



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Prospective mode of action of Ivermectin: SARS-CoV-2

Vaishali M. Patil^{a,*}, Saroj Verma^b, Neeraj Masand^c^a KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad, India^b Department of Pharmaceutical Chemistry, SGT University, Gurugram, Haryana, 122505, India^c Department of Pharmacy, Lala Lajpat Rai Memorial Medical College, Meerut, Uttar Pradesh, India

ARTICLE INFO

Keywords:

Ivermectin
SARS-CoV-2
Drug repurposing
Mechanism of action
Molecular docking/dynamic simulation

ABSTRACT

The well-known anti-helminthic drug ivermectin (IVM) has been established as an example of drug repurposing for the management of SARS-CoV-2 infection. Various study has been done to understand the inhibitory mechanism of IVM against SARS-CoV-2 targets. Broadly, IVM has been categorized as a host-directed agent and the proposed mechanism involves inhibition of the IMP α /B1-mediated nuclear import of viral proteins. In addition, *in vitro/in vivo* and molecular docking/dynamic simulation studies suggested multitargets mechanism of IVM against SARS-CoV-2. Present manuscript attempts to provide an overview of the detailed mechanism of action based on experimental and computational studies. The knowledge of binding interaction of IVM and SARS-CoV-2 targets will give the direction to developed new and potential anti-COVID agents.

1. Introduction

The well-known anti-helminthic drug ivermectin (IVM) was introduced in late 1970s [1]. Few years after its approval for use in animals, it was approved for clinical use in human and received a Nobel Prize (Physiology/Medicine) in 2015 [1–3]. Initially it was named as avermectin. Naturally occurring avermectins consists of four analogs, avermectin A1, A2, B1, and B2, each of exist as two variants, a and b. During the progress and synthetic derivatization, it focused as 22,23-dihydro-avermectins. Chemical structure of IVM consists of homologues mixture of 5-O-dimethyl-22,23-dihydro-avermectin B1a (80%) and B1b (20%) [4]. IVM have been reported for high lipid solubility and broad-spectrum of activity [1–3]. It has been investigated for biological activity on parasites, nematodes, arthropods, flavivirus, mycobacteria, and mammals. IVM has been reported for its multiple mechanisms such as antiparasitic, antiviral and host immunomodulatory properties. In cancer cell, IVM could show antiproliferative action and glucose and cholesterol regulator in animals [5]. It may possess some secondary toxic effects on cells (see Fig. 1).

The broad antiviral spectrum for IVM has been explored by using experimental and theoretical studies against RNA and DNA viruses (Fig. 2). Some of the RNA viruses for which the antiviral profile of IVM has been investigated includes Dengue virus, Yellow fever virus, West Nile virus, Hendra virus (10 μ M), Newcastle virus (100 μ g/ml), Venezuelan equine encephalitis virus (1 μ M), Chikungunya virus, Semliki

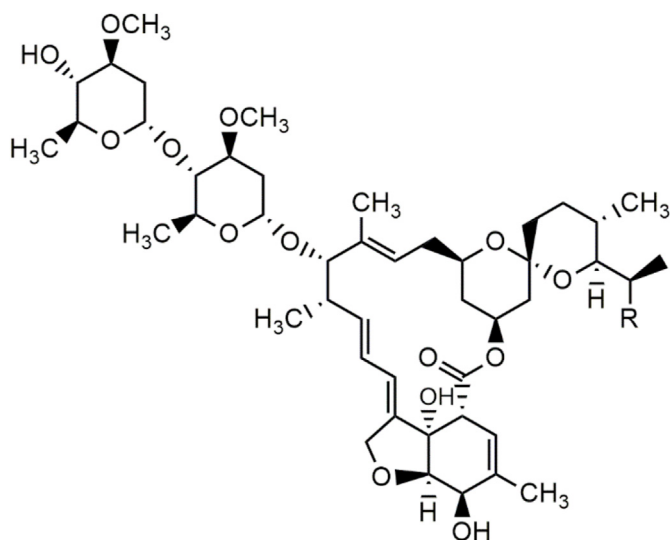
Forest virus, Sindbis virus, Avian influenza A virus (10 μ M), Porcine Reproductive and Respiratory Syndrome virus (1–15 μ M), and Human immunomodulatory virus type 1 (IC₅₀ = 4.8 μ M). IVM has exhibited inhibitory profile against DNA viruses namely, Equine herpesvirus type 1, Pseudorabies virus (1.5 or 2.5 μ M, delayed proliferation and no inhibition of virus adsorption in cells), BK polyomavirus, Porcine circovirus 2 (concentrations = 50 or 100 μ g/ml, no cytotoxicity & reduced viral load by 41% and 28.2%), and Bovine herpesvirus 1 virus (IVM concentration = 25 μ M) [6]. In case of DENV, during phase III (2014–2017) trials IVM was found to be safe (single daily oral dose) and has reduced the levels of viral NS1 protein. But it failed to produce any change in viremia [7]. The viral replication inhibitory properties of IVM in SARS-CoV-2 have been evaluated using TaqMan Real-Time RT-PCR assay resulting into IC₅₀ of ~0.2 μ M [8].

2. Mechanism of action for IVM

The efficacy of IVM in the treatment of broad spectrum of parasitic infections as well as other viruses and bacteria is well established, but the mode of action is less clear (Table 1) [9]. IVM at nanomolar concentrations affects nematode motility, feeding, and reproduction and acts via ligand-gated chloride channels, specifically those gated by glutamate [9]. In vertebrates the absence of Glutamate-gated chloride channels (GluCl_s) confers the broad safety margin of IVM. When administered at micromolar concentrations, IVM can interact with a wider range of

* Corresponding author.

E-mail addresses: vaishuwise@gmail.com (V.M. Patil), ss2016@gmail.com (S. Verma), neerajmasand@gmail.com (N. Masand).



R: CH₃ (22,23-dihydroavermectin B1b)
 R: CH₂ CH₃ (22,23-dihydroavermectin B1a)

Fig. 1. Chemical structure of Ivermectin.

