




## REVIEW

# The role of autophagy in controlling SARS-CoV-2 infection: An overview on virophagy-mediated molecular drug targets

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## Abstract

Autophagy-dependent cell death is a prominent mechanism that majorly contributes to homeostasis by maintaining the turnover of organelles under stressful conditions. Several viruses, including coronaviruses (CoVs), take advantage of cellular autophagy to facilitate their own replication. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a beta-coronavirus ( $\beta$ -CoVs) that mediates its replication through a dependent or independent ATG5 pathway using specific double-membrane vesicles that can be considered as similar to autophagosomes. With due attention to several mutations in NSP6, a nonstructural protein with a positive regulatory effect on autophagosome formation, a potential correlation between SARS-CoV-2 pathogenesis mechanisms and autophagy can be expected. Certain medications, albeit limited in number, have been indicated to negatively regulate autophagy flux, potentially in a way similar to the inhibitory effect of  $\beta$ -CoVs on the process of autophagy. However, there is no conclusive evidence to support their direct antagonizing effect on CoVs. Off-target accumulation of a major fraction of FDA-approved autophagy modulating drugs may result in adverse effects. Therefore, medications that have modulatory effects on autophagy could be considered as potential lead compounds for the development of new treatments against this virus. This review discusses the role of autophagy/virophagy in controlling SARS-CoV-2, focusing on the potential therapeutic implications.

## KEYWORDS

autophagy, beta-coronavirus, coronavirus disease 2019, severe acute respiratory syndrome coronavirus 2, virophagy

## 1 | INTRODUCTION

Through the perpetual course of evolution, living organisms have adopted several mechanisms by which the ultimate fate of the cell is determined under unfavorable conditions (Green, 2011). An evolutionary

phenomenon, cell death, has become an integral part of life as it is the prime factor behind the cornerstone of all living activities, that is, homeostasis (Galluzzi et al., 2016). It is through the intricate clockwork of cell death that the living cells may extend their lifetime by exterminating the redundant components inside the boundary of their membranes. To

**Abbreviations:** ACE2, angiotensin-converting enzyme 2; AMPK, AMP-protein activated kinase; ARDS, acute respiratory distress syndrome; ATGs, autophagy-related-genes;  $\beta$ -CoV, beta-coronavirus; cART, combined antiretroviral therapy; CMA, chaperone-mediated autophagy; CoVs, coronaviruses; COVID-19, coronavirus disease 2019; ER, endoplasmic reticulum; HCQ, hydroxychloroquine; HIV-1, human immunodeficiency virus 1; Hsp90, heat shock protein 90; LC3, light chain 3; mTORC1, mammalian target of rapamycin complex 1; NSP6, nonstructural protein 6; PCD, programmed cell death; PI3K, phosphatidylinositol 3-kinases; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SKP2, S-phase kinase-associated protein 2; TCZ, tocilizumab.

this end, several mechanisms of cell death have been adopted by cells, the most distinguished of which are necroptosis, apoptosis, and of course, autophagy-dependent cell death (Su et al., 2015).

Known alternatively as type II cell death (Gozuacik & Kimchi, 2004), autophagy-dependent cell death is a prominent mechanism mainly concerned with modulation of dysfunctional or otherwise superfluous intracellular materials, for example, damaged organelles and misfolded proteins (Choi, 2012). Shreds of evidence have suggested that several viral pathogens such as the measles virus, Chikungunya virus, and human immunodeficiency virus 1 (HIV-1) may induce autophagy in the host cell (Joubert et al., 2012; Rozières et al., 2017; X. Wang et al., 2012). In contrast, there are Macacine alpha-herpesvirus 1 and Murine gammaherpesvirus 68 that are capable of inhibiting autophagic activity (Shojaei, Suresh et al., 2020). Several other groups, like picornaviruses, coxsackieviruses, and coronaviruses (CoVs), take advantage of cellular autophagy to accelerate their replication (Cottam et al., 2011; Kembal et al., 2010). However, not all viruses are as elusive. In the majority of cases, autophagy restricts the replication of viruses through a specific process called “virophagy,” hence, the containment of infection (Mao et al., 2019).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative pathogen behind the novel coronavirus disease 2019 (COVID-19) pandemic (Jabbari et al., 2020; Rokni, Ghasemi et al., 2020; Sheervailou et al., 2020), is a beta-coronavirus ( $\beta$ -CoV) (Banaei et al., 2020; Rokni, Hamblin et al., 2020). Notably, SARS-CoV and MERS-CoV, the culprits for the severe acute respiratory syndrome coronavirus (SARS), and the Middle East respiratory syndrome (MERS) outbreaks in the past, also belong to the same category (Lotfi & Rezaei, 2020; Rokni, Ahmadikia et al., 2020; Shafique et al., 2020). With a positive-sense RNA molecule at their core (Banaei et al., 2020; Vickers, 2017),  $\beta$ -CoVs mediate their replication through dependent or independent ATG5 pathway (Prentice et al., 2004) through specific double-membrane vesicles (DMVs) that are mostly similar to autophagosomes (Fung & Liu, 2019).

SARS-CoV and MERS-CoV are known to produce viral membrane-anchored papain-like protease/PLpro-TM polyprotein. An accomplice to the pathogenesis, this polyprotein accelerates the formation of autophagosomes while disrupting their maturation. As a consequence, functional autolysosomes are no longer generated to keep the infection contained (Chen et al., 2014). However, there are several accounts on autophagy-independent pathogenesis of  $\beta$ -CoVs, two of which were published in 2007 and 2010 (Reggiori et al., 2010; Z. Zhao et al., 2007). In this particular case, the replication is mediated by DMVs coated with nonlipidated microtubule-associated protein 1 light chain 3 (LC3)-I (Benvenuto et al., 2020). Nevertheless, in 2019 it was reported that MERS-CoV infection resulted in inhibition of autophagy in the host cell (Gassen et al., 2019).

A health issue of global concern, the COVID-19 pandemic, soon became the motive for scientists to explore the mechanisms behind the peculiar effects of SARS-CoV-2 infection on patients. Several mutations in NSP6 were reported by analyzing the genetic sequence of the SARS-CoV-2 virus (Benvenuto et al., 2020). A nonstructural

protein, NSP6, has a positive regulatory effect on autophagosome formation, suggesting a potential correlation between the SARS-CoV-2 pathogenesis mechanism and autophagy (Benvenuto et al., 2020). As of today, there are still no specific effective medications either for the prevention or treatment of COVID-19 (Lotfi et al., 2020). However, promising results have been reported following a series of attempts at repurposing the clinical application of 12 different FDA-approved drugs (COVID-19; [https://www.who.int/blueprint/priority-diseases/key-action/Table\\_of\\_therapeutics\\_Appendix\\_17022020.pdf?ua=1](https://www.who.int/blueprint/priority-diseases/key-action/Table_of_therapeutics_Appendix_17022020.pdf?ua=1)). There are several medications that are thought to have modulatory effects on autophagy; however, there is no evidence indicative of their direct antagonizing effect on  $\beta$ -CoVs. However, they can inhibit autophagy flux in a way similar to  $\beta$ -CoVs (Shojaei, Suresh et al., 2020).

