PSM
Microbiology

Review Article **2025 | Volume 10 | Issue 1 | 159-176**

Revolutionizing Hepatitis C Treatment: The Cutting-Edge World of Hepatitis C Virus Inhibitors

Quazi M I Haq*[1], Ghulam Abbas[1]

Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, Nizwa, Oman.

Abstract:

Hepatitis C Virus (HCV) inhibitors have emerged as a beacon of hope in the battle against a persistent and potentially life-threatening global health threat. This review article delves into the expansive landscape of HCV inhibitors, showcasing their remarkable progress and the transformative impact they have had on the management of HCV infection. The HCV inhibitors can be broadly categorized into three main classes: direct-acting antivirals (DAAs), host-targeting agents, and immunotherapies. DAAs have gained significant attention due to their high efficacy and tolerability. Host-targeting agents, such as cyclophilin inhibitors and microRNA-based therapeutics, present alternative approaches to combat HCV infection by targeting host factors essential for the virus's life cycle. Immunotherapies, including therapeutic vaccines and monoclonal antibodies, have opened new frontiers in HCV treatment, enhancing viral clearance and reducing the risk of reinfection. Throughout this review, we also address ongoing challenges, including drug resistance, access disparities, and emerging HCV genotypes. As HCV inhibitors continue to evolve, their role in eliminating hepatitis C as a global health threat becomes increasingly prominent. In conclusion, this comprehensive overview of HCV inhibitors underscores their pivotal role in reshaping the landscape of hepatitis C treatment. The ongoing development and diversification of these inhibitors offer optimism for achieving a world free from the burden of HCV infection.

Open Access

Article Information

Received: October 3, 2025

Accepted: October 19, 2025

Published: November 8, 2025

Keywords

Hepatitis C Virus (HCV),
HCV Inhibitors,
Direct-Acting Antivirals (DAAs),
Immunotherapy,
Therapeutic Vaccines,
Monoclonal Antibodies.

Authors' Contribution

QMIH conceptualised the study; GA participated in data collection and the conduct of the experiments. QMIH, contributed to writing the manuscript. All authors read and approved the final version of the manuscript.

How to cite

Haq, Q.M.I., Abbas, G., 2025. Revolutionizing Hepatitis C Treatment: The Cutting-Edge World of Hepatitis C Virus Inhibitors. PSM Microbiol., 10(1): 159-176.

***Correspondence**

Quazi M I Haq, Ghulam Abbas
Email:
quaziimran@unizwa.edu.om
abbashej@unizwa.edu.om

1 These authors contributed equally to this work.

INTRODUCTION

Millions of individuals worldwide suffer from hepatitis C virus (HCV) infection, which has long been considered a serious public health threat. HCV infection frequently results in severe liver disorders such cirrhosis and hepatocellular carcinoma. The discovery of HCV in 1989 marked a crucial milestone in understanding the etiology of non-A, and non-B hepatitis and paved the way for the development of effective antiviral therapies (Choo *et al.,* 1989). Over the past few decades, the field of HCV research has witnessed remarkable progress, particularly in the discovery of HCV inhibitors, which have revolutionized the management of this viral infection.

A sustained virological response (SVR), which denotes the absence of measurable HCV RNA in the blood, is the main objective of HCV therapy and is considered a cure for the infection. Historically, interferon-based regimens were the mainstay of HCV treatment, but there is an immediate need for new effective and well-tolerated alternatives due to their low efficacy and serious adverse effects (Pawlotsky, 2014).

This review article provides a comprehensive overview of the landscape of HCV inhibitors, including direct-acting antivirals (DAAs), host-targeting agents, and immunotherapies. We examine the development, mechanisms of action, and clinical impact of these inhibitors in the context of HCV treatment. Furthermore, we explore the economic and public health implications of the widespread adoption of HCV inhibitors and their role in the global effort to eliminate HCV as a major health threat.

The advent of DAAs, such as sofosbuvir and ledipasvir, has transformed HCV therapy, providing cure rates exceeding 90% in many cases and minimizing the side effects associated with interferon-based treatments. Host-targeting agents, including cyclophilin inhibitors and microRNA-based therapeutics, offer innovative approaches to HCV treatment by targeting host factors essential for the virus's life cycle. Immunotherapies, such as therapeutic vaccines and monoclonal antibodies, have expanded the therapeutic arsenal, enhancing viral clearance and reducing the risk of reinfection.

As the world strives to achieve the World Health Organization's goal of eliminating viral hepatitis as a public health threat by 2030, HCV inhibitors stand as key players in this ambitious endeavor (World Health Organization, 2016). However, challenges such as drug resistance and disparities in access to these therapies persist and warrant ongoing attention.

In this review, we synthesize the state of the art in HCV inhibitors, shedding light on their transformative potential and underscoring their critical role in reshaping the future of hepatitis C treatment.

Review of Literature

Direct-Acting antivirals (DAAs) revolutionizing HCV treatment

With the development of direct-acting antivirals (DAAs), the therapy landscape for HCV has changed. DAAs are tiny compounds that interfere with the HCV life cycle by targeting particular viral proteins. Sofosbuvir, a uridine analog, and ledipasvir, a non-structural protein 5A inhibitor, have been particularly instrumental in achieving remarkable cure rates (SVR) exceeding 90% in most cases (Charatcharoenwitthaya *et al.,* 2020). Their high specificity and reduced side effects compared to interferon-based therapies have made them the cornerstone of current HCV treatment regimens.

A comparison of approved DAAs with emerging inhibitors

A number of DAAs have been approved by health agencies in recent years and play an important role in the treatment of hepatitis C. These drugs are tolerable, highly effective, and work against all genotypes of the disease. Several drug combinations have demonstrated very positive effects, for example, sofosbuvir, ledipasvir, glecaprevir/pibrentasvir (which was approved by the FDA in 2017), and sofosbuvir/velpatasvir/voxilaprevir (Feld *et al.,* 2015; Jacobson *et al.,* 2017). In most cases, these types of treatment result in more than 95% successful elimination of the virus, take less time, and have fewer side effects than interferon treatment.

New drugs continue to improve their ability to treat a wide range of genotypes and difficult-to-cure diseases, according to the FDA's Drug Approval Database. In addition, the antiviral compounds we mentioned, which inhibit NS2 PROTEASE, NS3, and NS5B, are still in early stages of development, but interesting results have been found regarding their effectiveness, their resistance, and how they work.

In order to assist you, we have outlined the differences between FDA-approved DAAs and newly reported inhibitors in terms of their targets, effectiveness, and stage of development (Table 1). By doing so, it is possible to uncover that new druglike molecules may be combined with or enhanced by current treatments. Latest studies have again shown that sofosbuvir-based therapy is very effective in different patient groups (Zhang *et al.,* 2024).