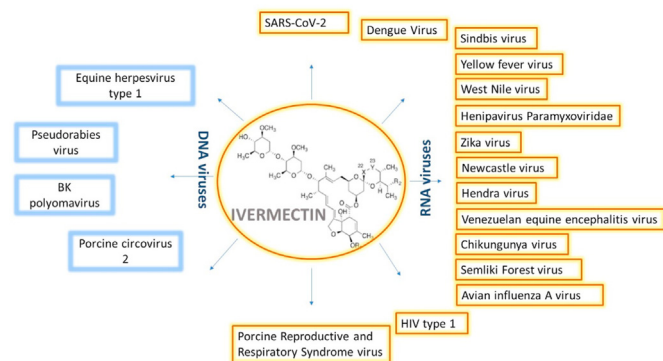


Fig. 2. Antiviral spectrum of activity for IVM.

Table 1

Reported targets for IVM and their effects.

Species/ systems	Target	Effect
Mammals	Farnesoid X receptor, WNT-TCF pathway, RNA helicase, tubulin	Glucose, cholesterol and bile homeostasis, cancer chemotherapy, immune-modulation
Nematodes	Ligand-gated chloride channels	Inhibition of feeding, motility, reproduction and host immune-modulation
Arthropods	Ligand-gated chloride channels	Inhibition of feeding, motility, reproduction, interruption of vector-borne disease transmission
Flaviviruses	Viral RNA helicase	Inhibition of replication

ligand-gated channels found in both invertebrates and vertebrates, including GABA, glycine, histamine, and nicotinic acetylcholine receptors [10].

In recent years efforts have been put to investigate the anti-viral mechanism of IVM against a broad range of viruses including RNA viruses (human immunodeficiency virus-1 (HIV-1), dengue virus (DENV), West Nile virus, Venezuelan equine encephalitic virus (VEEV), influenza,

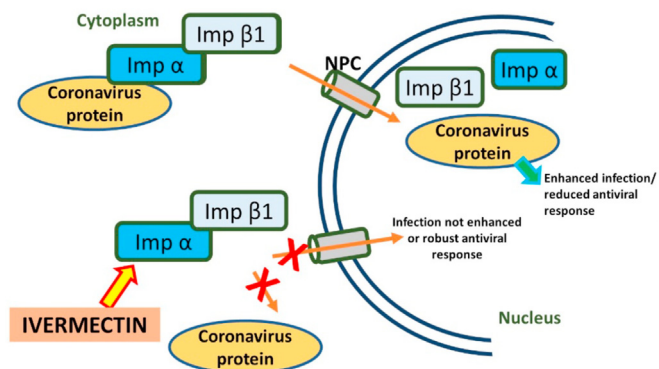


Fig. 3. Antiviral mechanism of action for IVM against SARS-CoV-2.

pseudorabies virus (PRV), Zika virus (ZIKV) and the recent coronavirus SARS-CoV-2 [11–17].

Taken together the reported studies, the anti-viral mechanism of IVM against SARS-CoV-2, the RNA virus the proposed mechanism involves inhibition of the IMP α / β 1-mediated nuclear import of viral proteins. Importins are a type of karyopherins, soluble transport receptors involved in nucleo-cytoplasmic transit of the various substrates [8, 18–20]. IMP α / β 1 binds to the coronavirus cargo protein in the cytoplasm and translocates it through the nuclear pore complex (NPC) into the nucleus where the complex falls apart and the viral cargo can reduce the host cell's antiviral response, leading to enhanced infection. IVM binds to and destabilizes the IMP α / β 1 heterodimer thus prevents IMP α / β 1 from binding to the viral protein and prevents its entry into the nucleus. This results in reduced inhibition of the antiviral responses and thus normal and more efficient antiviral response. Based on this conjecture and the reported *in vitro* studies it can be assumed that IVM has role in eliminating SARS-CoV-2 [21,22].

IVM can be categorized as a host-directed agent (HDA) as in mammalian cells IVM targets a host protein important for intracellular transport irrespective of the viral component [22]. Use of IVM as a HAD can reduce the viral load through inhibition of key cellular processes in host cells which are controlled by virus to enhance infection and thus suppress the host antiviral response. Use of IVM as HAD even at low concentration in the early stage of infection can enable the body's immune system for antiviral response before the infections takes control. A schematic presentation explaining the antiviral mechanism against SARS-CoV-2 is provided in Fig. 3.

3. SARS-COV-2 targets for binding of IVM

The very well reported targets for IVM includes glycine receptor subunit α -3 acting as a transmitter-gated ion channel activator and γ -amino butyric acid receptor subunit β -3 which controls GABA-gated chloride ion channel activity [23,24]. For SARS-CoV-2 inhibitory potential, various potential targets are under investigation. In addition to *in vivo/in vitro* studies, *in silico* studies of IVM have been reported for following SARS-CoV-2 targets [25].

1. SARS-CoV-2 Spike receptor binding domain attached to ACE2 [26].
2. 3CL protease and HR2 domain [27].
3. SARS-CoV-2 helicase [28].
4. SARS-CoV-2 S-protein [29].