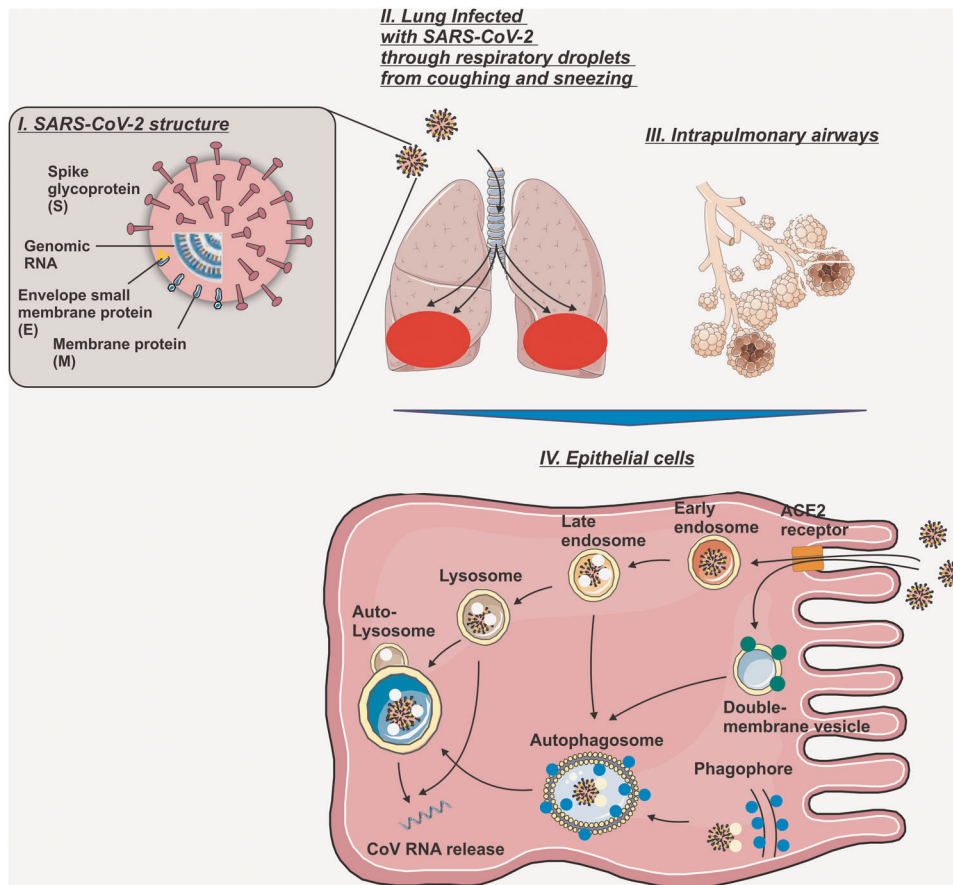
This updated review discusses the role of autophagy/virophagy in the pathogenesis of SARS-CoV-2, focusing mostly on the potential therapeutic implications. Figure 1 represents the process of the lung infection by SARS-CoV-2, followed by the autophagy pathway. Entry of SARS-CoV-2 into the lung cells is mainly mediated by the angiotensin-converting enzyme 2 (ACE2) receptor, meanwhile, autophagy has also been implicated in the viral replication in the cells, a process partly related to the formation of a DMV in the lung cells.

## 2 | AUTOPHAGY

### 2.1 | What is autophagy?

Through the last decade, a significant amount of new findings recommended that apoptosis and necrosis are often modulated by common pathways, occurring in similar subcellular organelles and compartments (Nikoletopoulou et al., 2013). The link between programmed cell death (PCD) pathways is a hot topic area (Kang et al., 2011; Rikiishi, 2012). Apoptosis, as type I programmed cell death (PCDI), is triggered by intra- or extracellular stimuli via activation of a cascade of protease enzymes (Nagata, 2018).

Recently, type II programmed cell death (PCDII), or autophagy, has been recognized as a cellular “self-eating” mechanism through which cytoplasm is surrounded by a DMV (Brest et al., 2020). Afterward, during this evolutionarily conserved process, other intracellular compartments and damaged organelles are engulfed into a double-membrane structure called phagophore that originates from cell membranes and/or cell organelles such as the endoplasmic reticulum (ER), Golgi complex, mitochondria, endosomes, and becomes an autophagosome (Salimi & Hamlyn, 2020) which ultimately fuses with the lysosome to form a degradative system, called autolysosome (Yang & Shen, 2020). Autophagy can be classified as macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) (Y. J. Li et al., 2017). More recently, the term “necroptosis” was introduced to describe a condition where necrosis occurs in a regulated and programmed manner, as an alternative to accidental death, and yet is distinctive from apoptosis (Galluzzi & Kroemer, 2008).



**FIGURE 1** The lung infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), followed by autophagy pathway. Entry of SARS-CoV-2 into the lung cells is mainly mediated by the angiotensin-converting enzyme 2 (ACE2) receptor, meanwhile the autophagy has also been implicated in the viral replication in the cells, a process partly related to the formation of a double-membrane vesicle in the lung cells

