A and HIS-41A, and hydrophobic interactions with residues HIS-41A and MET-165A (Figure 24).

Pyrimidine Derivatives as Anti-SARS-CoV-2 Agents

Nesaragi et al,[98] synthesized a series of pyrano[2,3-*d*]pyrimidinone 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 −75.076kcal/mol, respectively, whereas (6-chloro-7-((2-morpholinoethyl)amino)quinoline-5,8-dione) was used as a reference compound with a docking score of −77.045kcal/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,[99] prepared azoloazines and tested them as inhibitors of the SARS-CoV-2 $3CL^{pro}$. Among these derivatives, compounds **71** and **72** showed the highest docking scores against SARS-CoV-2 3C-Like proteinase ($3CL^{pro}$) (PDB code: LU7), with values of −7.529 and −7.517kcal/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-chloro-pyrimidine hybrids was synthesized by Qureshi et al,[100] and screened for their anti-SARS-CoV-2 activity using a computational approach. Out of these, compound **73** demonstrated good affinity against $3CL^{pro}$ (PDB ID: 6LU7) of SARS-CoV-2 with a binding free energy score of −8.12kcal/mol as compared to the standard drug remdesivir with a binding free energy score of −6.41kcal/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).

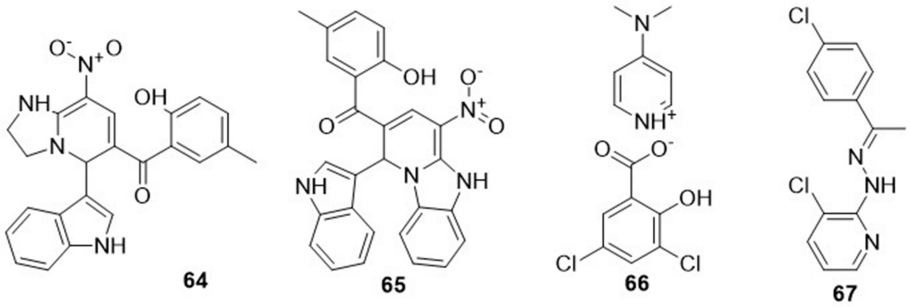


Figure 24 Pyridine derivatives (**64–67**) as anti-SARS-CoV-2 agents.

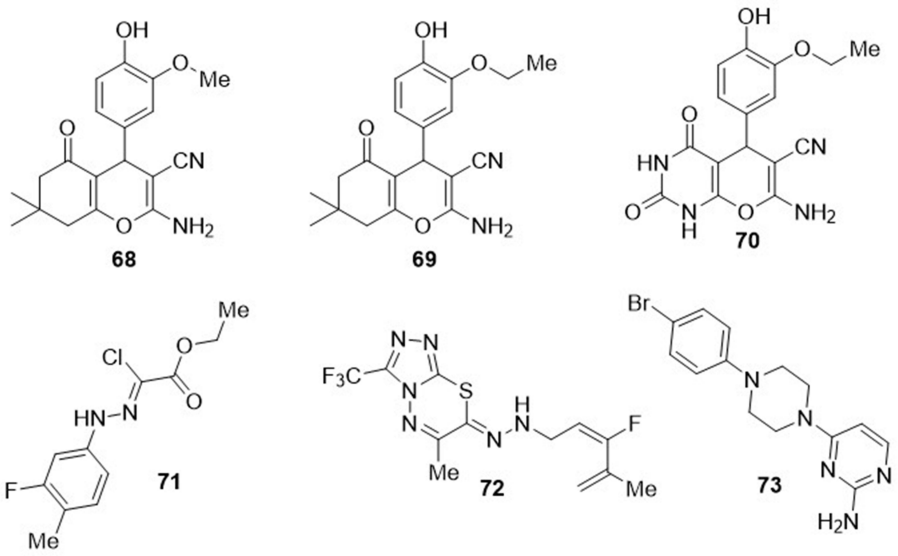


Figure 25 Pyrimidine derivatives (**68–73**) as anti-SARS-CoV-2 agents.

Quinoline Derivatives as Anti-SARS-CoV-2 Agents

Nepolraj et al,[7] synthesized 3-(hydroxymethyl)-2-phenyl-2,3-dihydroquinolin-4(1*H*)-one and tested it as an inhibitor of SARS-CoV-2 main protease (M^{pro}) (PDB code: 6LU7) through in silico studies. Compound **74** showed the highest binding affinity against SARS-CoV-2 main protease (−6.70kcal/mol by AutoDock) and (−7.52kcal/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,[101] 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^{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(−5.6kcal/mol) and M^{pro}(−8.5kcal/mol). Compound **75** preferentially binds M^{pro} to RdRp. It displayed H-bonding interactions with amino acid residues SER-144, GLU-166, LEU-141, and HIS-163 of M^{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 $5NO_2TAAPP$) **76** was prepared by Divya et al,[102] and tested its anti-SARS-CoV-2 main protease $3CL^{pro}$ (PDB code: 6LU7) activity using molecular docking studies. The compound ($5NO_2TAAPP$) **76** showed a lower binding energy towards SARS-CoV-2 main protease $3CL^{pro}$ (−7.95Kcal mol^{-1}) as compared to the reference drugs remdesivir (−4.96Kcal mol^{-1}) and hydroxychloroquine (−6.06Kcal mol^{-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).