The hypothesized molecular target for Ivermectin i.e., Importin- α has functional diversity and acts as a multifunctional protein like spindle assembly, lamin polymerization, nuclear envelope formation, protein degradation, mRNA-related function, cell surface function, gene expression and cytoplasmic retention [29]. Adaptor proteins bind the nuclear

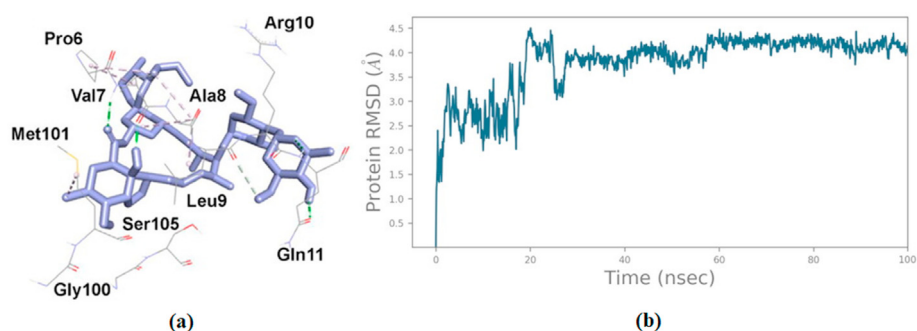


Fig. 4. Docked pose (a) and RMSD (b) of C α during 100 nsec MD simulation of IVM in complex with Nsp9 [10].

localization sequence (NLS) of import cargoes while recruiting importin β via an N-terminal importin β binding (IBB) domain. The use of adaptors greatly expands and amplifies the repertoire of cellular cargoes that importin β can efficiently import into the cell nucleus and allows for fine regulation of nuclear import [30]. It is proposed that, in addition to cellular factors, certain viral proteins may have developed IBB-like domain to efficiently enter the cell nucleus of infected cells [16].

3.1. Molecular docking/dynamic simulation interaction analysis of IVM and SARS-COV-2 targets

Drug repurposing is a very hopeful therapeutic strategy to explore drug molecules against COVID-19 [31]. Number of FDA approved drugs are presently under investigation and are in various stages of clinical development for COVID-19 treatment [32,33]. According to WHO report 2021, broad spectrum antiparasitic drug IVM advice to treatment of

Table 2

Molecular docking/dynamic simulation study of IVM with different SARS-CoV-2 protein targets.

S.N.	Targets	PDB	Docking software	MD Simulation Software and time	IVM binding interaction residues	Docking/MDS interpretation	Ref.
1	Main Protease	6LU7/6Y2E/6Y2F	AutoDock/MVD 6.0/DockThor (blind docking)	Desmond (100ns)/Gromacs (30ns)/myPresto	Gln189, Pro168, Met 165, Pro 168, Met 49, Leu50, His41/Asn151, Asp153, Asn203/Glu 19, Thr 25, Glu 47, Leu 50/Arg4, Lys5, Leu282, Ser284/	Strong and stable binding interactions/IVM B1a binds with a good affinity than IVM B1b	[41, 42,33, 47]
2	Papain-like protease	6WUU/6W9C	AutoDock/MVD 6.0	Desmond (100ns)/Gromacs (30ns)	Tyr 264, Tyr268, Pro248, Met208, Pro247/Thr74, Asn128,	Moderate affinity	[41, 42]
3	RdRp (RTP site)	7BV2	AutoDock	Desmond (100ns)	Ser549, Lys551, Lys621, Pro620, Lys798	Minimum affinity	[41]
4	RdRp (RNA site)	7BV2	AutoDock	Desmond (100ns)	Lys545, Ala688, Gln573, Val557, Leu576, Lys577		[41]
5	Helicase (Nsp13; ADP site)	6JYT	AutoDock	Desmond (100ns)	Gln537, Ala312, Ser539, Ala313, Glu540, Ala316, Lys320	Moderate affinity	[41]
6	Helicase (Nsp13; NCB site)	6JYT	AutoDock	Desmond (100ns)	Arg212, Arg178, Arg339 Ala312, Cys309, Met378	Moderate affinity	[41]
7	Nsp14 (ExoN) 5C8S	5C8S	AutoDock	Desmond (100ns)	Gln145, Ala187, Pro141, Pro142 His95, Phe146, Trp186, Phe190	Moderate affinity	[41]
8	Nsp14 (N7-MTase)	5C8S	AutoDock	Desmond (100ns)	Ala307, Arg310, Cys340, Pro342, Pro335, Lys336, Trp292, His314	Strong and stable hydrophobic/hydrophilic binding interactions	[41]
9	Spike RBD	6M0J/6M17	AutoDock	Desmond,/Gromacs (100ns)	Leu455, Gln493, Ser494, Glu484, Tyr449, Phe456, Tyr505,/Thr 500, Asn 501, Tyr 505	Moderate affinity/highly Stable during simulation	[41, 45]
10	Spike monomer (close)	6VXX	AutoDock/MVD 6.0	Desmond (100ns)/Gromacs (30ns)	Arg403, Val350, Pro507/Thr307, Glu309, Ile3,12, Asn953	Moderate affinity	[41, 42]
11	Spike trimer (open)	6VYB	AutoDock	Desmond (100ns)	Asn334, Pro337, Arg357, Val171, Arg357, Phe168	Moderate affinity	[41]
12	S2 (post fusion state)	6LXT/	AutoDock	Desmond (100ns)	Leu1166, Ala972, Ser 968/Asn 969, Gly 971 (HR1 domain)	Moderate affinity/highly Stable during simulation	[42, 43]
13	N protein (C domain)	6YUN	AutoDock	Desmond (100ns)	Gln35, Thr36, Arg73, Trp84, Ala90	Moderate affinity	[41]
14	N protein (N domain)	6YI3/6M3M/6VYO	AutoDock/MVD 6.0	Desmond (100ns)/Gromacs (30ns)	Ala50, Arg52/Arg 69, Tyr 124, Asn 127 Glu 137/Gln160, Leu161, Gly164, Thr166	Moderate affinity/highly Stable during simulation	[41, 42,43]
15	Nsp9	6WXD	AutoDock	Desmond (100ns)	Val7, Pro6, Val7, Ala8, Ala8 70, Gln11, Ala8 85, Pro6 49, Met101	Strong and stable hydrophobic/hydrophilic binding interactions	[10]
16	Nsp7, Nsp8, Nsp12 and Nsp13	6XEZ/67M1	AutoDock	Desmond (100ns)	Very week interactions	Minimum affinity	[12]
17	M Protein	Homology model	MVD 6.0	Gromacs (30ns)	Trp31, Ala 81	Moderate affinity	[11]