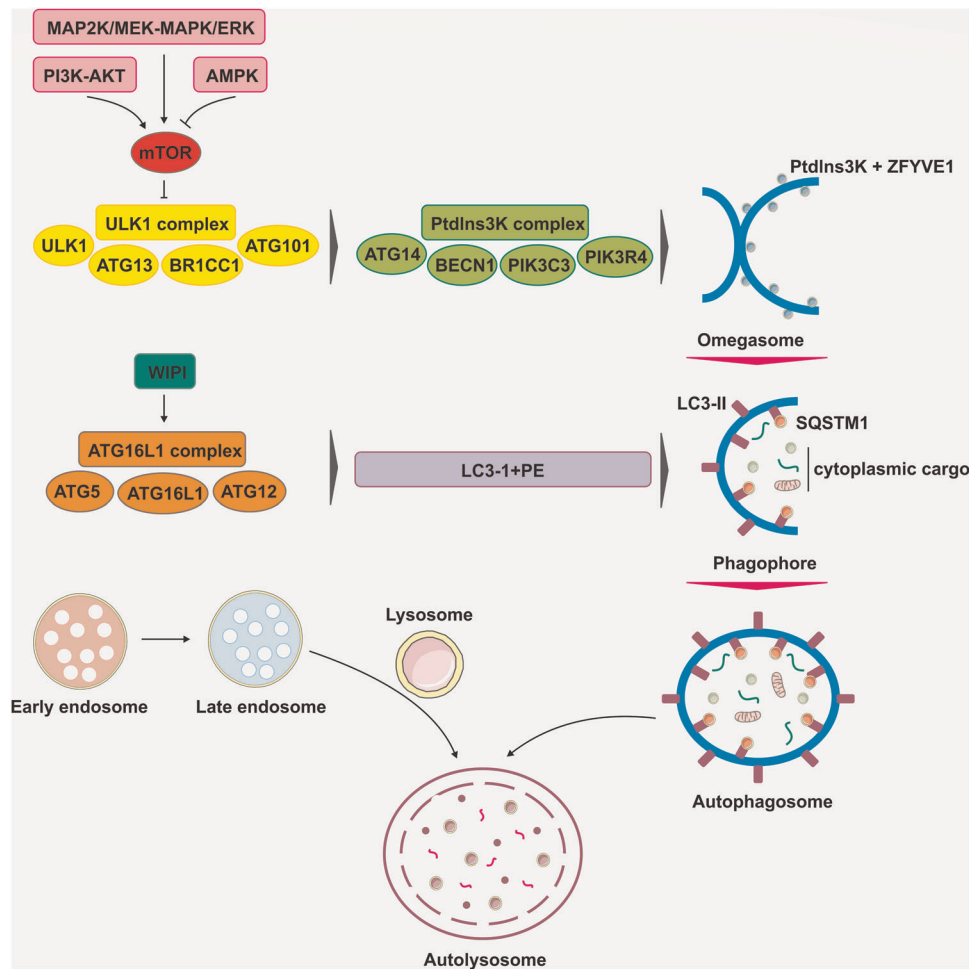
This process, in several consecutive stages, is tightly monitored by some proteins encoded by autophagy-related genes (ATGs) (Mizushima, 2020; Yin et al., 2016). The initiation stage is monitored by the ULK1/ATG1 complex, located downstream of rapamycin complex 1 (mTORC1). In contrast, the nucleation/expansion stage is controlled by the ATG14-Beclin-1-hVPS34/class III phosphatidylinositol 3-kinases (PI3K) complex and the two ubiquitin-like conjugation systems (ATG5-ATG12 and LC3/ATG8) as well. As explained here, ATG5 and ATG12 are conjugated and related with ATG16L1, through an ubiquitin-like conjugating system involving ATG7 as an E1-like activating enzyme and ATG10 as an E2-like conjugating enzyme needed for its function. The combination of ATG12-ATG5 acts as an E3-like enzyme, which is required for lipidation of ATG8 family proteins and their relationship to the vesicle membranes. ATG3 can also indirectly affect the formation of the ATG12-ATG5 complex as mature LC3 seems to be required for ATG12-ATG5-ATG16 conjugation, and deficiency of ATG3 in the cell indicates extremely reduced ATG12-ATG5 conjugation. In the final stage of this process, the autophagosome fuses with the lysosome to form an autolysosome for the effective degradation of the engulfed contents. In lung inflammation, deletion of ATG protein products results in autophagy-deficiency and can be studied for

various applications (Bello-Perez et al., 2020; Painter et al., 2020). Some studies demonstrated that cells with deletion of either ATG5 or ATG7 failed to impair the SARS-CoV replication rate (Yang & Shen, 2020). Nevertheless, another studies show that  $\beta$ -CoVs mediate their replication through the ATG5 pathway (Figure 2) (Prentice et al., 2004).

Degradation of cellular organelles or long-lived proteins through autophagy can provide an innate defense against viral pathogens. By contrast, autophagosomes can enhance infection by accelerating the formation of replicase proteins needed for viral RNA replication (Cottam et al., 2011).

## 2.2 | Cross-talk between autophagy and viral infections

For their survival, as obligate intracellular parasites, viruses can manipulate some host cell processes, such as metabolism, cellular trafficking, and host immune responses (Brest et al., 2020; Chiramel et al., 2013). It has been well-established that autophagy has multiple immunological roles that affect infection, inflammation, and immunity. In this scenario, autophagy regulates inflammation by removing



**FIGURE 2** Autophagy involved genes. Autophagy included three protein complexes: ULK1 complex, including ULK1, ATG13, RB1CC1, and ATG101; PtdIns3K complex, including the ATG14, BECN1, PIK3R4/VPS15, PIK3C3/VPS34 and ATG16L1 complex, including ATG16L1, ATG5, and ATG12. During starvation and stress the cell is in an emergency condition, when the mTOR protein is inactivated, allowing ULK1 complex formation, then activation of the PtdIns3K complex, as the result produces the PtdIns3P-rich areas on the surface of the omegasome. WIPI proteins realize this process and recruit the ATG16L1 complex and facilitate lipidation of LC3-I to make LC3-II. Expansion of the phagophore through membrane addition sequesters some of the cytoplasm and upon closure forms the autophagosome. After formation autophagosome from phagophore, autophagosomes are fused with lysosomes to form the autolysosomes, where the cargo is digested (Bello-Perez et al., 2020). ATG5, autophagy-related gene 5; ATG12, autophagy-related gene 12; ATG13, autophagy-related gene 13; ATG14, autophagy-related gene 14; ATG16L1, autophagy-related gene 16L1; ATG101, autophagy-related gene 101; BECN1, Beclin-1; BR1CC1, RB1 inducible coiled-coil 1; ERK, extracellular signal-regulated kinase; LC3-I, microtubule-associated proteins 1A/1B light chain 3B type I; LC3-II, microtubule-associated proteins 1A/1B light chain 3B type II; MAP2K, mitogen-activated protein kinase kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; PE, phosphatidylethanolamine; PIK3C3, phosphatidylinositol 3-kinase catalytic subunit type 3; PIK3R4, phosphatidylinositol 3-kinase regulatory subunit type 4; PtdIns3K, phosphoinositide 3-kinase; SQSTM1, sequestosome 1; ULK1, unc-51-like kinase 1; WIPI, WD-repeat protein interacting with phosphoinositides; ZFYVE1, zinc finger FYVE domain-containing protein 1

endogenous inflammasome agonists and influences the secretion of immune mediators; therefore, it interacts with the innate immune signaling pathway. Furthermore, autophagy serves a pivotal role in antigen processing by enhancing antigen presentation to T cells and thus initiates the adaptive immune response (Deretic et al., 2013).