Table 1. Newly reported HCV inhibitors compared with FDA-approved DAAs. Chatel-Chaix et al. (2012)

Drug/Compound	Target	Clinical Status	SVR/Efficacy	Unique Features
Sofosbuvir	NS5B Polymerase	FDA-approved	>95% (pan-genotypic)	First-in-class nucleotide analog
Ledipasvir	NS5A	FDA-approved	~95% (GT 1, 4–6)	High potency, often co-formulated with sofosbuvir
Glecaprevir/Pibrentasvir	NS3/4A + NS5A	FDA-approved	>97% (all genotypes)	Short duration (8 weeks), pan-genotypic
Voxilaprevir	NS3/4A Protease	FDA-approved (combo)	>95% (re-treatment)	Used in combination for resistant cases
Compound 1/2/3	NS2 PROTEASE INHIBITORS Protease	Preclinical	EC_{50} ~39–43 µM	First-in-class NS2 PROTEASE INHIBITORS auto-protease inhibitors
Compound 17–23	NS3 Protease	Preclinical	EC_{50} 5–70 nM	Optimized SAR for potent enzymatic inhibition
Compound 30/31	NS3 Helicase	Preclinical	IC_{50} ~1.5–5.6 µM	Novel dsRNA-intercalating mechanism
Compound 39	NS3/4A Protease	Preclinical	EC_{50} 7–9 nM	Effective against wild-type and mutant proteases

Note: SVR = Sustained Virological Response; EC_{50}/IC_{50} = Effective/Inhibitory concentration for 50% activity; GT = Genotype

It is possible that HCV clearance brought on by direct-acting antivirals (DAAs) improves outcomes at every stage of liver disease. However, the information that is currently available indicates that treating patients before they develop compensated advanced chronic liver disease (cACLD) yields the greatest quantifiable benefit. All patients with chronic hepatitis C should ideally receive treatment before they progress to advanced cirrhosis or fibrosis because, even if sustained virologic response (SVR) improves survival and lowers the risk of hepatic events (such as decompensation and hepatocellular carcinoma

[HCC]), it cannot completely prevent further liver disease progression and unfavorable outcomes, such as hepatic deaths (Calvaruso and Craxì, 2020).

A study explored the mechanisms involved in liver damage resolution following hepatitis C virus (HCV) clearance. After the virus is eradicated through specific antiviral treatments, patients aim to restore their metabolic functions in an environment previously marked by significant cellular destruction. The recovery route of the liver can vary, ranging from fibrosis to decompensated cirrhosis. Consequently, with the use of direct-acting antiviral (DAA) therapy and assuming sustained virological response (SVR) is achieved, the administration of antifibrotic drugs is increasing to enhance liver regeneration by reducing fibrotic tissue generated by HCV. Additionally, the use of antifibrotic drugs during HCV treatment helps prevent the increase of extracellular matrix (ECM) deposition, thereby decreasing hepatic stellate cell (HSC) activation. This approach in liver regression and restoration of liver damage offers promising prospects for patients who resolve HCV infection at advanced stages of fibrosis or cirrhosis (Salas-Villalobos *et al.,* 2017).

In another study, a notable reduction in inflammation in patients with SVR was observed. After treatment, there was no cirrhosis regression in any of the patients. Forty livers, including 21 with hepatocellular carcinoma (HCC), tested showed no signs of occult HCV infection. Increased fibrosis and inflammation were linked to the development of HCC, and the number of patients who developed HCC was comparable in the SVR and non-SVR groups (Celli *et al.,* 2021).

Host-Targeting agents: Innovative approaches to HCV treatment

Beyond DAAs, host-targeting agents have emerged as promising alternatives. Cyclophilin inhibitors, such as alisporivir, target host cell factors essential for the HCV life cycle. These agents offer potential advantages in terms of reduced viral resistance development (Foster *et al.,* 2013). Additionally, microRNA-based therapeutics are being explored, presenting an innovative approach to modulating host cellular responses to HCV infection, which may hold significant promise in the future.

Compared to direct-acting antivirals (DAA), HTAs are effective against many genotypes and frequently occurring resistance-related changes (RASs).

In recent years, it has become clear that several unusual HTAs have achieved positive results in early animal or human research, such as those against cyclophilin A, miR-122 inhibitors, or those that interfere with lipid metabolism. Researchers are currently developing cyclophilin inhibitors that target specific cells and have fewer side effects (Zhou *et al.,* 2023a). Several researchers have also suggested that the use of both HTAs and DAAs may reduce the likelihood of resistance and facilitate the successful treatment of difficult-to-treat patients. Consequently, HTAs could play an important role in future treatment plans, especially in regions that do not yet have access to DAA combinations or where the treatment has failed in the past.

In recent years, advances in medicine have highlighted the importance of HTAs in the management of hepatitis C. As a result of the low likelihood of resistance to alisporivir and similar drugs targeting cyclophilin, they continue to be effective against many kinds of viruses. Furthermore, miRNA drugs, particularly those targeting miR-122, are advancing in the clinical trial stage. In Phase II trials, Miravirsen and its relatives continue to suppress the hepatitis C virus (Kim *et al.,* 2022).

Immunotherapies: Enhancing HCV clearance and preventing reinfection

Immunotherapies are another exciting dimension of HCV treatment. Therapeutic vaccines and monoclonal antibodies offer potential adjuncts to antiviral therapy, enhancing the host's immune response and reducing the risk of reinfection.

Early studies show promise in using monoclonal antibodies against HCV envelope proteins, preventing viral entry into host cells (Fafi-Kremer *et al.,* 2010).

According to recent studies, immunotherapies are playing a greater role in the treatment of HCV, improving and extending the period during which the virus is cleared (Walker *et al.,* 2023). Furthermore, several studies are exploring how therapeutic vaccines based on mRNA and vectors may enhance T-cell activity (Garcia *et al.,* 2024).

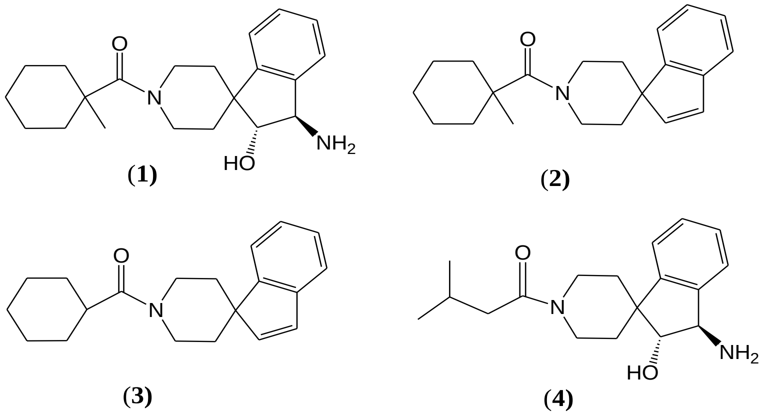
Global efforts toward HCV elimination

The widespread adoption of HCV inhibitors has not only transformed individual treatment but also played a pivotal role in global endeavors to eradicate HCV as a hazard to public health. HCV inhibitors are essential to reaching the World Health Organization's (WHO) lofty target of eradicating viral hepatitis by 2030 (World Health Organization, 2016). Their efficacy, safety profile, and accessibility are central factors in making HCV elimination feasible.

A. Inhibitors of NS2 PROTEASE INHIBITORS

The non-structural protein 2 (NS2 PROTEASE INHIBITORS) of the hepatitis C virus (HCV) encodes an auto-protease activity that is necessary for viral replication, making it a desirable antiviral target.

A library of compounds created using structure-guided virtual high-throughput screening against NS2 protease inhibitors and autoprotease was examined in a study by Joseph Shaw and colleagues. A series of chemicals were first tested *in vitro* to assess whether they might block the NS2 protease inhibitors auto-protease from producing one of its substrate products. In order to specifically test the ability to disrupt NS2 protease inhibitors-dependent HCV genome replication, a cell-based assay was conducted. In each of these tests, compound **1** was recognized to be a "hit," with an EC_{50} of 39.0 µM and 43.4 µM, respectively. Among the numerous analogs of compound 1 studied, the active analogs, compounds **2** and **3,** represent an intriguing chemotype (Figure 1). According to a structure-activity relationship (SAR) analysis, for NS2 protease inhibitors auto auto-protease inhibitors to work, compound **1** primary alcohol and amine groups at the stereocenters of the indene core are not required. However, compound **4** showed that the presence of the cyclohexane part was extremely important for action (Shaw *et al.,* 2015a).