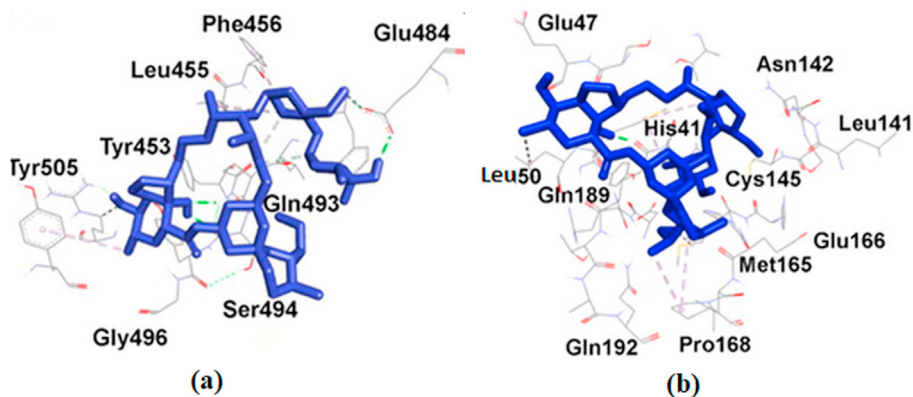


Fig. 5. Docked pose of IVM with (a) Spike RBD and (b) main protease of SARS-CoV-2 [10].

COVID-19 [34]. Various *in silico* approaches utilized to find out the binding mechanism of IVM and covid 19 drug targets [35,36]. Among these approaches, Molecular dynamics [MD] simulation is a powerful computational approach for study of conformational flexibility of water molecules, salt and entropy factors on the forces of binding interactions drug and targets. It gives virtual motion picture (video) of drug and targets interaction [37]. To promote research on covid 19 drug development, Shaw and group released the long run MD simulation trajectory and their videos of COVID-19 targets [38]. The critical analysis of the released MD simulation trajectory at atomistic level will be a big leap in the design and discovery of anti-covid drugs.

COVID-19 is an enveloped, positive-sense, single-stranded RNA beta-coronavirus consist of number of targets [39]. The IVM virtually explored against different targets to find out its binding interaction mechanism [40–45]. These targets are main proteases (M^{pro} /3CL pro), papain-like protease, RNA-dependent RNA polymerase (RdRp: RTP site/RNA site), Helicase (Nsp13; NCB/ADP site), Nsp14 (ExoN), Nsp14 (N7-MTase), nonstructural protein (Nsp9), RdRp components (nsp7, nsp8, nsp12), receptor binding domain (RBD) of the surface spike (S)/SRBD, Spike monomer (close), Spike trimer (open), S2 (post fusion state), Membrane (M) protein and C/N domains of Nucleocapsid (N) protein [41–45].

Several groups performed 100 ns MD simulation of docked complex of IVM and COVID-19 reported targets to comprehend the binding energy scenarios with aim of therapeutic drug development. Among these, IVM showed highest affinity and stability with Nsp9 (PDB: 6WXD) and Spike RBD (PDB: 6M0J) during simulation experiment [41,45]. The binding interaction stability of IVM-Nsp9 (Fig. 4) and IVM-Spike RBD docked complex confirmed by RMSD and RFMS plots [40,43]. Initially binding interaction validated by binding energy generated by docking experiment and further stability of docked model confirmed by MD simulation. The binding free energy ($\Delta G_{bind} = -84.85$ kcal/mol) showed that Nsp9 possesses highest binding affinity compared to other COVID-19 targets [40]. The SARS-CoV-2 symptoms (lowering in blood pressure followed by coma, increase in blood coagulation) associated with Nsp9 [46]. Targeted therapy of Nsp9 may be a potential therapy for COVID-19 infection. IVM showed binding interaction with LEU492, GLN493, GLY496, and TRY505 amino acid residues of spike protein which support binding of hACE2 in the active site [44]. Therefore, IVM can be a drug candidate for SARS-CoV-2 to enter the human biological milieu via hACE2 [44] (see Table 2).

Proteases also showed significant interaction and stability with IVM during MD simulation experiment [41,44,47]. In very recent, Sungur and group performed FDA approved drug repurposing against active site/allosteric binding sites of COVID-19 main protease. The obtained results indicated that IVM showed high binding affinity against main protease of COVID-19 [48]. Gonzalez-Paz et al. carried out a blind-docking experiment to predict the binding interactions of the B1a and B1b forms of IVM (Fig. 1) to main protease (3CLpro), indicating that

IVM B1a binds with a good affinity than IVM B1b [44]. Recently Mody et al. carried out computational molecular modeling study on 3987 FDA approved drugs, and 47 drugs were filtered to study their inhibitory effects on covid-19 specific 3-chymotrypsin like protease 3CLpro enzyme *in vitro*. Among filtered drug, IVM showed good inhibitory effect (IC_{50} : 21.53 μ M) against 3CLpro. The 100 ns MD simulation explained that the IVM may involve active form homodimeric of 3CLpro for its inhibitory activity [49]. Molecular docking/dynamic simulation results indicated that IVM is a multi-targeted drug. Binding interactions result suggested, Nsp9, spike protein and 3CLpro/ M^{pro} are the most probable targets for defining mode of action of IVM (Figs. 4 and 5). MD simulation-based analysis (RMSD, RMSF, radius of gyration, different energy profile, etc.), will provide atomistic level insights and benefit in designing of IVM based derivatives against COVID-19 targets.

4. Future directions

The FDA approved antiparasitic drug IVM has been used in the treatment of some neglected tropical diseases. It has been investigated to evaluate its properties towards malaria transmission and has shown well tolerance. For antiviral properties approval from FDA is not granted [50–52]. Many clinical trials have been initiated to establish anti-COVID use of IVM due its promising effects like reduced mortality rate, reduced level of inflammatory markers, and early recovery among infected individuals [53–59]. IVM belongs to Biopharmaceutical classification system (BCS) Class II having high permeability and low solubility. The limitations associated with IVM approval as anti-COVID therapeutics are smaller sample size in most of the trials, varying dose and dosage schedule, lack of double or single blinded study, coadministration of other drugs affects evaluation of safety and efficacy of IVM, lack of details about SARS-CoV-2 infection, and improper explanation of study outcomes. In addition to this, IVM is available as only oral dosage and need to be improved for pharmacokinetics as well as for targeted delivery.