As discussed before, autophagy safeguards the host cells against viral pathogens through different mechanisms, such as xenophagy or, more specifically, virophagy. Autophagy receptors can initiate virophagy through ubiquitin-dependent or independent manners

(Khaminets et al., 2016). It was previously demonstrated that ubiquitin-positive substrates, such as old protein aggregates, cellular organelles, and invading pathogens, are selectively targeted to lysosomes through this cell death mechanism (Fujita & Yoshimori, 2011). In a phenomenon termed “lysophagy,” the lysosome is selectively sequestered by autophagy when its membrane is disrupted (Hasegawa et al., 2015). On the other hand, xenophagy is an evolutionarily conserved mechanism responsible for eliminating nonhost entities after the cellular invasion (Yuk et al., 2012). Recently, it has

been proposed that lysosomal damage induces AMP-protein activated kinase (AMPK) through galectin-9 while suppressing mTOR via a galectin-based system termed GALTOR (Jia et al., 2018). In addition, some autophagy-related proteins, such as beclin-1 and LC3, are key regulators of virus-induced autophagy, and their lysosomal degradation or poly-ubiquitination reduces viral infection (Gassen et al., 2019). Besides this, through forming autophagosomes and membranes, some viruses exploit ATGs for the creation of new virions. Afterward, these pseudoenveloped virions are released from host cells and penetrate neighboring cells (Keller et al., 2020). There is also another theory suggesting that specific vesicles involved in ER-associated degradation, called EDEM1-containing organelles (EDEMosomes), are invaded by  $\beta$ -CoVs and used as membranes for the enclosure of newly generated virions (Reggiori et al., 2010).

Disruption of mitochondrial function and autophagy may affect the host cells' immune response during viral infections (Singh et al., 2021). Although it has been proposed that autophagy protects host cells against viruses, there is evidence indicating that this highly conserved cellular degradative pathway might act as a double-edged sword by serving either as an antiviral defense mechanism, or instead, as a proviral process during acute virus infection (Chiramel et al., 2013). In this regard, it has been observed that autophagy increments the genomic replication of some single-stranded RNA viruses (Fakher et al., 2020).

### 2.3 | The role of autophagy in COVID19 infection: Virophagy

Autophagy can reprocess everything. Hence, it might be inexorable that future hypothesis-driven studies will delineate the function of virophagy against COVID-19, yielding the most needed therapeutic interventions (Dalibor & Danie, 2020). Autophagy contributes to the antiviral responses as it is involved in the direct elimination of invading viruses, viral antigen presentation, suppression of excessive inflammatory reactions, and fitness of immune cells (Carmona-Gutierrez et al., 2020).

Virophagy might come into play either as a proviral or antiviral process, depending on the viral infection in the human host cell (Chiramel et al., 2013). As the name might suggest, proviral autophagy occurs when the invading virus has adopted various mechanisms to evade autophagy and manipulate it in a way that would entirely favor the replication of the virus (Kumar et al., 2020). This is highly important in terms of pathogenesis because certain groups of viruses, particularly  $\beta$ -CoVs, are known to benefit from an inappropriately induced autophagic process (Silvas et al., 2020). Thus, it would not be plausible to externally induce autophagy in hopes of mitigating the replication of the virus as it might further accelerate the progression of the infection.

There are several proteins expressed by CoVs that, despite their insignificant role in the replication process, are actively engaged in the immune escape of the virus, possibly utilized as inhibitory agents against virophagy. Findings reported by several investigations, albeit

preliminary, suggest that it might be possible to therapeutically target autophagy/virophagy in CoV infections (Ahmad et al., 2018; Dalibor & Danie, 2020; Dong & Levine, 2013; Silvas et al., 2020).

SARS-CoV-2 is a highly pathogenic virus (Wu et al., 2020). Indeed, there is a complex interaction between the virus and the autophagic pathway. Xiong et al. (2020) reported the upregulation of cell death pathways, that is, apoptosis, autophagy, and p53 pathways in peripheral blood mononuclear cells of patients infected with SARS-CoV-2. SARS-CoV-2 also downregulates autophagy-inducing spermidine and promotes the degradation of autophagy-initiating Beclin-1 (Gassen et al., 2020). According to Gorshkov et al. (2020), the cytopathic effect of SARS-CoV-2 is blocked with autophagy modulators. Moreover, results from another study have supported the hypothesis that SARS-CoV-2 upregulates the expression of genes involved in inflammation processes and cytokine signaling while downregulating the genes in mitochondria/respiration and the autophagic pathway (Singh et al., 2021).

In 2015, Y. Li et al. (2015) showed that ACE2, the cellular receptor for SARS-CoV entry (also for SARS-CoV-2), could suppress cell death, emphasizing that this protein can hinder apoptosis and autophagic pathways in pulmonary systems. Gassen et al. (2020) showed that SARS-CoV-2 infection inhibits the autophagic pathway by interfering with multiple metabolic pathways. Based on this investigation, SARS-CoV-2 prevents glycolysis by reducing the activation of AMPK and mammalian target of rapamycin complex 1 (mTORC1). Upon lysophagy, the viral genome is released, and autophagy is triggered by suppressing mTOR (rapamycin) by the GALTOR complex. In the absence of stress, the mTORC1/ULK1/2 complex suppresses phagophore formation. In the case of viral infection or under stressful situations, AMPK inhibits mTORC1 and instead activates PI3K/protein kinase B (AKT1), which triggers the autophagic pathway and results in virion encapsulation. Activation of these downstream signaling pathways helps in forming autolysosomes, followed by the fusion of autophagosomes with lysosomes to degrade the viral contents (S.-W. Tang et al., 2012). In this regard, according to Salimi and Hamlyn (2020), impaired autophagy might be the reason for COVID-19 infection, as autophagy also plays a role in the introduction of endogenous viral antigens to CD8<sup>+</sup> T cells by means of antigen-presenting cells (APCs).

Based on another hypothesis, SARS-CoV-2 might encode virulence factors that inhibit the host cell's autophagy machinery, leading to escape lysis (Brest et al., 2020). Considerably, almost all members of the respiratory CoVs have somehow evolved to selectively impede autophagy to promote their replication (Richards & Jackson, 2013). Moreover, most SARS-CoV-2 proteins are evolutionarily conserved, except for the S protein. During a process termed antibody-dependent enhancement of disease, antibodies against the S protein (spike) of SARS-CoV-2 trigger FC receptor-mediated uptake, which results in the infection of APCs. It has been proposed that APC infection might be responsible for dramatic T-cell depletion observed in some cases of SARS-CoV-2 infection (Fakher et al., 2020).

