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REVIEW

Targeting the intestinal TMPRSS2 protease to prevent SARS-CoV-2 entry into enterocytes-prospects and challenges

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Abstract

The transmembrane protease serine 2 (TMPRSS2) is a membrane anchored protease that primarily expressed by epithelial cells of respiratory and gastrointestinal systems and has been linked to multiple pathological processes in humans including tumor growth, metastasis and viral infections. Recent studies have shown that TMPRSS2 expressed on cell surface of host cells could play a crucial role in activation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein which facilitates the rapid early entry of the virus into host cells. In addition, direct suppression of TMPRSS2 using small drug inhibitors has been demonstrated to be effective in decreasing SARS-CoV-2 infection in vitro, which presents TMPRSS2 protease as a potential therapeutic strategy for SARS-CoV-2 infection. Recently, SARS-CoV-2 has been shown to be capable of infecting gastrointestinal enterocytes and to provoke gastrointestinal disorders in patients with COVID-19 disease, which is considered as a new transmission route and target organ of SARS-CoV-2. In this review, we highlight the biochemical properties of TMPRSS2 protease and discuss the potential targeting of TMPRSS2 by inhibitors to prevent the SARS-CoV-2 spreading through gastro-intestinal tract system as well as the hurdles that need to be overcome.

Keywords SARS-CoV-2 · TMPRSS2 · Serine protease · Enterocytes · Drug inhibitor

Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of current pandemic coronavirus disease 2019 (COVID-19). The virus is primarily thought to infect the lungs to provoke severe acute respiratory syndrome. However, recent reports have suggested that the virus could infect other organs such as gastrointestinal tract, kidneys and liver [1–3].

The SARS-CoV-2 entry mechanism in host cells is mediated by two main pathways which involved two key proteins located on the surface of epithelia of the lung and small intestine. The first pathway is occurred by engagement of SARS-CoV-2 spike (S) glycoprotein with angiotensin converting enzyme II (ACE2), whereas the second is induced by the transmembrane protease serine 2 (TMPRSS2) protease that cleaves the (S) glycoprotein of SARS-CoV-2 to generate unlocked fusion- catalyzing form of the virus and facilitates its entry to host cells via direct fusion of the viral and plasma membrane leading to release of the viral ssRNA into the cytoplasm [4].

Recent reports have shown that SARS-CoV-2 could potentially infect enterocytes of gastrointestinal tract in humans [5]. Indeed, several clinical studies have demonstrated gastrointestinal manifestations including diarrhea, vomiting and abdominal pain in patients infected with SARS-CoV-2 [6–8]. In this review, we shed some light on the biochemical properties of TMPRSS2 protease and the potential use of therapeutics to specifically target TMPRSS2 and block its function to abrogate the entry of SARS-CoV-2 into enterocytes of gastrointestinal system.

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Biochemistry of TMPRSS2 protease

The transmembrane protease serine 2 (TMPRSS2) is a member of Hepsin/TMPRSS subfamily of type II transmembrane serine proteases (TTSP) which also includes TMPRSS1 (Hepsin), TMPRSS3, TMPRSS4, TMPRSS5 (Spinesin) and TMPRSS13 [Mosaic serine protease large form (MSPL)] [9]. TMPRSS2 is thought to play a key role in prostate epithelial cell biology, and its prominent association with prostate carcinogenesis has led to the proposal that it may be a therapeutic or diagnostic marker for prostate cancer [10].

The gene encoding TMPRSS2 resides at chromosome 21, and has 15 exons and an open reading frame of 492 amino acids [11]. *TMPRSS2* gene expression has been shown to be positively regulated by androgen hormone in prostate cancer cells, where the expression of *TMPRSS2* gene was significantly reduced during androgen deprivation [10]. Later studies conducted to understand mechanisms behind the androgen regulation of the *TMPRSS2* gene expression have identified key androgen receptor binding sites (ARBS) at ~ 13 Kb upstream of the *TMPRSS2* gene transcription start site [12].