IVM and associated adverse/side effects have been documented [60–63]. Further studies on use of IVM in SARS-CoV-2 infection are necessary to overcome the adverse effects mainly neurological, safety in pediatric patients and at various stages of pregnancy.

In addition to the ongoing efforts towards establishment of IVM for its antiviral properties against SARS-CoV-2, medicinal chemistry groups are focusing on structural optimization of the lead nuclei. Literature has documented investigation of IVM hybrids (*in vivo* and *in vitro* antimalarial agents), synthesis of Avermectin B1a, improved hydrophilicity for dipeptide and carbohydrate IVM B1 derivatives, and sodium 5-sulfate-IVM and disodium 4',5-disulfate-IVM [64–67]. Some of the possible strategies which can be adopted includes preparation of IVM hybrid analogs with aim to improve pharmacokinetic and pharmacodynamic profile against SARS-CoV-2. Furthermore, use of IVM as inhalation will help to provide high concentration of drug where viral load can be

Table 3

Clinical trials reporting use of IVM and its formulations for SARS-CoV-2 treatment.

ClinicalTrials.gov Identifier	Details	Ref.
NCT04510233	Ivermectin Nasal Spray for COVID-19 patients	[69]
NCT04920942	Ivermectin treatment efficacy in COVID-19 high risk patients (0.4 mg/kg/day for 5 days)	[70]
NCT04425850	Topical Ivermectin and Carrageenan to prevent contagion of COVID-19	[71]
NCT04523831	Clinical trials of Ivermectin plus Doxycycline for the treatment of confirmed COVID-19 infection	[72]
NCT04381884	Ivermectin effect on SARS-CoV-2 replication in patients with COVID-19 (600 µg/kg/once daily plus standard care)	[73]
NCT047234459	Efficacy of nano-Ivermectin impregnated masks in prevention of COVID-19 among healthy contacts and medical staff	[74]
NCT04646109	Ivermectin for severe COVID-19 management (200 µg/kg/day)	[75]
NCT04681053	Inhaled Ivermectin and COVID-19 (6 mg BID for 3 days)	[76]
NCT04739410	Effectiveness of Ivermectin in SARS-CoV-2/ COVID-19 patients (12 mg, oral stat)	[77]

effectively controlled (lungs and airways). IVM can be used in combination with other therapeutic agents having different mechanism of action for effective control of SARS-CoV-2 infection.

5. Clinical outcomes

Worldwide for effective management of SARS-CoV-2 infection various agents are in use based on their *in vitro* or observational studies. Among the reported more than 300 clinical trials evaluating efficacy of anti-COVID therapeutics, 81 report use of IVM and its formulations as therapeutic option for the treatment of SARS-COV-2 infection are reported (Table 3). For these trials results or primary end points are published and few will be reported in the coming months. Few trails report no significant improvement in the time required to resolve the symptoms [68]. The associated limitation with reported trials such as significantly different results for primary and secondary end points, and instead of virological assessments clinical characteristics reflecting viral activity were measured. More trials may be required in the coming time to understand the mechanism of IVM for other clinically relevant outcomes.

6. Conclusion

The anti-helminthic drug IVM having diverse biological activity profile has been investigated for its therapeutic potential against SARS-CoV-2 infection. IVM has been classified as host-directed agent. It shows host immunomodulatory properties and has been found to inhibit the IMP α / β 1-mediated nuclear import of viral proteins. For the efficient management of COVID-19 and its possible future outbreaks it is required to understand the new variants of SARS-CoV-2 and the possibility for target-based drug design. Over the past 1½ year more than 1400 structures of SARS-CoV-2 proteins have been deposited in the Protein Data Bank (PDB) and have contributed significantly to provide biological/virtual insight to design anti-COVID drugs. Using protein structures data, various computational studies have been done to explore IVM binding mechanism against SARS-CoV-2 targets. The experimental and molecular docking/dynamic simulation of IVM with different targets suggested NS7, spike protein and main proteases are the possible targets for defining mechanism of IVM. We hope this review will improve the understanding the mechanism of IVM against SARS-CoV-2.

Author contribution

V.M.P. and S.V. designed the review, analyzed the data and articles,

performed the analyses, and wrote the manuscript, with input from N.M. All authors reviewed the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors acknowledge the infrastructure and facilities provided by KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad, India, Department of pharmaceutical chemistry, SGT University, Gurugram, Haryana, India, and Department of Pharmacy, Lala Lajpat Rai Memorial Medical College, Meerut, Uttar Pradesh, India for completing the work.