(1) (2)

(3) (4)

Fig. 1. Hepatitis C virus (HCV) non-structural protein 2 (NS2 protease inhibitors) autoprotease inhibitors.

In a different investigation, J. Shaw and colleagues revealed a number of small compounds that include epoxides that can inhibit NS2 protease inhibitors-NS3 proteolysis *in vitro* and show the possibility of selectivity towards the NS2 protease inhibitors autoprotease. Compound **5** (EC_{50} = 93 µM) was found to potentially impede in vitro NS2 protease inhibitors-NS3 processing in a dose-dependent manner in this investigation. With an EC_{50} value of 55 µM, a 4-benzyloxy, 3-chloro substituted derivative (**11**) was the most effective inhibitor. Numerous chemical alterations, like the removal of benzene's 3-chloro substituent (**6**) and the addition of a 4-bromo substituent without (**7**), decreased the activity of compound 5 to below the level of detection. Compound **8** with the 3-chloro group, and compounds **10**, with a 4-amino-phenoxy substituent respectively, exhibited some activity (96 to >300 µM) but to a lesser extent to that shown by **5**, though a 3-phenyloxy substituent (**9**) as shown in Figure 2, did enhance activity of 70 µM. According to these results, inhibiting the NS2 protease inhibitor's auto-protease could be a viable way to prevent HCV polyprotein processing and have an antiviral effect (Shaw *et al.,* 2015b).

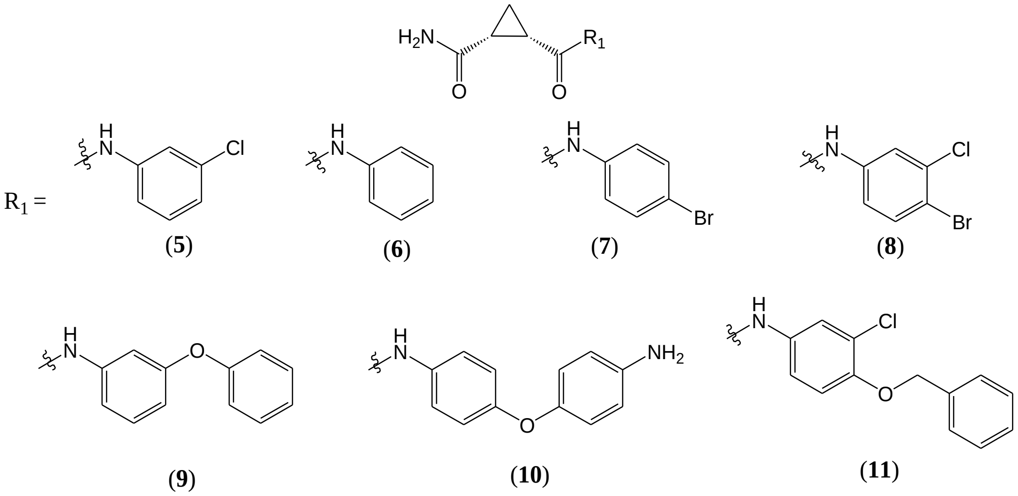


Fig. 2. A series of epoxide-containing small molecules as inhibitors of NS2 protease inhibitors auto-protease.

B. Inhibitors of NS3

One of the first viral proteins to be examined as a target for antiviral research was the NS3 protease, a 180 amino acid serine protease that resembles chymotrypsin and is found in the N-terminal third of the NS3 protein. Compound **12** served as the foundation for additional optimization in a study conducted by Llinàs-Brunet and his co-workers. A cursory evaluation of compound **12**'s structure-activity relationship (SAR) analysis at the aminothiazol moiety showed that an acetyl group, akin to compound 13, was well tolerated, resulting in a compound with improved potency and an IC_{50} value = 5± 10 nM. Additionally, the corresponding inhibitor's cell-based potency increased to EC_{50} = 34.20 over the reference compound **12** (IC_{50}= 14± 2 nM and EC_{50}= 45± 19 nM) bearing an isopropyl, as shown in Figure 3. Similarly, compound 14, 15, 16 and 17 exhibited significant enzyme activity with IC_{50} values of 17±8, 15±3, 8±2, 10±2 nM and EC_{50} = 68±26,40±6, 19±7 and 17±8 nM respectively (Llinàs-Brunet *et al.,* 2010).

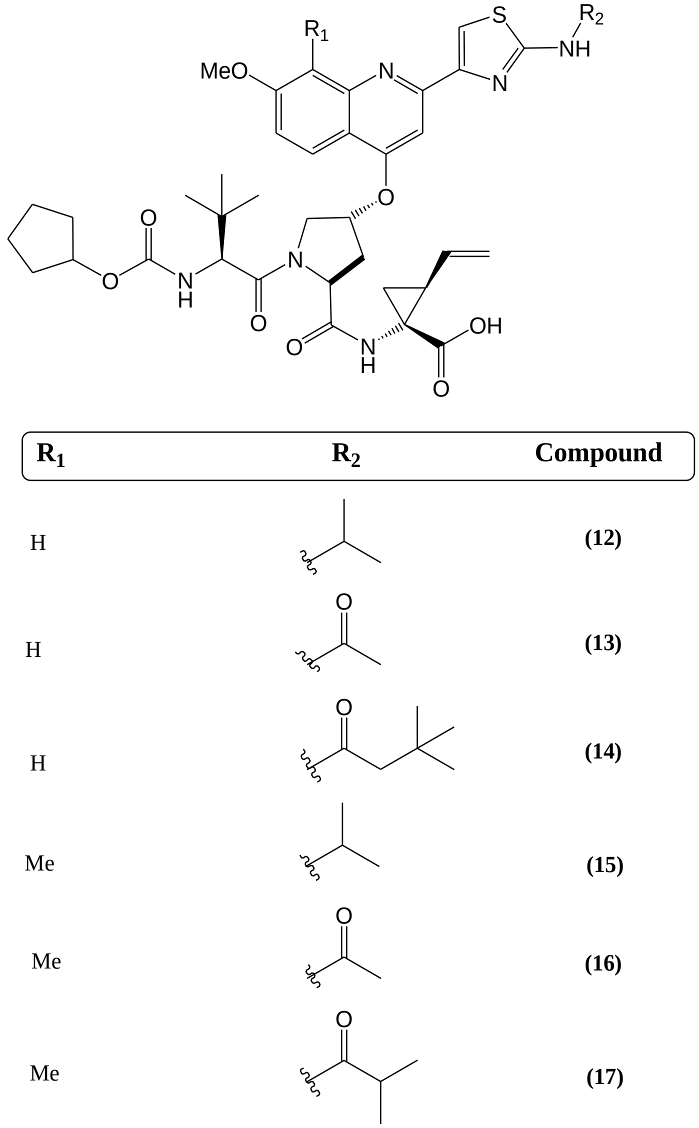
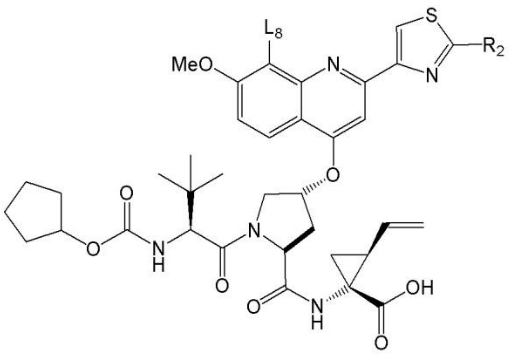


Fig. 3. Inhibitors of NS3 protease.