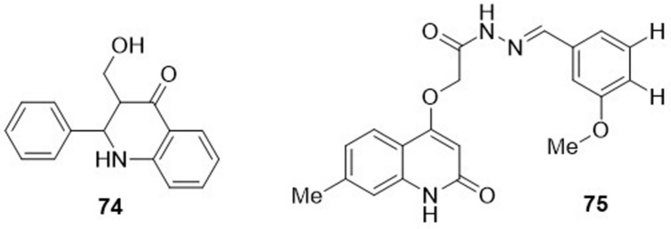


Figure 26 Quinoline derivatives (**74** and **75**) as anti-SARS-CoV-2 agents.

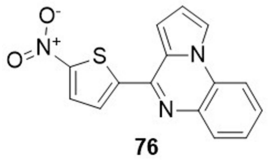


Figure 27 Quinoxaline derivative (**76**) as an anti-SARS-CoV-2 agent.

Thiadiazole Derivatives as Anti-SARS-CoV-2 Agents

A series of novel 1,3,4-thiadiazole hybrids was prepared by Rashdan and Abdel monsef,[103] 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^{pro}), RNA-dependent RNA polymerase (RdRp), and papain-like protease (PL^{pro}) of SARS-CoV-2 with docking scores of −6.8, −11.4, −8.2, and −9.4Kcal mol^{-1}, respectively. Compound **77** showed the best bonding interactions with main protease (M^{pro}) (−11.4Kcal mol^{-1}) as compared to the standard drug darunavir (−7.5Kcal mol−1). It showed hydrogen bond interactions with amino acid residues THR-24 and SER-144 of the targeted M^{pro}; pi-stacked interactions with residues LYS-218, TYR-251, and PHE-258 of the targeted PL^{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 monsef,[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 (−8.3Kcal/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,[105] synthesized novel thiazole derivatives and screened them for SARS-CoV-2 $3CL^{pro}$ 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 −7.6Kcal/mol. It displayed van der Waals interactions with amino acid residues HIS-41, CYS-145, and GLU-166. Adel Alghamdi et al,[106] synthesized a new series of thiazole-clubbed pyridine hybrids and examined them as potential inhibitors of COVID-19 M^{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 (−8.0kcal/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,[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 (−8.9Kcal/mol).

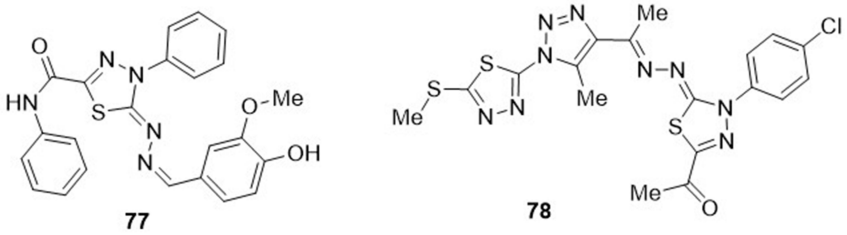


Figure 28 Thiadiazole derivatives (**77** and **78**) as an anti-SARS-CoV-2 agent.

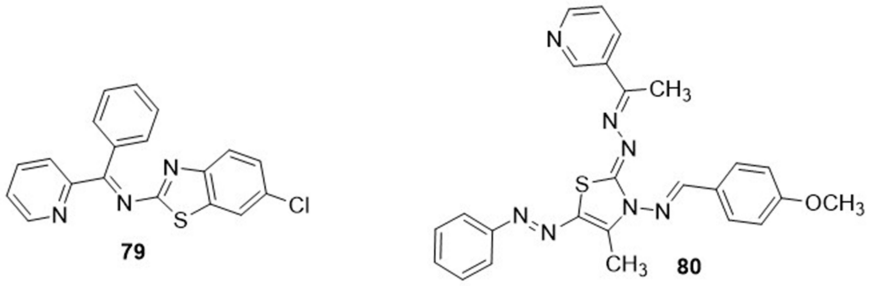


Figure 29 Thiazole derivatives (**79** and **80**) as anti-SARS-CoV-2 agents.