References

- [1] F.L. Njoo, C.E. Hack, J. Oosting, L. Luyendijk, J.S. Stijl, A. Kijlstra, C-reactive protein and interleukin-6 are elevated in onchocerciasis patients after ivermectin treatment, *J. Infect. Dis.* 170 (1994) 663–668.
- [2] E. Mastrangelo, M. Pezzullo, T.D. Burghgraev, et al., Ivermectin: a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug, *J. Antimicrob. Chemother.* 67 (2012) 1884–1894.
- [3] F.S. Varghese, P. Kaukinen, S. Gläsker, et al., Discovery of berberine, abamectin and ivermectin as antivirals against chikungunya and other alphaviruses, *Antivir. Res.* 126 (2016) 117–124.
- [4] R. Laing, V. Gillan, E. Devaney, Ivermectin - old drug, new tricks? *Trends Parasitol.* 33 (2017) 463–472.
- [5] T.M. Tessier, M.J. Dodge, et al., Viral appropriation: laying claim to host nuclear transport machinery, *Cells* 8 (2019) 1–23.
- [6] F. Heidary, R. Gharebaghi, Ivermectin: a systematic review from antiviral effects to COVID-19 complementary regimen, *J. Antibiot.* 73 (2020) 593–602.
- [7] E. Yamasmith, et al., Efficacy and Safety of Ivermectin against Dengue Infection: a Phase III, Randomized, Double-Blind, Placebo-Controlled Trial. He 34th Annual Meeting the Royal College of Physicians of Thailand, Internal Medicine and One Health, Chonburi, Thailand, 2018.
- [8] L. Caly, J.D. Druce, M.G. Catton, D.A. Jans, K.M. Wagstaff, The FDA approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro, *Antivir. Res.* (2020) 104787.
- [9] R. Laing, V. Gillan, E. Devaney, Ivermectin- Old drug, new tricks? *Trends Parasitol.* 33 (2017) 463–472.
- [10] A.J. Wolstenholme, A.T. Rogers, Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics, *Parasitology* 131 (Suppl) (2005) S85–S95.
- [11] V. Gotz, L. Magar, D. Dornfeld, et al., Influenza A viruses escape from MxA restriction at the expense of efficient nuclear vRNP import, *Sci. Rep.* 6 (2016) 23138.
- [12] L. Lundberg, C. Pinkham, A. Baer, et al., Nuclear import and export inhibitors alter capsid protein distribution in mammalian cells and reduce Venezuelan Equine Encephalitis Virus replication, *Antivir. Res.* 100 (3) (2013) 662–672.
- [13] M.Y.F. Tay, J.E. Fraser, W.K.K. Chan, et al., Nuclear localization of dengue virus (DENV) 1–4 non-structural protein 5; protection against all 4 DENV serotypes by the inhibitor Ivermectin, *Antivir. Res.* 99 (3) (2013) 301–306.
- [14] K.M. Wagstaff, S.M. Rawlinson, A.C. Hearps, et al., An AlphaScreen(R)-based assay for high-throughput screening for specific inhibitors of nuclear import, *J. Biomol. Screen* 16 (2) (2011) 192–200.
- [15] K.M. Wagstaff, H. Sivakumaran, S.M. Heaton, et al., Ivermectin is a specific inhibitor of importin alpha/beta mediated nuclear import able to inhibit replication of HIV-1 and dengue virus, *Biochem. J.* 443 (3) (2012) 851–856.
- [16] E. Mastrangelo, M. Pezzullo, T. De Burghgraev, et al., Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug, *J. Antimicrob. Chemother.* 67 (8) (2012) 1884–1894.
- [17] N. Freitas, C. Cunha, Mechanisms and signals for the nuclear import of proteins, *Curr. Genom.* 10 (2009) 550–557.
- [18] K. Lott, G. Cingolani, The importin β binding domain as a master regulator of nucleocytoplasmic transport, *Biochim. Biophys. Acta* 1813 (2011) 1578–1592.
- [19] T.M. Tessier, M.J. Dodge, et al., Viral appropriation: laying claim to host nuclear transport machinery, *Cells* 8 (2019) 1–23.
- [20] M. Oka, Y. Yoneda, Importin α : functions as a nuclear transport factor and beyond, *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 94 (2018) 259–274.
- [21] M.Y. Tay, J.E. Fraser, W.K. Chan, et al., Nuclear localization of dengue virus (DENV) 1–4 non-structural protein 5; protection against all 4 DENV serotypes by the inhibitor Ivermectin, *Antivir. Res.* 99 (3) (2013) 301–306.
- [22] S.N.Y. Yang, S.C. Atkinson, C. Wang, et al., The broad spectrum antiviral ivermectin targets the host nuclear transport importin α/β heterodimer, *Antivir. Res.* 2 (2020) 104760.