Following their observation that the C8-Me increased potency, Llinàs-Brunet and colleagues looked into the impact of various substituents, such as halogen, at this location. When the C8-methyl moiety of **17** was replaced with a fluoro, chloro, or bromo substituent as shown in Figure (4), inhibitors with comparable efficacy were produced in the enzymatic assay (IC_{50} = 7±8 nM). While the C8-chloro and C8-bromo analogs (**19** and **20**) both showed improved activity with EC_{50} values = 11±2 and 5±1 nM, the C8-fluoro analog (**18**) was 2-fold less powerful (EC50=36±10). These substituents had somewhat diverse effects on the cellular potency. Analog **20** demonstrated an EC_{50} of 5 nM, which was their first observation of single-digit nano-molar activity for a linear tripeptide inhibitor in the replicon experiment. Eventually, the C8-bromo substituent was determined to be the most powerful addition (Llinàs-Brunet *et al.,* 2010).



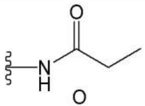
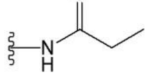
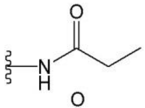
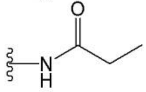
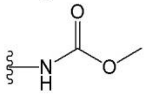
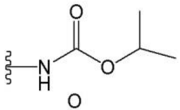
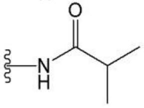
L8	R2	Compound
Me		(17
F		(18)
Cl		(19)
Br		(20)
Br		(21)
Br		(22)
Br		(23)

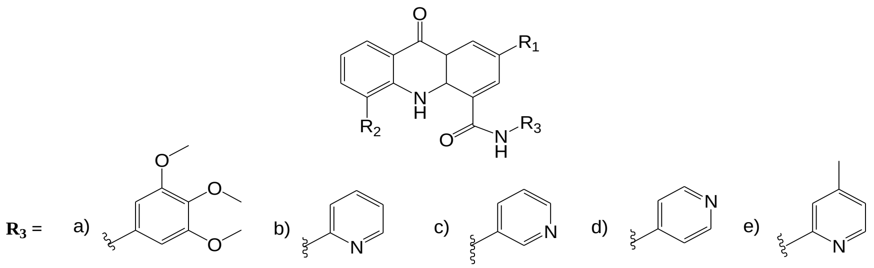
Fig. 4. Different analogs as inhibitors of NS3.

Three of these inhibitors, including the methyl and isopropyl carbamate derivatives **21** and **22**, had IC_{50} values of 6±1, 6±2, and EC_{50} values of 3±1, 6±2, respectively, and the acyl analogue **23**. The most effective compound overall was compound 23, with IC_{50} and EC_{50} values of 3 nM (Llinàs-Brunet *et al.,* 2010).

To ascertain the degree to which a new class of acridone derivatives could block the NS3 helicase of the hepatitis C virus (HCV), Stankiewicz-Drogo and colleagues investigated them. At low concentrations, six of the compounds under investigation reduced the activity of the NS3 helicase (IC_{50} range: 1.5 - 20 μM). For the activity of acridone derivatives, although contact with the enzyme is likely, it happens by intercalation into double-stranded nucleic acids, with a high preference for double-stranded RNA. The presence of the pyridyl ring and the location of the nitrogen atom at the ring dictated the anti-helicase activity strength, as shown by compound **24** almost complete lack of activity and compound 25 three-fold lower IC_{50} value than compound **26**. This was discovered through analysis of the structure-activity relationship between acridone derivatives as shown in Figure (5).

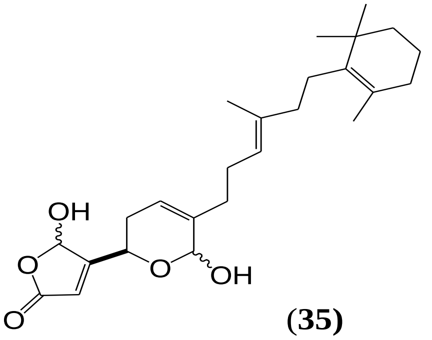
Furthermore, the C2 position of the acridone structure of **25** was modified by adding either a fluoride (**31**) or a chloride atom **(28)**, resulting in two compounds with inhibitory potentials that ranged by more than a factor of 10, with an IC_{50} of 5.6 μM for **31** and 64.7 μM for **28** while substituting a fluoride atom for a hydrogen atom resulted in more potent helicase inhibitors than prototype compounds in both scenarios, doing so also decreased the anti-HCV effectiveness of MACA derivatives. Comparable activity changes were seen between **32** (IC_{50} = 8.6 μM) and **29** (IC_{50} = 54.6 μM) when **25** was halogenated at the same site. A fluoride or chloride molecule added to C4 at the pyridyl ring altered the anti-helicase activity of the compounds in a similar manner. Combining the substitution of chloride at this location with the chloride present at the acridone molecule's C2 position yielded the most effective helicase inhibitor, **30** (IC_{50} = 1.5 μM). The acridone compounds utilized in this study as helicase inhibitors have a mechanism of action that suggests they interact with the dsDNA substrate rather than the enzyme (Stankiewicz-Drogon *et al.,* 2010).

Using high-throughput screening photo-induced electron microscopy, mannoalide (**35**) from marine sponge extracts was discovered to be an RNA helicase inhibitor in a study as shown in Figure 6. In this study, compound 35 exhibits a dose-dependent inhibition of NS3's RNA helicase and ATPase activities, with IC_{50} values of 15 μM and 70 μM, respectively. According to the results of a biochemical kinetic analysis, 35 does not affect the apparent Km value (0.31 mM) of NS3 ATPase activity, suggesting that it acts as a non-competitive inhibitor of the NS3 Helicase of the hepatitis C virus (Salam *et al.,* 2012).



R1	R_2	R3	Compound	IC_{50}±SD (μM)
H	OCH_3	a	**(24)**	751.0±33.7
H	OCH_3	b	**(25)**	6.2±2.1
H	OCH_3	c	**(26)**	20.6±6.3
H	$OCH_2C_6H_5$	e	**(27)**	35.8±10.2
Cl	OCH_3	b	**(28)**	64.7±44.6
Cl	OCH_3	c	**(29)**	54.6±4.0
Cl	OCH_3	e	**(30)**	1.5±0.9
F	OCH_3	b	**(31)**	4.7±1.3
F	OCH_3	c	**(32)**	8.6±2.7
F	OCH_3	e	**(33)**	46.3±17.9
H	$OCH_2C_6H_5$	d	**(34)**	8.0±2.2

Fig. 5. Inhibitory potential of Acridone derivatives toward the NS3 helicase of hepatitis C virus.



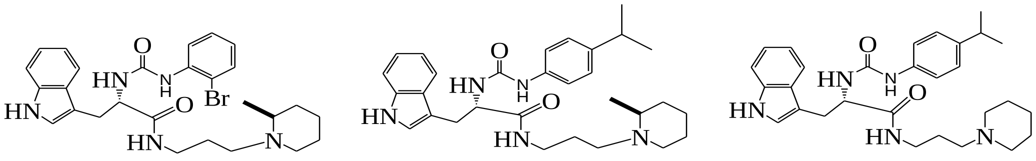
(35)

Manaolide (1) was reported as the first marine natural product inhibiting the phospholipases A2 (PLA2s).

Fig. 6. Inhibitory potential of Manaolide toward the NS3 helicase of hepatitis C virus.