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,[107] 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,[108] 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,[109] synthesized zinc complexes of hydroxychloroquine and chloroquine and evaluated them as potential inhibitors of COVID-19's M^{pro} (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

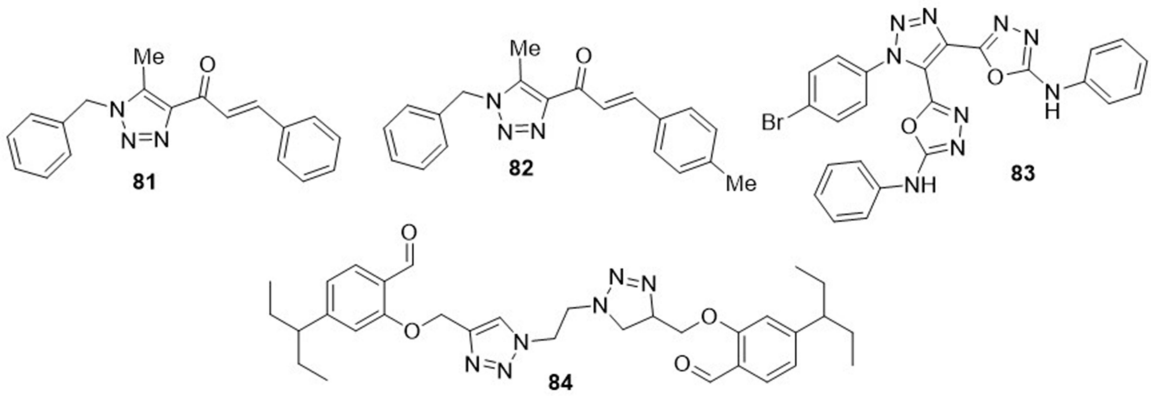


Figure 30 Triazole derivatives (**81–84**) as Anti-SARS-CoV-2 agents.

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).

Chromene Derivatives as Anti-SARS-CoV-2 Agents

A series of dihydrobenzofuro[3,2-*b*]chromene hybrids were prepared by Gorai et al,[110] and screened as SARS-CoV-2 $3CL^{pro}$ (PDB code: 6lu7) inhibitors through molecular docking analysis. From these, compound **87** demonstrated lower binding energy towards the $3CL^{pro}$ 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,[111] synthesized *N*-methyl-2-[(4-oxo-4*H*-chromen-3-yl)methylidene]-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^{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,[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^{pro} (PDB code: 6W9C), M^{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^{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^{pro} (−7.4kcal/mol), M^{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-217Aof PL^{pro}, HIS-164A and GLY-166A of M^{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^{pro}). Chidambaram et al,[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

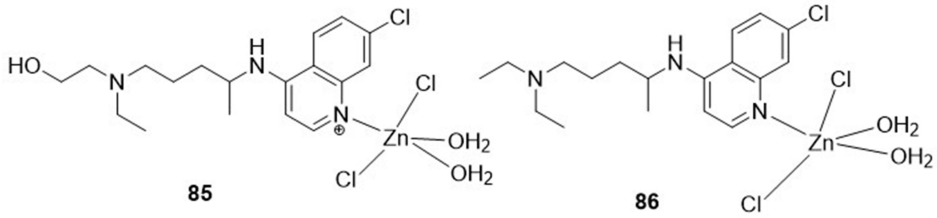


Figure 31 Chloroquine derivatives (**85** and **86**) as anti-SARS-CoV-2 agents.

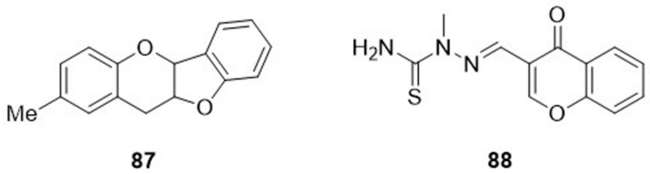


Figure 32 Chromene derivatives (**87** and **88**) as anti-SARS-CoV-2 agents.

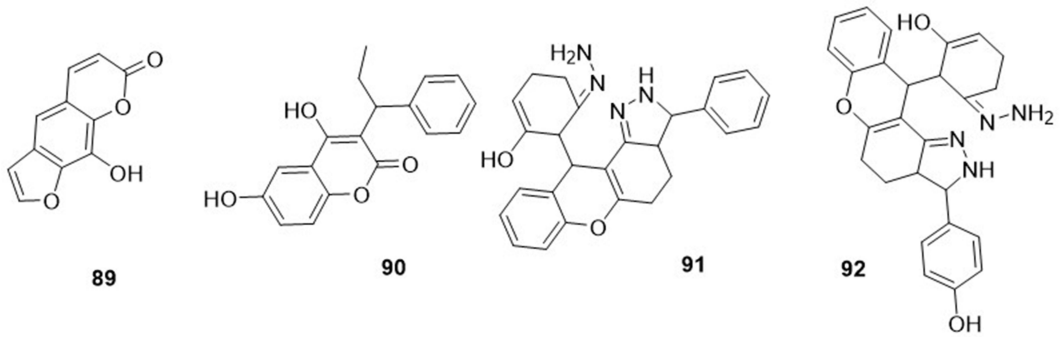


Figure 33 Coumarin derivatives (**89–92**) as anti-SARS-CoV-2 agents.

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.

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