TMPRSS2 protein is ~ 70 kDa and comprises several domains (Fig. 1): an N-terminal intracellular cytoplasmic domain (amino acid residues 1–84), a transmembrane region (residues 85–105), and a C-terminal extracellular region (residues of 106–492) that contains an LDL receptor class A- like domain (it represents a binding site for calcium), a scavenger receptor cysteine-rich (SRCR) domain (involved in binding to extracellular molecules), and a serine protease domain that cleaves at arginine (Arg) or lysine (Lys) (residues 256–489) [9, 13]. The 70 kDa TMPRSS2 is made as a precursor protein (zymogen) which has been shown to undergo autoproteolytic activation in prostate cancer cells [14]. The protease domain of TMPRSS2 belongs to the S1 family of serine proteases that cleave at Arg or Lys residues, and it shares a high degree of amino acid sequence identity with other members of TTSP, in particular, the histidine, aspartate, and serine residues which are necessary for catalytic activity [15]. Furthermore, the protein sequence of TMPRSS2 reveals that it has three Arg residues (Arg240, Arg252, and Arg255) near the N-terminus of the protease domain of TMPRSS2 [14]. Previous experiments performed using site-directed mutagenesis showed that an autoproteolytic cleavage of TMPRSS2 could occur primarily at Arg-255 and resulted in the release of the protease domain (32 kDa) to extracellular space [14]. However, the autocleavage process of TMPRSS2 has not been reported in other tissues than prostate cancer cells, and whether the mechanism is tissue specific or it is generally required for TMPRSS2 activation in various tissues still to be defined.

TMPRSS2 mediates entry of SARS-CoV-2 into human cells

TMPRSS2 protease activity is currently considered as a key mechanism for SARS corona virus entry and pathogenesis in host cells [16, 17]. Indeed, it has been demonstrated that TMPRSS2 cleaves the coronavirus (S) glycoprotein to generate unlocked, fusion-catalyzing forms of the (S) glycoprotein at the cell surface of host cells which facilitate rapid entry of the virus into cells [18]. Also, Yoshikawa and his

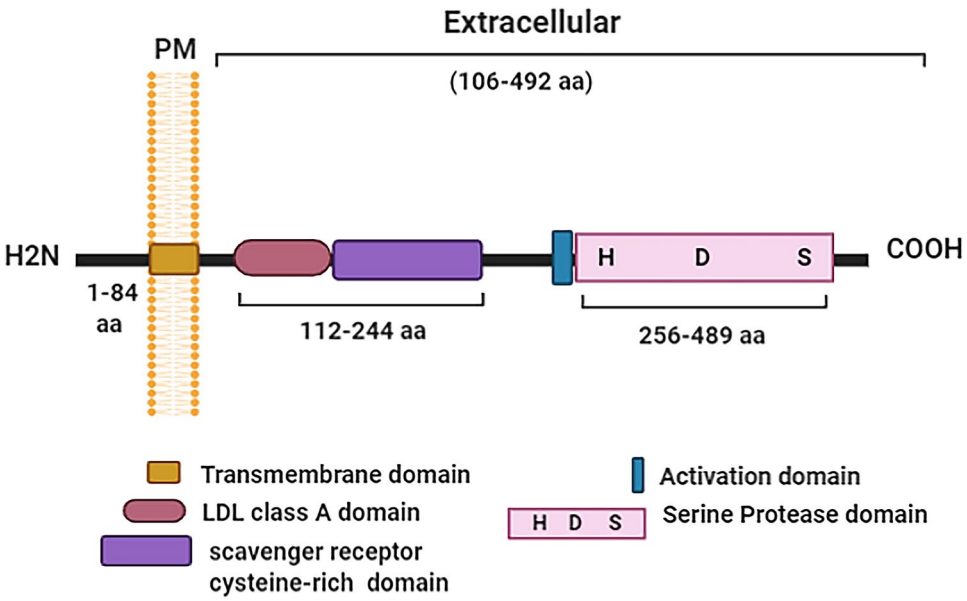

Fig. 1 Structural domains of TMPRSS2 protein. A linear map of structural domains of TMPRSS2 protein. The C-terminus (COOH end) contains the key domains; serine protease domain, required for cleavage of the virus (S) protein, and the scavenger receptor cysteine rich domain and LDL class A like receptor which are required for binding to extracellular molecules and calcium binding, subsequently. It also contains a transmembrane domain (TM) for membrane anchoring and an intracellular N-terminus (NH2 end) cytoplasmic tail for appropriate intracellular trafficking. The figure was created using BioRender.com

colleagues have used TMPRSS2-knockout (KO) mice which experimentally infected with SARS-CoV and MERS-CoV, and their results suggested that the lack of TMPRSS2 in the respiratory airways reduced the severity of lung immunopathology after infection by SARS-CoV and MERS-CoV [19]. Just recently, it has been shown that the TMPRSS2-expressing kidney epithelial cell line (VeroE6) was highly susceptible to SARS-CoV-2 infection [20], indicating that the TMPRSS2 protease activates the viral (S) glycoprotein for direct membrane fusion mechanism and is crucial for virus entry into host cells.