C. NS3/4A Inhibitors

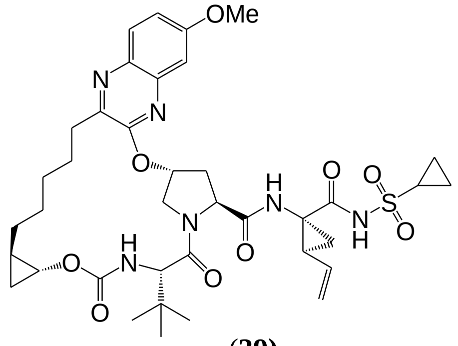
Ittipat Meewan investigated a new class of small compounds known as HCV NS3/4A protease inhibitors, which are effective against both the wild type and mutant forms of the protease, and are based on a scaffold derived from tryptophan as shown in Figure (7). Replicant and enzymatic assays were employed to evaluate the antiviral efficacy. Twenty-two compounds were discovered that suppress HCV, with compound 38 having an EC_{50} value of 0.64 to 63 μM. Particularly, **38** exhibited an LC_{50} of 47 μM for mammalian cells and an EC_{50} of 0.64 μM in a viral replication experiment, resulting in an overall therapeutic index of 73.4. When tested with the D168A mutant, it had an IC_{50} value of 4.60 μM and directly inhibited the protease. There was no discernible loss of potency. Additionally, compound **36** revealed 14.68±1.23 μM and 11.37±0.45 μM, compound **37** exhibited 13.08±2.77 μM and 11.45±0.55 0.9 μM, and compound **38** showed 4.60±1.26 μM and 5.98±0.43 μM. The wild type and the D168A mutant were both sensitive to the simeprevir standard inhibitor at concentrations of 0.043±0.79 μM and 0.247±0.39 μM, respectively (Meewan *et al.,* 2019).



(36) **(37)** **(38)**

Fig. 7. Tryptophan derivative scaffold identified as HCV NS3/4A protease inhibitors.

A new class of HCV NS3/4a protease inhibitors was discovered by Steven Harper and colleagues (Harper *et al.,* 2012). In preclinical animals, this series was tuned for liver exposure and enzyme activity. In this series, compound **39** was synthesized (Figure 8), by replacing the highly basic quinolone P2 heterocycle with a slightly basic quinoxaline P2 heterocycle to address disproportionation problems. Compound **39** demonstrated exceptional performance in rat liver exposure, superb potency in the replicon system (gt 1b IC_{50} (50% NHS) = 7.4 nM; gt 1a IC_{50} (40% NHS) = 7 nM), and high efficacy against a wide range of mutant enzymes (Meewan *et al.,* 2019).



(39)

Fig. 8. Slightly basic quinoxaline P2 heterocycle inhibitor of HCV NS3/4a protease.

D. NS5A Inhibitors

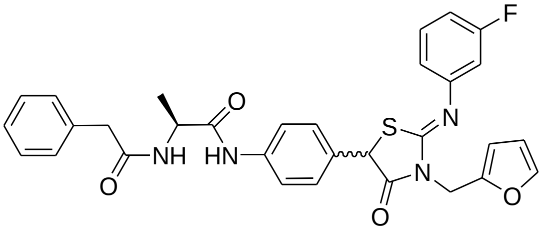
A new target of highly successful HCV treatments, NS5A is a multifunctional protein encoded by the virus that is necessary for HCV RNA replication and virion assembly. The role of NS5A in the viral life cycle has been extensively studied due to its involvement in interactions with various cellular proteins and lack of known enzymatic activities. Both the basally phosphorylated (p56) and hyperphosphorylated (p58) forms of the phosphoprotein NS5A are thought to play a role in distinct phases of the viral life cycle. The 447-amino-acid protein NS5A has three structural domains (**I**, **II**, and **III**) and an N-terminal amphipathic alpha helix (amino acids AA, 5–25). Because it defines a new class and is involved with various stages of the HCV life cycle, NS5A is an appealing target for anti-HCV therapy. This feature may enhance the antiviral effect by inhibiting several vital functions (Gao, 2013).

The multifunctional phosphoprotein NS5A is essential for the replication and assembly of the hepatitis C virus's (HCV) genome. However, it is still unclear how NS5A phosphorylation affects HCV biology. Using a phosphoablative mutant (S225A), researchers were able to determine the function of serine 225 (S225), a major phosphorylation site within the low complexity sequence 1 (LCS**I**) of NS5A, in subcellular localization, NS5A-host interactions, and genome replication (Goonawardane *et al.,* 2025).

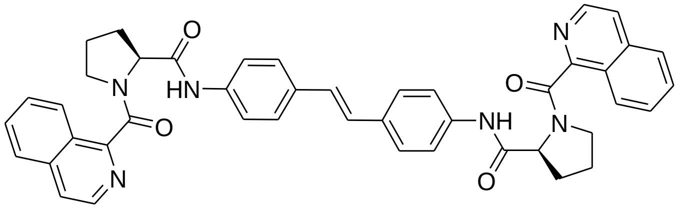
NS5A with bound inhibitor elucidated the distinct susceptibilities of resistance mutations to NS5A-targeting inhibitors in genotypes 1a and 1b, especially for genotype 1a. With this knowledge, one could also employ rationally medicinal chemistry strategies to synthesize modified compounds that lessen the effects of resistance polymorphisms. Numerous investigations showed that the compound's activity was unique to HCV and that it produced a wide therapeutic window; CC_{50} values were greater than 50 µM. BMS researchers later found that BMS-824 (**40**) converted into other inhibitory species after being incubated in cell culture medium.

The active compound species was a dimer of BMS-824 that resulted from an intermolecular radical-mediated reaction of the parent compound, according to mass spectral and nuclear magnetic resonance data. The stilbene derivative BMS-665 (**41**) was created after the structural components of the dimer necessary for anti-HCV activity were examined. Although BMS-665 potency for HCV genotype 1b was comparable to that of BMS-824, the molecule now had activity against genotype 1a, with an EC_{50} of 393 nM. The stilbene dimer was further refined to produce BMS-346 (**42**) (Figure 9), which shifted potency into the picomolar range with an impressive EC_{50} of 86 pM and enhanced activity against the HCV genotype 1b replicon by 70 times.

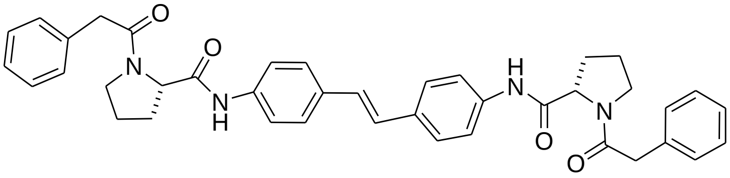
Finding the stable, active pharmacophore linked to these substances provided the basis for creating stronger inhibitors, which ultimately led to the discovery of the symmetrical homodimeric biphenyl-based molecule BMS-790052 (43). With highly potent mean EC_{50} values of 9 pM and 50 pM against HCV genotype 1b and 1a replicons, respectively, BMS-790052 was later renamed as daclatasvir (Belda and Targett-Adams, 2012).



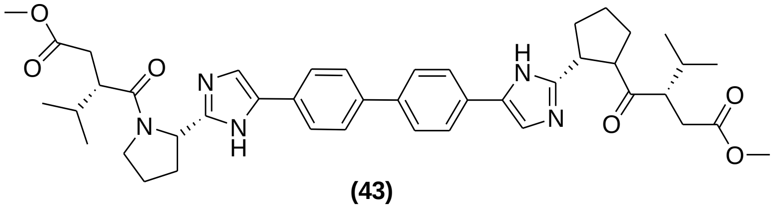
(**40**)



(41)



(42)

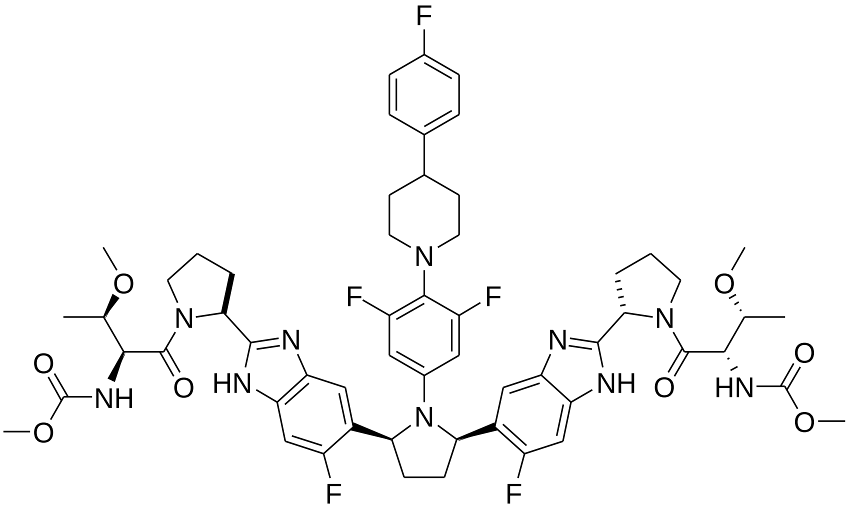


(43)

Fig. 9. Potent inhibitors of NS5A.