Chen et al. (2014) showed that the membrane-associated papain-like protease PLP2 (PLP2-TM) of CoVs functions as a

specific autophagy-inducing protein through interacting with LC3 and Beclin-1 (Chen et al., 2014). In this case, PLP2-TM exacerbates autophagosome accumulation and inhibits the binding of autophagosomes to lysosomes. Brest et al. (2020) suggested that the accumulation of autophagosome like DMVs also occurs during CoVs infection, providing these viruses a membrane source for their envelope and a platform for genome replication. Generally, CoVs interact with autophagy pathway components to exploit them to replicate their genetic material and suppress the autophagic flux (Gassen et al., 2019). Like other CoVs, the formation of pseudo-enveloped vesicles during SARS-CoV-2-induced autophagy relies on ER-derived membranes (Fakher et al., 2020).

Yang and Shen (2020) proposed that endocytosis of cathepsin proteases might be an essential mediator of viral entry for several CoVs, such as SARS-CoVs, MERS-CoVs, and perhaps SARS-CoV-2. As cysteine proteases, cathepsins serve pivotal roles in protein degradation in many cellular processes, specifically autophagy (Schrezenmeier & Dörner, 2020).

Benvenuto et al. (2020) performed an evolutionary analysis of 351 available sequences of the SARS-CoV-2 genome, aiming to discover any mutations developed during the COVID-19 pandemic. They found that one synonymous mutation in nonstructural protein 6 (NSP6) significantly altered the relationship of SARS-CoV-2 with its host, explicitly concerning a decisive host antiviral defense, such as the autophagic lysosomal machinery. In light of these findings, it has been reported that NSP6 of the avian coronavirus produces autophagosomes from the ER of the host cell. This protein impedes autophagosome expansion, and therefore, prevents the delivery of viral components to lysosomes for degradation (Lippi et al., 2020). NSP6 also appears to restrict the expansion of autophagosomes, thus, perturbing the naturally expected conveyance of viral components to degradative organelles, ultimately favoring CoV infection (Cottam et al., 2014).

On the other hand, CMA is a lysosomal pathway that accounts for the degradation of about one-third of cytosolic proteins under stressful conditions (Dice, 2007). The heat shock protein 90 (HSP90) is a protein relevant to viral infections (Wyler et al., 2020). It has been suggested that HSP90 might enable the SARS-CoV-2 to hijack infected cells via autophagy (Sultan et al., 2020). A previous study has reported that HSP90 and Beclin-1 functionally interact with each other to control toll-like receptor-mediated autophagy (Xu et al., 2011). Likewise, Nabirotkin et al. (2020) analyzed possible interactions between the autophagic pathways and unfolded protein response (UPR) in host cells. Interestingly, they concluded that UPR/autophagy was essential for the viral cycle of SARS-CoV-2. In this perspective, they found that activation of the NLRP3 inflammasome, another pathway associated with UPR/autophagy, was also regulated by the autophagy flux. Altogether, this overwhelming evidence suggest that autophagy plays an important role in the pathophysiology of SARS-CoV-2.

It is assumed that autophagy/virophagy modulation might eventually be part of the “disposal strategies” in the feisty fight against SARS-CoV-2. However, it is necessary to foster and extend

robust research involving the potential function of this pathway as it concerns infectious diseases, such as CoVs (Dalibor & Danie, 2020).

### 3 | DRUGS AFFECTING THE AUTOPHAGIC PATHWAY IN COVID-19 INFECTION

Table 1 presents a list of drugs used in the treatment of COVID-19, and candidate therapeutic agents (“[https://www.who.int/blueprint/priority-diseases/key-action/Table\\_of\\_therapeutics\\_Appendix\\_17022020.pdf?ua=1](https://www.who.int/blueprint/priority-diseases/key-action/Table_of_therapeutics_Appendix_17022020.pdf?ua=1)”), with potential regulatory effects on autophagy (Shojaei, Suresh et al., 2020; Yang & Shen, 2020). It should be noted that the greater proportion of these medications do not have any kind of direct antagonizing effects on SARS-CoVs or SARS-CoV-2, but rather, they inhibit the autophagy flux (Shojaei, Suresh et al., 2020). In light of this knowledge, it has been theorized that these therapeutic agents can potentially work by overaccumulating in autophagosomes, which may trigger apoptosis and result in the death of cells infected by the virus (Shojaei, Koleini et al., 2020).

#### 3.1 | Autophagy inhibitors

There is no medication to have been officially approved for selective treatment of COVID-19; however, preclinical studies have suggested repurposing a few FDA-approved drugs to this end. Almost half of these well-recognized medications are thought to be inhibitors of the pathways involved in autophagy. Thus, they are speculated to counteract the spread of infection through an indirect effect on the virus that should be mediated through the fabric of autophagy. With due attention to the cross-talk between autophagy and apoptosis, it is postulated that intracellular precipitation of autophagosomes might activate apoptosis, which would result in the death of the infected cells and cessation of virus replication. Nonetheless, one should not overlook the adverse effects that might be brought about by the mere administration of these drugs as a direct consequence of their off-target accumulation (Shojaei, Suresh et al., 2020).

##### 3.1.1 | Hydroxychloroquine/chloroquine

Despite their sought-after inhibitory effects on autophagy, hydroxychloroquine (HCQ) and chloroquine (CQ) are known to have a narrow therapeutic index that results in their dose-limiting toxicity. This poses a challenge to evaluate their mechanism of action in patients with COVID-19 (Gorshkov et al., 2020).

The pharmacological induction of autophagy with rapamycin might promote the replication of SARS-CoV-2. HCQ on the other hand, can potentially counteract this effect and result in a slower replication (Brest et al., 2020). This is a promising finding, owing to the fact that as an analog to CQ, HCQ is associated with fewer adverse effects, making it an intriguing contender for the treatment of COVID-19.