On the other hand, it is widely accepted that the human angiotensin converting enzyme II (ACE2) is involved in SARS-CoV-2 binding and entry into human target cells [21]. Briefly, the receptor-binding domain (RBD) of the SARS (S) glycoprotein binds to the tip of subdomain I of ACE2 [22], which then induced endocytosis of the virus that ends up in endosomal compartments, where an increase in H + influx into the endosome activates cathepsin L enzymes which activate viral (S) glycoprotein and facilitate viral membrane fusion and release of ssRNA out of the endosome [4].

It has been suggested that TMPRSS2 may also play a role in ACE2-mediated entry of SARS-CoV. Indeed, Heurich and his colleagues have shown that the co-expression of TMPRSS2 and ACE2 in 293T cells resulted in cleavage of ACE2 with a generated C-terminal ACE2 fragment of ~ 13 kDa which can be detectable in cell lysates, and the cleavage of ACE2 by TMPRSS2 resulted in augmented SARS-CoV entry into host cells [18]. Interestingly, SARS-CoV (S) glycoprotein binding to ACE2 could also induce cleavage of ACE2 by TMPRSS2, and it has been suggested that the SARS-CoV (S)-mediated shedding of ACE2 may increase the cellular uptake mechanism of virus particles by endocytosis [18, 23].

In conclusion, upon SARS-CoV-2 binding to the cell surface of a host cell, TMPRSS2 could induce viral entry into the cell by two proposed mechanisms; firstly by direct SARS-(S) glycoprotein cleavage, which activates the (S) glycoprotein for membrane fusion. Secondly by cleavage of ACE2, which then augments viral uptake through the receptor mediated endocytosis/cathepsin L-dependent pathway (Fig. 2).

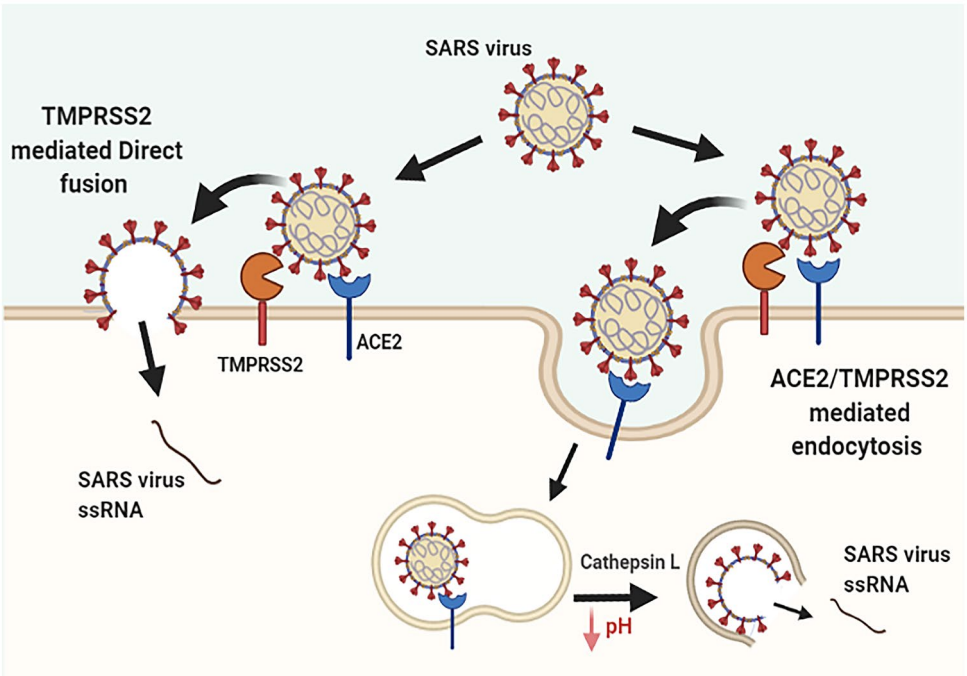

Fig. 2 TMPRSS2 mediated entry of SARS-CoV-2 into host cells. Upon SARS-CoV-2 binding to the cell surface, TMPRSS2 could potentially activate the virus entry into host cells by at least two main pathways. (Left) TMPRSS2 on the host cell surface mediates the proteolytic cleavage of the viral (S) protein which induces direct fusion of the viral and plasma membrane leading to release of the viral ssRNA into the cytoplasm. (Right) Alternatively, TMPRSS2 may cooperate with host cell receptor ACE2 in activation of SARS-CoV-2 (S) protein which then stimulates receptor mediated endocytosis, subsequently SARS-CoV-2 ends in endosomal compartments, where a decrease in endosomal pH stimulates cathepsin L enzymes which further cleave and activate viral (S) glycoprotein and facilitate the release of the viral ssRNA into the cytosol. The figure was created using BioRender.com