Pibrentasvir (ABT-530) is a novel and pan-genotypic NS5A inhibitor that exhibits 50% effective concentrations (EC50) ranging from 1.4 to 5.0 pM against HCV replicons containing NS5A from genotypes 1 to 6. Similar activity to pibrentasvir (44) was demonstrated by a panel of chimeric replicons containing HCV NS5A of genotypes 1 to 6 from clinical samples. Resistance selection studies were conducted using HCV replicon cells with NS5A of genotypes 1a, 1b, 2a, 2b, 3a, 4a, 5a, or 6a. The EC_{50} for the corresponding replicon was 10 or 100 times lower than the pibrentasvir concentration that was used. In HCV genotypes 1 through 6, pibrentasvir (Figure 10) is effective against common resistance-conferring substitutions that were found for other NS5A inhibitors, such as those at crucial amino acid positions 28, 30, 31, or 93.

Pibrentasvir and other classes of HCV inhibitors inhibited HCV replication in a synergistic manner. In conclusion, pibrentasvir is a next-generation HCV NS5A inhibitor that exhibits strong and pan-genotypic activity. It stays active against common amino acid substitutions of HCV genotypes 1 through 6 that are known to confer resistance to NS5A inhibitors that are currently approved (Teresa *et al.,* 2017).



(44)

Fig. 10. Pibrentasvir as NS5A inhibitor.

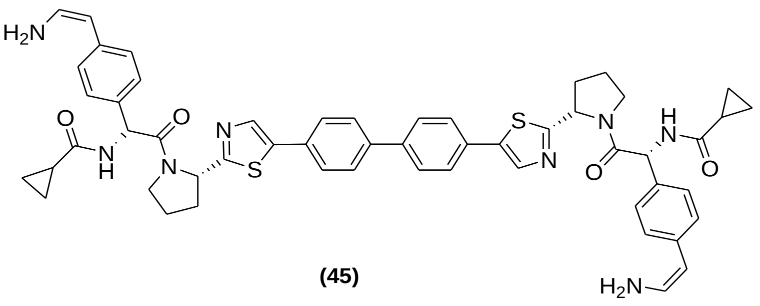
In a study, a quantitative structure–activity relationship (QSAR) was used to design novel compounds with improved inhibitory activity against HCV. Identification and development of novel inhibitors that target NS5A was the primary objective of this study. In this case, a QSAR model was built using the Monte Carlo optimization technique with a set of 82 phenylthiazole derivatives. This model provides important information about the precise structural features that either increase or decrease the inhibitory activity. New NS5A inhibitors were created using these discoveries. Additionally, the newly designed inhibitors' binding affinity within the NS5A protein was predicted using molecular docking, and the dynamic interactions over time were examined using molecular dynamics simulations.

With predicted EC_{50} values of 0.0006, 0.0003, and 0.0002 nM, respectively, three of the designed compounds, **45**, **46**, and **47** (Figure 11) showed good conformational and structural stability as well as favorable binding profiles. These compounds also fulfilled pharmacological safety requirements and demonstrated non-toxic profiles, highlighting their potential as therapeutic agents for the treatment of HCV. To verify these compounds' safety and effectiveness, however, experimental validation (in vitro and in vivo assays) is required (Liman *et al.,* 2024).

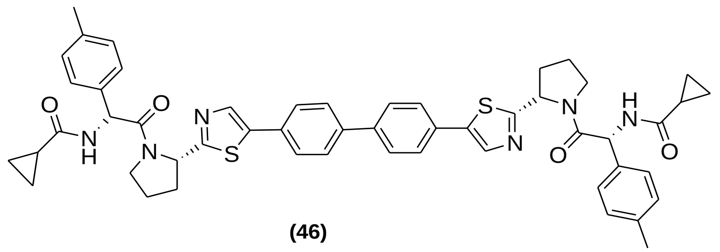
E. NS5B inhibitors

Different hepatitis C virus genotypes, and even the same genotype, have different binding sites in terms of form and structure. Finding compounds that can inhibit in a variety of binding locations is a difficulty for antiviral medication research due to these variations. To address this, Pauwels et al. (2007) created a hepatitis C virus mutant and genotypic recombinant polymerase panel to direct medicinal chemistry through the clarification of new inhibitors' sites of action and genotype profiling.

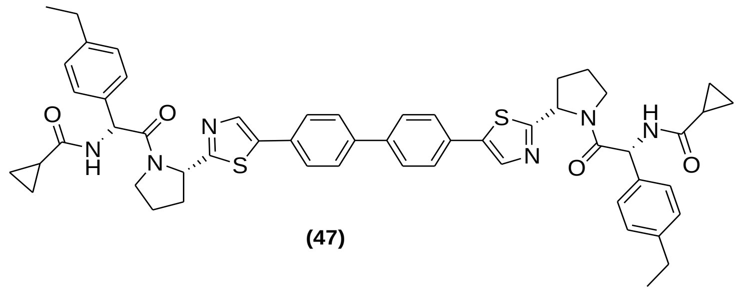
In general, they discovered that benzimidazole **40** maintained effective inhibitions across genotypes, where it had an IC_{50} that was around 100 times higher. The only genotypes that thiophene **41** could block were 1a, 1b, and 5a. Benzothia-diazine 3 was only able to inhibit genotypes 1a and 1b, much as thiophene **41**.



(45)



(46)



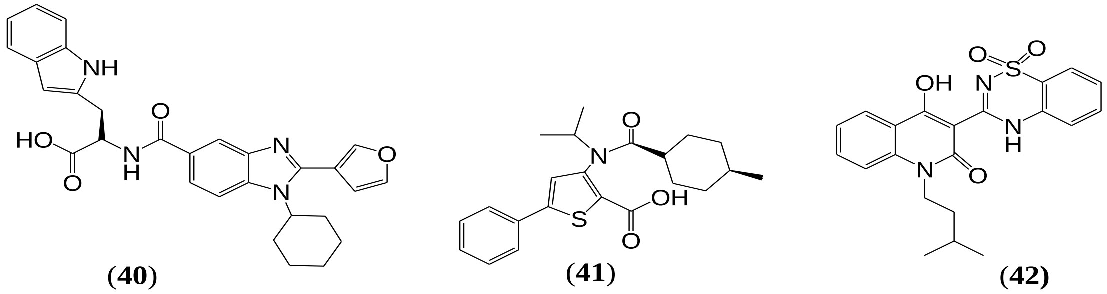
(47)

Fig. 11. Phenylthiazole derivatives as NS5A inhibitors.