**TABLE 1** In vitro/in vivo studies and clinical trials on drugs affecting COVID-19-related autophagy genes

Product name	Description	Status of clinical development for CoV/in vivo/in vitro studies	Proposed dose for COVID-19	Autophagy-affected mechanism	Side effects
CQ/HCQ	Antimalarial agent/ Heme polymerase inhibitor	Clinical trial COVID-19 (Lu, 2020), In vitro study: COVID-19 (Gao et al., 2020; M. Wang, Cao, & Zhang, 2020) MERS (Coleman et al., 2016; Cong et al., 2018; De Wilde et al., 2014) SARS (Barnard et al., 2006; De Wilde et al., 2014; Keyaerts et al., 2004; Vincent et al., 2005)	HCQ 400 mg per day for 5 days	Inhibiting autophagy flux by decreasing autophagosome- lysosome fusion (Mauthe et al., 2018)	Retinopathy, gastrointestinal effects, cardiomyopathy, myopathy (Schrezenmeier & Dörner, 2020)
Corticosteroids	Steroid hormone	Clinical trial COVID-19 (ClinicalTrials.gov, 2020, <a href="https://clinicaltrials.gov/ct2/show/NCT04244591?draw=3">https://clinicaltrials.gov/ct2/show/NCT04244591?draw=3</a> , Clinical studies SARS (Auyeung et al., 2005; Lee et al., 2004), Clinical studies MERS (Arabi et al., 2018), Phase III clinical trial H1N1 (ClinicalTrials.gov, 2020, <a href="https://clinicaltrials.gov/ct2/show/NCT04244591?draw=3">https://clinicaltrials.gov/ct2/show/NCT04244591?draw=3</a> )	Methylprednisolone 40 mg q12 for 5 days	Inhibiting autophagy by blocking LC3 recruitment (Kyrnizi et al., 2013)	Myopathy, osteopenia/osteoporosis, decreased sex hormones (Langhammer et al., 2009)
Emtricitabine/ tenofovir (Truvada)	Nonnucleoside reverse transcriptase inhibitor + nucleoside reverse transcriptase inhibitor	Clinical trial COVID-19 (ChiCTR, 2020c)	Not available	Increasing expression/accumulation of SQSTM1/p62 (Rodriguez et al., 2019), Decreasing fusion of autophagosomes with lysosomes (Tripathi et al., 2019)	Renal toxicity (Todd Stravitz et al., 2012)
Interferon $\alpha$ -2b (Pegasis® and others PEGylated IFN $\alpha$ 2a)	Type I interferone made by leukocytes during viral infection	Clinical study MERS	180 $\mu$ g subcutaneously per week for 2 weeks	Inducing autophagy and accumulation of autolysosomes (J. Zhao et al., 2014)	Flu-like symptoms, nausea, anorexia, depression, confusion, myalgia, fatigue, joint pain (Pestka, 2007), retinopathy, neuropsychopathy (Hejny et al., 2001)
Ritonavir/ lopinavir (Kaletra)	Protease inhibitors	Clinical trial COVID-19 (ChiCTR, 2020a, 2020b, 2020c, 2020d, 2020e, 2020f, 2020g; Ning, 2020) Clinical trial SARS (Chu et al., 2004) Case report COVID-19 (B. Tang et al., 2020) Clinical study SARS (Chu et al., 2004; Que et al., 2003)	500 mg once, Twice a week, 2 weeks	Inducing autophagosome accumulation (Zha et al., 2013)	Gastrointestinal effects, headache, diabetes, hyperbilirubinemia, dizziness (Kim et al., 2015)

TABLE 1 (Continued)

Product name	Description	Status of clinical development for CoV/in vivo/in vitro studies	Proposed dose for COVID-19	Autophagy-affected mechanism	Side effects
Ruxolitinib (Jakavi®), Jakafi®)	Myelofibrosis and polycythaemia vera treatment	Retrospective cohort COVID-19 (Deng et al., 2020) Retrospective method cohort SARS (Que et al., 2003) In vivo: MERS (Sheahan et al., 2020) In vitro: SARS (Chu et al., 2004) Clinical trial COVID-19 (Cong et al., 2018)	Not available	Downregulating the mTORC1-RPS6KB-EIF4EBP1 pathway (Ishida et al., 2018), Inducing accumulation of autophagosomes (Kusoglu et al., 2020)	Anemia, pancytopenia (Alimam et al., 2015)

Abbreviations: COVID-19, coronavirus disease 2019; CQ, chloroquine; EIF4EBP1, eukaryotic initiation factor 4E-binding protein; H1N1, hemagglutinin type 1 and neuraminidase type 1 (influenza strain; aka swine flu); IFN $\alpha$ 2a, interferon alpha-2a; LC3, microtubule-associated protein 1A/1B-light chain 3; MERS, Middle East respiratory syndrome; mTORC1, mammalian target of rapamycin; P62, mouse sequestosome-1; PEGylated, polyethylene glycol; RPS6KB, ribosomal protein S6 kinase; SARS, severe acute respiratory syndrome; SQSTM1, sequestosome 1.

Several drugs are capable of altering the pH of the cell surface. This can disrupt the fusion of the virus to the host cell membrane (Fakher et al., 2020). For instance, CQ/HCQ with a dose of 400 mg per day for 5 days, affects the pH of the lysosomal membrane similarly, significantly impairing the fusion of lysosomes to autophagosomes in COVID-19 patient (Lu, 2020). A lysosomotropic agent, CQ can also prompt autophagy-independent severe disorganization in the function of the Golgi apparatus, which is suspected to be the primary mechanism behind its inhibitory effects on autophagosome-lysosome fusion (Mauthe et al., 2018). Several investigations have suggested the potential efficacy of CQ against coronavirus infections, which can be held true in the case of SARS-CoVs as well (Colson et al., 2020).

From a microscopic point of view, the action of CQ results in the accumulation of damaged mitochondria inside the cell as they can no longer be cleared due to the concomitant repression of mitophagy. This is a deleterious event, that along with the resulting oxidative stress might lead to renal tubular dysfunction in some cases (Festa et al., 2018). Thus, CQ cannot be regarded as an ideal option for the autophagy-wise treatment of COVID-19, as it may cause simultaneous tissue damage when the therapeutic goal was to decelerate replication (Edelstein et al., 2020).

### 3.1.2 | Corticosteroids

Corticosteroids are also capable of inhibiting autophagy through the blockade of LC3 recruitment. Some findings showing that 40 mg q12h methylprednisolone for 5 days could lower the mortality rate in patients with a severe form of the condition (Khosroshahi et al., 2021). Kyrmizi et al. (2013) established a crucial role in the autophagic pathway in prohibiting the intracellular growth of *Aspergillus fumigatus* within human phagocytes. Moreover, their observations on defective antifungal autophagy due to impaired Dectin-1/Syk kinase/ROS signaling provided a mechanistic explanation for the defective phagocyte function in two individual groups of patients, conferring an enhanced risk for invasive aspergillosis (Kyrmizi et al., 2013; Saghazadeh & Rezaei, 2020).

### 3.1.3 | Antivirals

Table 1 lists the in vitro/in vivo studies and clinical trials on drugs affecting COVID-19-related autophagy genes. A number of phase II, III, and IV clinical trials (NCT04261517, NCT04244591, ChiCTR2000029468, NCT04255017, ChiCTR2000029573 and NCT04414098) that aim to regulatory effects on autophagy in  $\beta$ -CoV/COVID-19 are underway.