TMPRSS2 and SARS-CoV-2 infection of gastrointestinal tract system

Having that both ACE2 and TMPRSS2 are highly expressed in the gastro-intestinal tract (GIT), in particular by intestinal epithelial cells, which makes this region as a target for many enteric viruses including SARS-CoV-2. Indeed, SARS-CoV-2 could potentially infect the GIT system in humans [5]. In fact, it has been reported that some patients infected with SARS-CoV-2 have demonstrated gastrointestinal manifestations such as diarrhea, vomiting and abdominal pain [6, 7, 24]. Additionally, in the first case of COVID-19 infection confirmed in the United States, Holshue et al., 2020 have shown the detection of SARS-CoV-2 RNA in a stool specimen collected from the patient on day 7 of the patient's illness [25]. In a recent study conducted on 73 hospitalized patients infected with SARS-CoV-2 in China, it has been reported that about half of the patients tested positive for SARS-CoV-2 RNA in stool samples [2]. Also, in the same study, using immunofluorescent microscopy imaging technique, Xiao and his colleagues have shown that ACE2 protein was abundantly expressed in the glandular cells of gastric, duodenal, and rectal epithelia of a hospitalized patient infected with SARS-CoV-2 [2], which further supports the entry of SARS-CoV-2 into host GIT cells.

In another study, Lee et al., have utilized the human intestinal cell line (C2BBe1), characterized by high levels of TMPRSS2 and ACE2, to study the role of ACE2 and TMPRSS2 in SARS-CoV-2 infection of GI tract. The authors found that the cells demonstrated persistent infection with SARS-CoV-2 and robust viral propagation [26]. It's noteworthy to mention that the C2BBe1 cells are brush border expressing cells with microvilli resembling the brush border of human intestinal epithelia [26].

On the other hand, Zang and colleagues have shown that TMPRSS2 and TMPRSS4 serine proteases could facilitate the SARS-CoV-2 infection of human duodenum enteroids, isolated from human subjects and cultured in vitro, by inducing cleavage of the (S) glycoprotein and enhancing membrane fusion [27]. Also, they showed that human intestinal epithelial cells were predominantly infected by SARS-CoV-2 from the apical surface compared to the basolateral side [27]. Moreover, the co-expression of TMPRSS2 with ACE2 resulted in enhanced infectivity of SARS-CoV-2 in HEK293 cells [27]. Strikingly, recent studies have found high degree of co-expression correlation between ACE2 and TMPRSS2 in different human tissues, including salivary and thyroid glands, kidney, gallbladder, colon duodenum, small intestine [28] and lung tissues [29].

To sum up, there are several evidences coming from different research labs and clinical studies which claim the potential capability of SARS-CoV-2 to infect the GIT by a specific mechanism, and it seems that ACE2 and TMPRSS2 are main players in this mechanism. But, how could SARS-CoV-2 provoke GIT disorders is still to be elucidated, it could be the binding of the virus on the apical surface of intestinal enterocytes mediated by ACE2-TMPRSS2 system may cause a deregulation of the sodium dependent transmembrane transporters such as Na + /H + exchangers (NHEs) and sodium-glucose transport protein (SGLT1) located along the intestine that results in GIT manifestations such as diarrhea and abdominal pain [30, 31]. However, further research is necessary to validate such hypothesis.

Targeting of TMPRSS2 to prevent SARS-CoV-2 entry to GI tract enterocytes—potential drugs

Inhibition of TMPRSS2 could prevent SARS-CoV-2 entry into human lung cells and hence the viral respiratory infection. Indeed, it has been found that knocking-out of mouse *tmprss2* gene protected against SARS-CoV infection [19]. Although multiple types of research investigated the influence of inhibiting TMPRSS2 on SARS-CoV-2 infection in the lung [32, 33], still there are no reported studies to show clearly the effect of targeting TMPRSS2 on the SARS-CoV-2 mediated GIT infection. However, it has been suggested that targeting of TMPRSS2 and TMPRSS4 could be potentially used to reduce the GIT infection induced by SARS-CoV-2 virus [27].