On the basis of published structures in literature the key hydrophobic interactions by which benzimidazole **40**, thiophene **41**, and benzothiadiazine **42** (Figure 12) interact with NS5B while hydrogen bonding and contacts with the polypeptide backbone are occasional. Small molecules should ideally use enthalpy- and entropy-driven factors such as hydrogen bonding with the polypeptide backbone or highly conserved side chains and flexible bonds that allow the hydrophobic groups to accommodate diversity within the pool of binding sites—as demonstrated by the human immunodeficiency virus (Pauwels *et al.,* 2007).

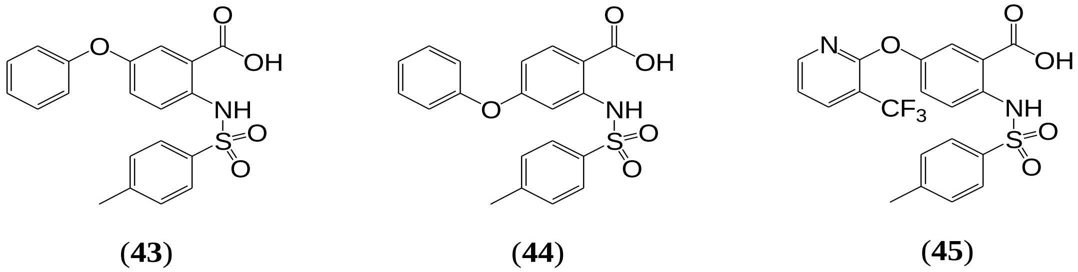
Compounds **43** and **44** were discovered to be selective inhibitors in the study of the isomeric phenoxy-substituted anthranilic acid sulphonamides (Figure 13) by Timothy A. Stammers and colleagues. Here, compounds **43** and **44** (with compound **43** having an IC_{50} value of 0.22 μM and EC_{50} > 30 μM) were demonstrated to exhibit sub-micromolar potency in enzymatic assays, but they lacked cell culture activity in the HCV subgenomic replicon assay. Compound **45**, an inhibitor having enzymatic (IC_{50}) and cell culture (EC_{50}) potencies of 0.08 ±0.04 μM and 0.095 ±0.03 μM, respectively, was created by combining these constituents (Stammers *et al.,* 2013).

Wei and colleagues explain how they used a virtual screening (VS) method that is based on random forest (RB-VS), e-pharmacophore (PB-VS), and docking (DB-VS) methodologies to find new, powerful HCV NS5B polymerase inhibitors. Five compounds (compounds **46** to **50**) were chosen from the final hits for additional anti-HCV activity and cellular cytotoxicity testing as shown in Figure (14).



(40) (41) (42)

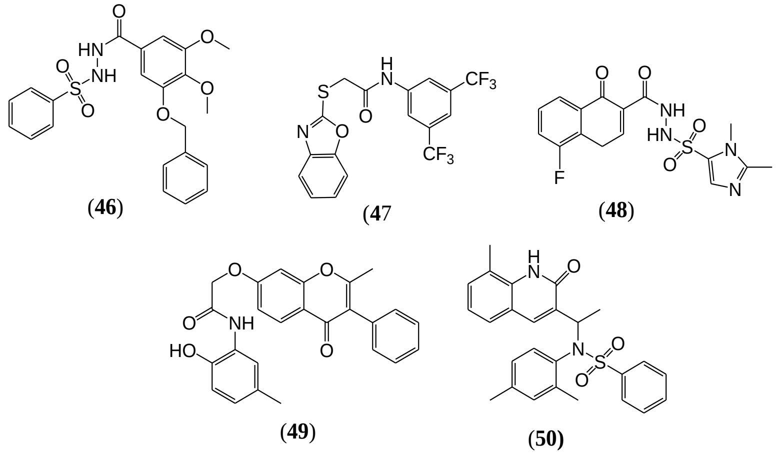
Fig. 12. Benzimidazole, thiophene, and benzothiadiazine as NS5B inhibitors.



(43) (44) (45)

Fig. 13. Phenoxy-substituted anthranilic acid sulphonamides inhibitors of NS5B.

All five compounds were found to demonstrate anti-HCV actions with EC_{50} values ranging from 1.61 to 21.88 μM and to inhibit NS5B polymerase with IC_{50} values ranging from 2.01 to 23.84 μM, with the exception of compound **47**, which showed weak cellular cytotoxicity with an EC_{50} value of 51.3 μM. With a selectivity index of 32.1, the hit compound **47** exhibited the greatest antiviral effectiveness against HCV. Through further research and optimization, the five successful compounds with novel scaffolds may eventually function as NS5B polymerase inhibitors (Wei *et al.,* 2016).



(46) (47 (48)

(49) (50)

Fig. 14. Inhibitors of NS5B polymerase.

CONCLUSION

The remarkable progress in the field of hepatitis C virus (HCV) inhibitors has ushered in a new era in the battle against this persistent global health threat. Direct-acting antivirals (DAAs) have redefined HCV treatment, with cure rates exceeding 90% and vastly improved tolerability compared to older interferon-based regimens. Sofosbuvir and ledipasvir are emblematic of the success of DAAs, providing hope for millions who once faced the burden of chronic HCV infection. Beyond DAAs, host-targeting agents and immunotherapies have introduced innovative approaches to HCV therapy. Cyclophilin inhibitors and microRNA-based therapeutics target host factors, potentially reducing the risk of viral resistance and broadening the therapeutic arsenal. In recent years, therapeutic vaccines and monoclonal antibodies have grabbed the attention of many because of their ability to control the immune system of patients.

On a global scale, HCV inhibitors play a central role in the quest to eliminate HCV as a major public health concern by 2030, as outlined by the World Health Organization. Their efficacy, safety profile, and accessibility make this ambitious goal attainable. Nonetheless, challenges remain on the path to HCV elimination.

Drug resistance, while less common with DAAs, continues to be a concern, necessitating ongoing vigilance and the development of novel agents. Addressing disparities in access to HCV inhibitors is critical to ensuring that the benefits of these therapies are equitably distributed, particularly in regions with a high HCV burden.

In closing, the literature on HCV inhibitors highlights their pivotal role in transforming HCV treatment, bringing us closer to the vision of a world free from the threat of hepatitis C. As we look toward the future, continued research, international collaboration, and a commitment to universal access will be essential to surmount the remaining challenges and make the goal of HCV elimination a reality for all. The journey toward a hepatitis C-free world is underway, and HCV inhibitors are the beacon lighting the way.

Challenges and Future Perspectives

Despite the significant success of current DAAs in curing HCV, more work must be done to further enhance HCV treatment. Among these factors are RASs, patients who are not responding to treatment, advanced liver disease, HIV co-infection, and the reduced efficacy of current regimens for those with repeat infections? In recent years, medical journals have acknowledged the need for better and more comprehensive therapies (Sarrazin, 2022). Moreover, HTAs are becoming increasingly popular as a viable method of combating cancer (Zhou *et al.,* 2023b). Meanwhile, researchers are focusing on using vaccinations and immune strategies to treat the disease or prevent relapse among those at the highest risk, such as people who inject drugs (PWID). Although an HCV vaccine is not available on the market yet, ongoing trials are investigating possible vaccines that could be used alongside current antiviral therapies. Lastly, we continue to learn about the effectiveness of DAAs in different groups of people. By 2030, the WHO aims to cease considering HCV a public health threat due to new developments in drug design, therapies that block the virus, and increased access to HCV treatments.

ACKNOWLEDGMENTS

Dr. Quazi M. I. Haq is thankful to the University of Nizwa, Oman, for its generous support in carrying out the research project (ID# UoN/CAS/IF/2023/12), which also facilitated the writing of this review.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

Belda, O., Targett-Adams, P., 2012. Small-molecule inhibitors of the hepatitis C virus-encoded NS5A protein. Virus Res., 170(1–2): 1–4.