#### *Emtricitabine/tenofovir*

A combination of lopinavir, abacavir, and raltegravir, known as LAR, has been shown to modulate several histone-modifying enzymes associated with lower susceptibility to HIV infection. LAR reduces the viral load of HIV while regulating the inappropriately high release of



cytokines and chemokines. These effects, however, are negated once there is exposure to morphine. "ERA" is another combinatorial preparation that includes emtricitabine, ritonavir, and atazanavir. Similar to LAR, ERA has also been reported to be counteracted by morphine in terms of controlling the virus replication. It is speculated that the upregulation of p62/SQSTM1 caused by antiretroviral drugs and subsequent possible modulation of autophagy by these agents might be the culprit for the increased neurotoxicity witnessed in HIV-infected primary human astrocytes treated with antiretroviral agents (Rodriguez et al., 2019).

In a separate investigation, scientists explored the mechanism behind the activation of microglia through combined antiretroviral therapy (cART). They noticed that certain combinations, such as tenofovir–disoproxil–fumarate, increased the permeability of the lysosomal membrane, leading to the ultimate disruption of lysosomal function. Through the study, a time-dependent elevation in the concentration of autophagy markers was deemed to be a sign of an increased formation of autophagosomes. Despite the accelerated autophagosome formation, however, a simultaneous defect was noted in the fusion of lysosomes to autophagosomes. Overall, the study concluded that cART might dysregulate autophagy by impairing the function of lysosomes and result in an increased level of inflammation in the neurons (Tripathi et al., 2019).

### 3.1.4 | Other compounds

Several other therapeutic agents such as clomipramine, hycanthone, verteporfin, and mefloquine may potentially block the pathogenic effect of SARS-CoV-2 in Vero-E6 cells. With an  $EC_{50}$  value of 2–13  $\mu$ M, these drugs may have the potential to be further appraised for their efficacy in the treatment of SARS-CoV-2 infection through modulation of autophagic pathways (Gorshkov et al., 2020).

A small dimeric molecule, ROC-325, is an inhibitor of autophagy, which is administered orally. Containing core motifs of HCQ and lucanthone, this novel agent inhibits lysosomal-mediated autophagy. Compared to HCQ, ROC-325 is suggested to be ten times more potent in terms of anticancer and antiautophagic activity, the latter of which may counteract the cytopathic effects of SARS-CoV-2, with negligible inherent cytotoxicity (Carew et al., 2017; Carew & Nawrocki, 2017; Jones et al., 2019). Based on the cytopathic effects assay results reported by Gorshkov et al., the counter-autophagic activity of ROC-325 was significantly correlated with repression of the cytopathic effects of SARS-CoV-2, as measured by LC3B spot counts (Gorshkov et al., 2020).

## 3.2 | Autophagy enhancers

MERS-CoV multiplication is thought to cause the Beclin-1 levels to fall and block the lysosome-autophagosome fusion. A key regulatory factor of autophagy, Beclin-1, is poly-ubiquitinated and subsequently degraded by S-phase kinase-associated protein 2 (SKP2). The activity

of SKP2, on the other hand, is controlled through phosphorylation in a hetero-complex involving AKT1, PHLPP, FKBP51, and Beclin-1. Genetic inactivity or external inhibition of SKP2 results in reduced ubiquitination of Beclin-1 and slowing down its degradation while promoting the autophagic flux. SKP2 inhibition not only accelerates autophagy but also slows MERS-CoV replication by approximately 28,000-fold. The link between SKP2 and Beclin-1 makes the molecule a potential target for antiviral drugs. Thus, agents promoting autophagy through SKP2 could be considered potential candidates for the autophagy-mediated treatment of COVID-19 (Lydie, 2020).

### 3.2.1 | Interferon alfa-2b

Interferon alfa-2b or IFN- $\alpha$ 2b is a triggering factor for the accumulation of autolysosomes in HepG2 cells. Experimental treatment with IFN- $\alpha$ 2b was reported to positively regulate the expression of Beclin-1 and LC3-II. The upregulation of these molecules is associated with the induction of autophagy, indicating that IFN- $\alpha$ 2b mediated autophagy in HepG2 cells (J. Zhao et al., 2014). The combination of IFN- $\alpha$ 2b and ribavirin has decreased viral replication and inflammatory response in the  $\beta$ -CoV infected, that is, SARS and MERS (Khosroshahi et al., 2021).

### 3.2.2 | Lopinavir/ritonavir

The most effective therapy for HIV infection to date, highly active antiretroviral therapy, consists primarily of HIV protease inhibitors (PI). Lopinavir is one such PI that, combined with ritonavir or even alone, can induce an ER stress response, inhibit cell differentiation, and initiate apoptosis in adipocytes. This type of HIV PI-induced ER stress is highly likely to be associated with the inhibition of autophagy, especially in adipocytes that is very well recorded with lopinavir/ritonavir. Thus, there might be potential therapeutic targets within the autophagy signaling pathways that could be used to treat PI-induced metabolic adverse effects in patients with HIV infection (Zha et al., 2013). Multiple clinical trials are underway to examine any possible relations between lopinavir/ritonavir and COVID-19 more precisely (NCT04255017). In this clinical trial, an open, prospective/retrospective, randomized controlled cohort study was designed to compare the efficacy of three antiviral drugs in the treatment of SARS-CoV-2 pneumonia by studying the efficacy of abidol hydrochloride, oseltamivir, and lopinavir/ritonavir in the treatment of SARS-CoV-2 viral pneumonia, and to explore effective antiviral drugs for new coronavirus (Ning, 2020).