Generally, most of drugs available against TMPRSS2 can be classified into two main categories: drugs that inhibit TMPRSS2 activity by either direct chemical interaction between the drug inhibitor and TMPRSS [34] or down regulate the mRNA expression of the *TMPRSS2* gene [35]. Drugs that showed inhibitory activity against TMPRSS2 and are used currently as mucolytic, anti-inflammatory, and anticoagulant drugs. For example, bromhexine and its potent metabolite ambroxol are used clinically to suppress excess pulmonary mucosal secretions and hence suppress the productive cough [36]. Bromhexine and ambroxol reduce the secretion of inflammatory mediators, such as interleukins and tumor necrosis factor-alpha (TNF-α), therefore bromhexine and ambroxol have an anti-inflammatory effect [37]. Additionally, ambroxol was found to suppress the proliferation of influenza virus in mouse lungs [38]. Interestingly, bromhexine has been demonstrated to inhibit TMPRSS2 using both in vitro and in vivo methods [39], indicating that the drug could be utilized as protective agents against SARS-CoV-2 infection. Just recently, it has been shown that bromhexine reduced clinically the SARS-CoV-2 infection in a clinical trial conducted in Iran

[40]. Notably, the drug significantly reduced the intensive care unit (ICU) transfer, intubation, and the mortality rate in patients with COVID-19 [38]. However, it was observed elsewhere that both bromhexine and ambroxol can cause a GIT disturbance, such as nausea, vomiting, and diarrhea [41]. Unfortunately, these unwanted side effects of bromhexine and ambroxol may worse the clinical symptoms among SARS-CoV-2 infected patients, who may be already suffer from GIT problems [42].

Aprotinin, camostat, and nafamostat are anti-coagulant drugs that used clinically in the treatment of thrombotic diseases [43, 44]. In fact, camostat is used in Japan for treatment of pancreatitis [45]. These drugs inhibit plasmin, kallikrein, and thrombin and also have anti-inflammatory activity through reducing the levels of interleukin-6, interleukin-8 and TNF-α [46, 47].

Strikingly, in a recent study using in silico methods, it has been pointed out that aprotinin can inhibit the serine protease activity of TMPRSS2 [48]. Also, it has been reported that aprotinin inhibited the replication of SARS-CoV-2 in non-small-cell lung cancer (Clu-3) and colon carcinoma (Caco2) cells and primary bronchial epithelial cells [49]. Additionally, aprotinin decreased the rate of mortality caused by influenza infection using in vivo mouse models [50]. In fact, aprotinin is used clinically, in Russia, for treatment of mild to moderate influenza [50]. Since TMPRSS2 plays a major role in the entry of both influenza and SARS-CoV-2 virus, it can be speculated that aprotinin can protect clinically against SARS-CoV-2 infection by inhibiting the activity of TMPRSS2.

On the other hand, camostat and nafamostat can inhibit TMPRSS2 through chemical interaction with Asp435, Ser441, and His296 residues which are essential for proper protease activity of TMPRSS2 protein [51]. Also, the compounds were shown to reduce the rate of SARS-CoV-2 entry into Calu-3 lung cells, simian kidney Vero E6 cells, and cervical cancer HeLa cells [17]. Furthermore, it was found that nafamostat inhibited MERS-CoV (S) protein-mediated viral entry to the lung cells [52], which shares similar serine protease activity with the SARS-CoV-2 virus. Notably, both camostat and nafamostat drugs have mild to moderate disturbance to the gastro-intestinal tract [53]. Making these drugs as promising candidates to prevent SARS-CoV-2 infection of GIT system. However, camostat is considered relatively safer than nafamostat, which may cause agranulocytosis, hyperkalemia, anaphylaxis, and cardiac arrest [54, 55].

Searching for natural and safer drugs, Roomi and Khan, used in silico methods for discovering potential natural compounds that can inhibit TMPRSS2 [48]. They found several natural compounds, such as salannin, deacetylsalannin, nimbolin, nobiletin, pinostrobin, sakuranetin, umuhengerin and eucalyptin, which bind with variable affinity to different amino acid residues in TMPRSS2 protein. However, further in vitro and in vivo experiments are needed to confirm these in silico findings.