Calvaruso, V., Craxì, A., 2020. Hepatic benefits of HCV cure. J. Hepatol., 73(6): 1548–1556.

Celli, R., Saffo, S., Kamili, S., et al. 2021. Liver pathologic changes after direct-acting antiviral agent therapy and sustained virologic response in the setting of chronic hepatitis C virus infection. Arch. Pathol. Lab. Med., 145(4): 419–427.

Charatcharoenwitthaya, P., Wongpaitoon, V., Komolmit, P., et al., 2020. Real-world effectiveness and safety of sofosbuvir and nonstructural protein 5A inhibitors for chronic hepatitis C genotype 1, 2, 3, 4, or 6: a multicentre cohort study. BMC Gastroenterol., 20(1): 47. doi: 10.1186/s12876-020-01196-0.

Chatel-Chaix, L., Vuillier, F., Berger, C., et al. 2012. Direct-acting and host-targeting hepatitis C virus inhibitors: state of the art and future directions. Antivir. Res., 96(2): 272-276.

Choo, Q.L., Kuo, G., Weiner, A.J., Overby, L.R., Bradley, D.W., Houghton, M., 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science., 244(4902): 359–362.

Fafi-Kremer, S., Fofana, I., Soulier, E., et al. 2010. Neutralizing antibodies in hepatitis C virus infection. J. Viral. Hepat., 17(1): 18–31.

Feld, J.J., Jacobson, I.M., Hézode, C., et al. 2015. Sofosbuvir and velpatasvir for HCV genotype 1–6 infection. N. Engl. J. Med., 373(27): 2599–2607.

Foster, T.L., Verow, M., Wozniak, A.L., Bentham, M.J., 2013. Resistance mutations define specific antiviral effects for inhibitors of the hepatitis C virus p7 ion channel. J. Virol., 87(18): 12405–12413.

Gao, M., 2013. Antiviral activity and resistance of HCV NS5A replication complex inhibitors. Curr. Opin. Virol., 3(5): 514–520.

Garcia, M.L., Ahmed, S., Zhang, T., Nguyen, T., 2024. A phase I study of an mRNA-based therapeutic vaccine for chronic hepatitis C virus infection. Lancet Infect. Dis., 24(3): 187–196. https://doi.org/10.1016/S1473-3099(24)00032-5

Goonawardane, N., Yin, C., Roberts, G.C., Zothner, C., Harris, M., 2025. A key role for hepatitis C virus NS5A serine 225 phosphorylation revealed by super-resolution microscopy. Sci. Rep., 15(1): 9567.

Harper, S., McCauley, J.A., Rudd, M.T., et al. 2012. Discovery of MK-5172, a macrocyclic hepatitis C virus NS3/4a protease inhibitor. ACS Med. Chem. Lett., 3(4): 332–336.

Jacobson, I.M., Lawitz, E., Gane, E.J., et al. 2017. Efficacy of 8 weeks of glecaprevir and pibrentasvir in treatment-naive, HCV-infected patients without cirrhosis. N. Engl. J. Med., 378(1): 64–73.

Kim, C.W., Lee, J.H., Park, M.S., 2022. Clinical development of miR-122 antagonists: From Miravirsen to next-generation agents. J. Hepatol. Res., 75(2): 189–197. https://doi.org/10.1016/j.jhep.2022.01.014

Liman, W., Oubahmane, M., Lahcen. N.A., et al. 2024. Computational design of potent dimeric phenylthiazole NS5A inhibitors for hepatitis C virus. Sci. Rep., 14(1): 31655.

Llinàs-Brunet, M., Bailey, M.D., Goudreau, N., et al. 2010. Discovery of a potent and selective noncovalent linear inhibitor of the hepatitis C virus NS3 protease (BI 201335). J. Med. Chem., 53(17): 6466–6476.

Meewan, I., Zhang, X., Roy, S., et al. 2019. Discovery of new inhibitors of hepatitis C virus NS3/4A protease and its D168A mutant. ACS Omega., 4(16): 16999–17008.

Pauwels, F., Mostmans, W., Quirynen, L.M., et al. 2007. Binding-site identification and genotypic profiling of hepatitis C virus polymerase inhibitors. J. Virol., 81(13): 6909–6919.

Pawlotsky, J.M., 2014. New hepatitis C therapies: the toolbox, strategies, and challenges. Gastroenterol., 146(5): 1176–1192.

Salam, K.A., Furuta, A., Noda, N., et al. 2012. Inhibition of hepatitis C virus NS3 helicase by manoalide. J. Nat. Prod., 75(4): 650–654.

Salas-Villalobos, T.B., Lozano-Sepúlveda, S.A., Rincón-Sánchez, A.R., et al. 2017. Mechanisms involved in liver damage resolution after hepatitis C virus clearance. Med. Univ., 19(75): 100–107.

Sarrazin, C., 2022. Treatment failure with DAA-based therapies: Mechanisms and implications for retreatment. J. Hepatol., 76(2): 452–466.

Shaw, J., Harris, M., Fishwick, C.W., 2015a. Identification of a lead-like inhibitor of the hepatitis C virus non-structural NS2 autoprotease. Antivir. Res., 124: 54–60.

Shaw J, Fishwick CW, Harris M. 2015b. Epoxide-based inhibitors of the hepatitis C virus non-structural 2 autoprotease. *Antiviral Res*, 117, 20–26.

Stammers, T.A., Coulombe, R., Duplessis, M., et al. 2013. Anthranilic acid-based Thumb Pocket 2 HCV NS5B polymerase inhibitors with sub-micromolar potency in the cell-based replicon assay. Bioorg. Med. Chem. Lett., 23(24): 6879–6885.

Stankiewicz-Drogon, A., Dörner, B., Erker, T., Boguszewska-Chachulska, A.M., 2010. Synthesis of new acridone derivatives, inhibitors of NS3 helicase, which efficiently and specifically inhibit subgenomic HCV replication. J. Med. Chem., 53(8): 3117–3126.

Teresa Cruz, M., Ferreira, I., Liberal, J., D Martins, J., Silva, A., Miguel Neves, B., 2017. Inflammasome in dendritic cells immunobiology: implications to diseases and therapeutic strategies. Curr. Drug Targets., 18(9): 1003-18.

Walker, L.M., Thompson, J.A., Fafi-Kremer, S., Bailey, J.R., 2023. Broadly neutralizing monoclonal antibodies for HCV prevention and therapy. Nat. Rev. Gastroenterol. Hepatol., 20(4): 231–244. https://doi.org/10.1038/s41575-023-00792-6

Wei, Y., Li, J., Qing, J., et al. 2016. Discovery of novel hepatitis C virus NS5B polymerase inhibitors by combining random forest, multiple e-pharmacophore modeling and docking. PLoS One., 11(2): e0148181.

World Health Organization. 2016. Combating hepatitis B and C to reach elimination by 2030. https://www.who.int/hepatitis/publications/hep-elimination-by-2030-brief/en

Zhang, L., Wang, Y., Chen, L., et al. 2024. Real-world effectiveness of sofosbuvir-based regimens for HCV genotype 6: A retrospective study. Front. Med., 11: 1462706. https://doi.org/10.3389/fmed.2024.1462706

Zhou, Y., Li, M., Tanaka, Y., Yamamoto, N., 2023a. Non-immunosuppressive cyclophilin inhibitors as potent host-targeting antivirals against hepatitis C virus. Antivir. Res., 210: 105527. https://doi.org/10.1016/j.antiviral.2023.105527

Zhou, H., et al. 2023b. Host-targeting antiviral agents: Promising options for future HCV therapies. Antivir. Res., 205: 105408.