- [23] H.M. Berman, J. Westbrook, Z. Feng, et al., The protein Data Bank, *Nucleic Acids Res.* 28 (2000) 235–242.
- [24] X.P. Feng, J. Hayashi, R.N. Beech, et al., Study of the nematode putative GABA type-A receptor subunits: evidence for modulation by ivermectin, *J. Neurochem.* 83 (2002) 870–878.
- [25] M. Mudatsir, A. Yufika, F. Nainu, et al., Antiviral activity of Ivermectin against SARS-CoV-2: an old-fashioned dog with a new trick - a literature review, *Sci. Pharm.* 88 (2020) 36.
- [26] S. Lehrer, P.H. Rheinstein, Ivermectin docks to the SARS-CoV-2 spike receptor-binding domain attached to ACE2, *vivo* 34 (2020) 3023–3026.
- [27] L.A. Gonzalez-Paz, C.A. Lossada, F. Romero, et al., Molecular docking and molecular dynamic study of two viral proteins associated with SARS-CoV-2 with Ivermectin, *Preprints* (2020) 2020040334.
- [28] S. Khater, G. Das, Repurposing ivermectin to inhibit the activity of SARS CoV2 helicase: possible implications for COVID 19 therapeutics. <https://www.google.com/url?sa=t&rct=j&q=&src=s&source=web&cd=&mp;cad=rja&uact=8&ved=2ahUKEwjFwe2X2YbsAhWPzDgGHQhOCd4QFjADegQIBB&url=https%3A%2F%2Fosf.io%2F8f8dseq%2Fdownload&usq=AovVaw24D-SnFreolSp0WwGaLxqD>, 2020.
- [29] O.V. Oliveira, G.B. Rocha, A.S. Paluch, et al., Repurposing approved drugs as inhibitors of SARS-CoV-2 S-protein from molecular modeling and virtual screening, *J. Biomol. Struct. Dyn.* (2020) 1–10.
- [30] Y. Miyamoto, K. Yamada, Y. Yoneda, Importin α : a key molecule in nuclear transport and non-transport functions, *JB Review* 160 (2016) 69–75.
- [31] S. Dotolo, A. Marabotti, A. Facchiano, R. Tagliaferri, A review on drug repurposing applicable to COVID-19, *Briefings Bioinf.* 22 (2021) 726–741.
- [32] C. Gil, T. Ginex, I. Maestro, V. Nozal, L. Barrado-Gil, M.A. Cuesta-Geijo, J. Urquiza, D. Ramírez, C. Alonso, N.E. Campillo, A. Martínez, COVID-19: drug targets and potential treatments, *J. Med. Chem.* 63 (2020) 12359–12386.
- [33] T.U. Singh, S. Parida, M.C. Lingaraju, M. Kesavan, D. Kumar, R.K. Singh, Drug repurposing approach to fight COVID-19, *Pharmacol. Rep.* 72 (2020) 1479–1508.
- [34] <https://www.who.int/news-room/feature-stories/detail/who-advises-that-ivermectin-only-be-used-to-treat-covid-19-within-clinical-trials>.
- [35] P.K. Ojha, S. Kar, J.G. Krishna, K. Roy, J. Leszczynski, Therapeutics for COVID-19: from computation to practices-where we are, where we are heading to, *Mol. Divers.* 25 (2021) 625–659.
- [36] R. Banerjee, L. Perera, L.M.V. Tillekeratne, Potential SARS-CoV-2 main protease inhibitors, *Drug Discov. Today* 26 (2021) 804–816.
- [37] S. Verma, Y.S. Prabhakar, Target based drug design - a reality in virtual sphere, *Curr. Med. Chem.* 22 (2015) 1603–1630.
- [38] D.E. Shaw Research, Molecular Dynamics Simulations Related to SARS-CoV-2, D. E. Shaw Research Technical Data, 2020. http://www.deshawresearch.com/resources_sarscov2.html.
- [39] G. Li, E. De Clercq, Therapeutic options for the 2019 novel coronavirus (2019-nCoV), *Nat. Rev. Drug Discov.* 19 (2020) 149–150.
- [40] Kumar BK, Faheem, K.V.G.C. Sekhar, S. Kunjiappan, J. Jamalir, R. Balaña-Fouce, B.L. Tekwani, M. Sankaranarayanan, Druggable targets of SARS-CoV-2 and treatment opportunities for COVID-19, *Bioorg. Chem.* 104 (2020) 102469.
- [41] F. Azam, I.M. Taban, E.E.M. Eid, M. Iqbal, O. Alam, S. Khan, D. Mahmood, M.J. Anwar, H. Khalilullah, M.U. Khan, An *in-silico* analysis of ivermectin interaction with potential SARS-CoV-2 targets and host nuclear importin α , *J. Biomol. Struct. Dyn.* 2 (2020) 1–14.
- [42] A.F. Eweas, A.A. Alhossary, A.S. Abdel-Moneim, Molecular docking reveals ivermectin and remdesivir as potential repurposed drugs against SARS-CoV-2, *Front. Microbiol.* 11 (2021) 592908.
- [43] H. Kaur, N. Shekhar, S. Sharma, P. Sarma, A. Prakash, B. Medhi, Ivermectin as a potential drug for treatment of COVID-19: an in-syn review with clinical and computational attributes, *Pharmacol. Rep.* 73 (2021) 736–749.
- [44] L.A. Gonzalez Paz, C.A. Lossada, L.S. Moncayo, F. Romero, J.L. Paz, J. Vera-Villalobos, A.E. Perez, E. San-Blas, Y.J. Alvarado, Molecular Docking and Molecular Dynamic Study of Two Viral Proteins Associated with SARS-CoV-2 with Ivermectin. *Preprints*, 2020.
- [45] J.K. Saha, M.J. Raihan, The binding mechanism of ivermectin and levosalbutamol with spike protein of SARS-CoV-2, *Struct. Chem.* 12 (2021) 1–8.
- [46] A.K. Chakraborty, Corona virus ORF1ab-derived Nsp9 and Nsp10 non-structural proteins have homologies to S8/S10 ribosomal proteins as well as RlmG/ ErmDrRNAMethyltransferases and may inhibit host mitoribosome assembly and protein synthesis, *Viol. Mycol.* 9 (2020) 1–6.
- [47] T.D.S. Arouche, A.Y. Martins, T.C. Ramalho, R.N.C. Júnior, F.L.P. Costa, T.S.A. Filho, A.M.J.C. Neto, Molecular docking of azithromycin, ritonavir, lopinavir, oseltamivir, ivermectin and heparin interacting with coronavirus disease 2019 main and severe acute respiratory Syndrome coronavirus-2 3C-like proteases, *J. Nanosci. Nanotechnol.* 21 (2021) 2075–2089.
- [48] M. Yuce, E. Cicek, T. Inan, A.B. Dag, O. Kurkcuoglu, F.A. Sungur, Repurposing of FDA-approved drugs against active site and potential allosteric drug-binding sites of COVID-19 main protease, *Proteins* (2021).