On the other side, it can be proposed that drugs that down-regulate TMRPSS2 expression may be useful in decreasing SARS-CoV-2 entry and infection, compared with drugs that up-regulate TMRPSS2 expression may exacerbate SARS-CoV-2 infection. It is found that sexual hormones modulate the expression of *TMPRSS2* gene [56]. Usually, the sexual hormones are prescribed clinically in the treatment of hormonal disturbance, hypogonadism, and as contraceptives [57]. Besides, athletes used to take androgenic drugs, such as oxandrolone for performance enhancement [58]. It was found that estradiol, genistein and phytoestrogen could down regulate TMPRSS2 mRNA expression [59]. These drugs act by modulating the nuclear estrogen receptor expression. Additionally, it has been shown that the androgen receptor antagonist enzalutamide down regulated significantly the mRNA expression of the *TMPRSS2* gene [59]. On the other hand, testosterone, synthetic androgens, and estrogen receptor antagonist fulvestrant up-regulated significantly the mRNA expression of the *TMPRSS2* gene [59]. Moreover, Chu et al., have demonstrated that androgen receptor (AR) negative prostate cancer (PCa) cells showed hypermethylation and low expression levels of *TMPRSS2* gene, compared to AR-positive prostate cells which displayed hypomethylation and low expression levels of *TMPRSS2* gene [35]. Interestingly, treatment of the AR-negative prostate cells with the 5-Aza-2′-deoxycytidine (an inhibitor of DNA methylation) reversed the low expression levels of TMPRSS2 [35]. The authors found that the activation of nuclear androgen receptor reduced epigenetically the methylation of *TMPRSS2* gene which lead to an increase in *TMPRSS2* mRNA expression [35]. In another study, it was also observed through analyzing human post-mortem lung tissues that the level of *TMPRSS2* mRNA expression is inversely correlated with estrogen treatment [59]. Indicating that estrogen treatment may reduce the expression of TMPRSS2 and consequently inhibit the entry of the virus into cells. Interestingly, emerging global data shows that men appear to be at higher risk of SARS-CoV-2 infection and mortality than women [60, 61]. Thus, we think that sex hormones including estrogen and androgen may play a role in COVID-19 disease by at least the regulation of TMPRSS2 expression and subsequent effect on virus entry mechanism into host cells.

Conclusion

The recent findings of potential GIT infection by SARS-CoV-2 has opened a new door for potential fecal-oral transmission route of the virus and for developing new strategies to prevent the transmission of the virus, as well as finding new therapeutics for COVID-19 disease.

Table 1 Potential inhibitors of TMPRSS2 enzymes

Drugs	Family	Mechanism of action	Refs
Direct inhibitors of TMPRSS2 enzyme			
Bromhexine and ambroxol	Mucolytics and expectorants	Disrupts the structure of mucopolysaccharide fibres in mucoid sputum	[62]
Aprotinin	Antifibrinolytic	Pancreatic trypsin inhibitor	[63]
Camostat	Anti-inflammatory of pancreas	Serine protease inhibitor	[64]
Nafamostat	Anti-coagulant and Anti-inflammatory of pancreas	Serine protease inhibitor	[65]
Salannin, deacetylsalannin, nimbolin, nobiletin, pinostrobin, sakuranetin, umuhengerin and eucalyptin	Natural products	–Insecticidals –Anticancer –Anti-inflammatory –Antiallergic	[66–69]
Down-regulators of TMPRSS2 RNA expression			
Estradiol	Synthetic estrogen	Agonists of nuclear estrogen receptors	[70]
Genistein and phytoestrogen	Natural estrogens	Agonists of nuclear estrogen receptors	[71]
Enzalutamide	Androgen receptor antagonist	Preventing androgen to bind to the nuclear androgen receptor	[72]

The identification of compounds that specifically targets TMPRSS2 and selectively partition into the gastrointestinal tract would be of high interest given the recent evidences demonstrating the key mechanism of the virus entry mediated by TMPRSS2 localized in this region that can impact SARS-CoV-2 disease. There are many of promising potential drugs available that have been described in the literature with capability to inhibit TMPRSS2 (Table 1) either by direct inhibition of the enzyme such as bromhexine, ambroxol, camostat and nafamostate, or by deregulation of TMPRSS2 gene expression including enzalutamide, estradiol and genistein. However, there are issues and challenges before using these drugs clinically that need to be considered carefully such as safety and bioavailability of the drugs, as well as using of proper delivery methods to deliver the drugs successfully to specific target regions.

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Author contributions Dr. Ismail Mahmoud: Conception, designing and writing of the manuscript; Dr. Yazun Jarrar: Writing and revising of the manuscript.

Data availability All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Conflict of interest The authors declare no conflict of interest.

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