### 3.2.3 | Ruxolitinib

Ruxolitinib is a Janus kinase inhibitor (JAK inhibitor) with selectivity for subtypes JAK1 and JAK2. JAK1 and JAK2 recruit signal transducers and activators of transcription (STATs) to cytokine receptors

leading to modulation of gene expression. According to recent findings, ruxolitinib can downregulate the mTORC1/S6K/4EBP1 pathway and induce accumulation of autophagosomes. This is thought to be mediated, at least to some extent, through inhibition of the STAT5/Pim-2 pathway, with simultaneous downregulation of BCL-xL, c-Myc, MCL-1, that induce autophagy together. Ruxolitinib can inhibit cell proliferation more effectively than it induces apoptosis. However, synergistic activation of Bak and Bax with ruxolitinib was reported to activate caspase-dependent apoptosis in HEL cells. This was achieved in a study through inhibition of BCL-xL/BCL-2 by the BH3-mimetics, ABT-737, and navitoclax. However, these findings were not exclusive to these BH3-mimetics, as obatoclax, a putative pan-BH3-mimetic, along with chloroquine and bafilomycin A1 were also capable of inhibiting and inducing apoptosis in PVTL-2 cells when administered at the same time with ruxolitinib. This suggests that the antiapoptotic family of BCL-2 is perhaps partially regulated by the mTORC1 pathway, downstream of STAT5/Pim-2. Depending on the cell type, the BCL-2 family may have protective effects on JAK2-V617F-positive leukemic cells against apoptosis induced by ruxolitinib (Ishida et al., 2018). This may have future implications in the development of therapeutics, as one study indicated that treatment with ruxolitinib resulted in the downregulation of IL-6, IL-18, JAK-2, and TYK2 (Kusoglu et al., 2020). As many patients with severe respiratory disease due to COVID-19 have features consistent with the cytokine release syndrome and increased activation of the JAK/STAT pathway, it is postulated that ruxolitinib might have a useful role in treating these patients (NCT04359290).

Originally developed as an antiviral medication against RNA viruses, remdesivir showed promising therapeutic results against the Ebola virus in a clinical trial conducted amid the 2016 Ebola outbreak. Remdesivir is an intravenous nucleotide prodrug of an adenosine analog. The active metabolite of this interferes with the action of viral RNA-dependent RNA polymerase, inhibiting viral replication through premature termination of RNA transcription. It has showed in vitro activity against SARS-CoV-2. Accordingly, remdesivir can be a good candidate for the treatment of COVID-19 as the causative agent is also an RNA virus. Following the release of the findings of an in vitro investigation that suggested the potential antiviral activity of remdesivir against SARS-CoV-2 (M. Wang, Cao, Zhang et al., 2020), several other clinical trials were rapidly initiated. The drug was reported to be effective as a novel medical therapy for in-patient management of hospitalized COVID-19 patients. It was shown to reduce the recovery time of these patients from 15 days to 11 (Zhu et al., 2020).

### 3.2.4 | Tocilizumab

According to an in vitro study on macrophages, a considerable disruption in autophagic degradation is thought to be imminent upon the prolonged exposure of these phagocytosing cells to exogenous recombinant human IL-6. This was confirmed by the elevated levels of LC3B and p62 measured in both primary and transformed

macrophages. A humanized monoclonal antibody with anti-IL-6R activity (Rokni, Hamblin et al., 2020), tocilizumab (TCZ) was recently indicated to reverse the impairment of autophagic degradation and mitigate p62 accumulation in macrophages in a paracrine manner (Hsu et al., 2021). This particular type of antibody has also been proposed as an effective candidate therapeutic option for the treatment of COVID-19 (Delorme-Axford & Klionsky, 2020). The rationale behind this proposal lies within the COVID-19-associated induction of IL-6 in the form of a cytokine storm mediated by T cells and monocytes. Thus, selective targeting of the excess IL-6 by an agent, TCZ in this scenario, is speculated to alleviate this inflammatory storm. Besides this, an improvement in body temperature and respiratory function was reported in patients treated with TCZ. Therefore, we suggest that TCZ is an effective treatment in severe patients of COVID-19 to calm the inflammatory storm and reduce mortality (Fu et al., 2020). A clinical trial of TCZ on 15 COVID-19 patients, led by Luo, concluded that TCZ was significantly correlated with the remission of IL-6-dominant cytokine storm in these patients. Accordingly, the study recommends the administration of TCZ in repeated doses for patients critically affected by COVID-19 (Luo et al., 2020). Consistently, a cohort study on 301 patients in a phase II clinical trial denoted the markedly reduced mortality in patients who had undergone a 30-day treatment with TCZ, highlighting the negligible toxicity of this monoclonal antibody. Nonetheless, successful conclusion of the ongoing third phase of this clinical investigation is warranted to safely recommend administration of TCZ (Perrone et al., 2020).

Overall, despite the available evidence, if limited, regarding the efficacy and potential adverse effects of the therapeutic agents reviewed in this paper, further investigations are needed to illustrate the common molecular mechanisms involved both in the pathogenesis of SARS-CoV-2 and pharmacodynamics of these drugs, and the potential interactions between them despite, to give us a full picture on the most optimal approach.

## 4 | CONCLUSION

Through the last two decades, possible implications of virophagy in the pathogenesis of CoVs have become an area of interest for researchers, most probably due to the recent outbreaks of SARS and MERS. Corticosteroids, antivirals, and interferons with the capability of targeting autophagy pathways may be of therapeutic value in the treatment of COVID-19. Overall, the salutary effects of these drugs might lie in the accumulation of autophagosomes, which would result in the apoptotic death of the cells infected with viruses and impairment of the replication cycle. However, notions cannot replace actual trials, and further research is required to validate any points of view that might be suggestive of possible implications of these therapeutic agents in the development of new treatments for COVID-19. Nevertheless, further clinical trials are required to confirm the potential benefits and safety of these medications, either alone in combination with antivirals.

As the most recent studies have suggested, nanomaterials, as an entirely different class of compounds, can potentially be used for the purpose of autophagy modulation. Hence, nanoparticles may provide a promising platform for specific delivery of a drug to cells infected with SARS-CoV-2 with the ultimate goal of targeting autophagic pathways. In this particular instance, nanoparticles act as carriers for the proper conveyance of therapeutic agents to the infected site, indicating that these materials are a crucial component of any given platform that provides successful specific targeting of autophagy flux.

Clinical application of nanoparticles is associated with a promising novel approach called nanomedicine, which is chiefly concerned with precise targeting of active sites while avoiding the off-target accumulation of the therapeutic agent. Thanks to this groundbreaking advancement, it can soon be possible to closely monitor the interaction between any given drug and the target cell and restrict the final therapeutic outcome only to the target area as a direct consequence of several unique properties found exclusively in nanoparticles, such as high targeting capacity, large surface-area-to-volume ratio, substantial bioavailability, and capacity to be readily modified with ligands, whose receptors are found within the target sites. In this regard, nanotechnology can be a game-changing asset in the ongoing fight against COVID-19 by paving the road for developing novel therapies that can selectively hinder the replication of the virus in target cells.

## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

Nima Rezaei proposed the general concept and supervised the project. Saman Sargazi, Roghayeh Sheervalilou, Mohsen Rokni, Milad Shirvaliloo, and Omolbanin Shahraki contributed to the data gathering, writing the manuscript, and preparing the table and figures, while Nima Rezaei contributed to study design, scientific, and structural editing. All the authors critically revised the manuscript and approved the final draft of manuscript before submission.

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