- [49] V. Mody, J. Ho, S. Wills, A. Mawri, L. Lawson, M.C.C.J.C. Ebert, G.M. Fortin, S. Rayalam, S. Taval, Identification of 3-chymotrypsin like protease (3CLPro) inhibitors as potential anti-SARS-CoV-2 agents, *Commun. Biol.* 4 (2021) 93.
- [50] S. Omura, A. Crump, Ivermectin: panacea for resource-poor communities? *Trends Parasitol.* 30 (2014) 445–455.
- [51] M.L. Fritz, P.Y. Siegert, E.D. Walker, M.N. Bayoh, J.R. Vulule, J.R. Miller, Toxicity of bloodmeals from ivermectin-treated cattle to *Anopheles gambiae* s.l., *Ann. Trop. Med. Parasitol.* 103 (2009) 539–547.
- [52] L.H. Kircik, J.Q. Del Rosso, A.M. Layton, J. Schaubert, Over 25 years of clinical experience with ivermectin: an overview of safety for an increasing number of indications, *J. Drugs Dermatol. JDD* 15 (2016) 325–332.
- [53] S. Ahmed, M.M. Karim, A.G. Ross, et al., A five-day course of ivermectin for the treatment of COVID-19 may reduce the duration of illness, *Int. J. Infect. Dis.* 103 (2020) 214–216.
- [54] A.Z.K. Chachar, K.A. Khan, M. Asif, K. Tanveer, A. Khaqan, R. Basri, Effectiveness of ivermectin in SARS-COV-2/COVID-19 patients, *Intermt. J. Sci.* 9 (2020) 31–35.
- [55] A.T.M.M. Chowdhury, M. Shahbaz, M.R. Karim, J. Islam, D. Guo, S. He, A Randomized Trial of Ivermectin-Doxycycline and Hydroxychloroquine-Azithromycin Therapy on COVID19 Patients. *Research Square*, Preprint, 2020.
- [56] P. Soto-Becerra, C. Culquichicón, Y. Hurtado-Roca, R.V. Araujo-Castillo, Real-world Effectiveness of Hydroxychloroquine, Azithromycin, and Ivermectin Among Hospitalized COVID-19 Patients: Results of a Target Trial Emulation Using Observational Data from a Nationwide Healthcare System in Peru, *medRxiv*, 2020. Preprint.
- [57] H.A. Hashim, M.F. Maulood, A.W. Rasheed, D.F. Fatak, K.K. Kabah, A.S. Abdulamir, Controlled Randomized Clinical Trial on Using Ivermectin with Doxycycline for Treating COVID-19 Patients in Baghdad, *medRxiv*, Iraq, 2020. Preprint.
- [58] M.S. Niaeae, N. Gheibi, P. Namdar, et al., Ivermectin as an Adjuvant Treatment for Hospitalized Adult COVID-19 Patients: a Randomized Multi-Center Clinical Trial. *Research Square*, Preprint, 2020.
- [59] M.S.I. Khan, M.S.I. Khan, C.R. Debnath, et al., Ivermectin treatment may improve the prognosis of patients with COVID-19, *Arch. Bronconeumol.* 56 (2020) 828–830.
- [60] R.E. Chandler, Serious neurological adverse events after ivermectin—do they occur beyond the indication of onchocerciasis? *Am. J. Trop. Med. Hyg.* 98 (2018) 382–388.
- [61] M. Pacque, B. Munoz, G. Poetschke, J. Foose, B.M. Greene, H.R. Taylor, Pregnancy outcome after inadvertent ivermectin treatment during community-based distribution, *Lancet* 336 (1990) 1486–1489.
- [62] J.P. Chippaux, N. Gardon-Wendel, J. Gardon, J.C. Ernoult, Absence of any adverse effect of inadvertent ivermectin treatment during pregnancy, *Trans. R. Soc. Trop. Med. Hyg.* 87 (1993) 318.
- [63] J.O. Gyapong, M.A. Chinbuah, M. Gyapong, Inadvertent exposure of pregnant women to ivermectin and albendazole during mass drug administration for lymphatic filariasis, *Trop. Med. Int. Health* 8 (2003) 1093–1101.
- [64] L. Singh, D. Fontinha, D. Francisco, A.M. Mendes, M. Prudencio, K. Singh, Molecular design and synthesis of ivermectin hybrids targeting hepatic and erythrocytic stages of *plasmodium* parasites, *J. Med. Chem.* 63 (4) (2020) 1750–1762.
- [65] S. Yamashita, D. Hayashi, A. Nakano, Y. Hayashi, M. Hirma, Total synthesis of avermectin B1a revisited, *J. Antibiot. (Tokyo)* 69 (2016) 31–50.
- [66] Y.L. Sebyakin, A.A. Loseva, U.A. Budanova, A.V. Lubeshkin, Synthesis of dipeptide and carbohydrate ivermectin B1 derivatives, *Macrocyclics* 9 (3) (2016) 314–319.
- [67] M.A. Shchetinina, E.I. Chernoburova, N.G. Kolotyrykina, et al., Synthesis of sodium 5-sulfate-ivermectin and disodium 4',5-disulfate-ivermectin, *Russ. Chem. Bull.* 67 (2018) 836–839.
- [68] E. Lopez-Medina, P. Lopez, I.C. Hurtado, et al., Effect of Ivermectin on time to resolution of symptoms among adults with mild COVID-19. A Randomized clinical trial, *J. Am. Med. Assoc.* 325 (2021) 1426–1435.
- [69] (last accessed on 20th October 2021), <https://clinicaltrials.gov/ct2/show/NCT04510233?term=ivermectin&cond=covid&draw=1&rank=1>.
- [70] (last accessed on 20th October 2021), <https://clinicaltrials.gov/ct2/show/NCT04920942?term=ivermectin&cond=covid&draw=1&rank=2>.
- [71] (last accessed on 20th October 2021), <https://clinicaltrials.gov/ct2/show/NCT04425850?term=ivermectin&cond=covid&draw=1&rank=3>.
- [72] (last accessed on 20th October 2021), <https://clinicaltrials.gov/ct2/show/NCT04523831?term=ivermectin&cond=covid&draw=1&rank=4>.
- [73] (last accessed on 20th October 2021), <https://clinicaltrials.gov/ct2/show/NCT04381884?term=ivermectin&cond=covid&draw=1&rank=6>.
- [74] (last accessed on 20th October 2021), <https://clinicaltrials.gov/ct2/show/NCT04723459?term=ivermectin&cond=covid&draw=1&rank=7>.
- [75] (last accessed on 20th October 2021), <https://clinicaltrials.gov/ct2/show/NCT04646109?term=ivermectin&cond=covid&draw=1&rank=8>.
- [76] (last accessed on 20th October 2021), <https://clinicaltrials.gov/ct2/show/NCT04681053?term=ivermectin&cond=covid&draw=1&rank=10>.
- [77] (last accessed on 20th October 2021), <https://clinicaltrials.gov/ct2/show/NCT04739410?term=ivermectin&cond=covid&draw=1&rank=13>.