A Review on Therapeutic Approaches for Hepatitis C Virus Infection: Synthetic Methods and SARs of Direct Acting antivirals

By

Faria Nawrin
Student ID: 20146076

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Faria Nawrin
Student ID: 20146076

Approval

The thesis titled "A Review on Therapeutic Approaches for Hepatitis C Virus Infection: Synthetic Methods and SARs of Direct Acting antivirals" submitted by Faria Nawrin (20146076), of Spring, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

Supervised By:

_____

Dr. Humair Bin Md. Omer
Assistant Professor
School of Pharmacy
Brac University

Approved By:

Dean:

_____

A.F.M. Yusuf Haider, PhD
Acting Dean, School of Pharmacy
Professor, Department of Mathematics and Natural Sciences
Brac University

Ethics Statement

This project does not involve any kind of animal and human trial.

Abstract

This review provides risk factors that involves the modes of transmission as well as groups that are under risk of Hepatitis C virus (HCV). Moreover, lifecycle, pathogenesis and virulence of HCV is discussed. It also focuses on the prevalence of HCV worldwide as it is increasing day by day. The development of direct acting antivirals (DAAs) via total synthetic methods involves mechanisms that target NS3/4A protease, NS5A and NS5B. Also based on SAR modifications, improved selectivity, potency and efficacy of the drugs were achieved. Classification of DAA drugs involved studies of SVR based on clinical trials and highlights some combination therapy of HCV with inhibitors and other DAAs. Interestingly, DAAs proved having good clinical outcomes, if the comparison is done with therapies on interferons (IFN) that were employed beforehand. This review sums up the therapeutic approaches to eliminate Hepatitis C and the importance of synthetic methods in the development of DAAs.

Keywords: Hepatitis C virus; Direct acting antivirals; Synthesis; SAR; Treatment; Inhibitor

Dedication

This paper is dedicated to my respected supervisor who have shown guidance to me throughout these days and to my parents, friends as well as well as well-wishers of mine.

Acknowledgement

At first, all credit goes to Almighty. I am thankful to each and every one who are part of my journey and guided me in every step of my stressful days, provided me immense support and care. My sincere gratitude goes towards my kind supervisor Dr. Humair Bin Md Omer, Assistant Professor School of Pharmacy BRAC University. It's been a wonderful experience to work under such helpful person's supervision. I am really grateful towards my supervisor for making things more organized and easier for me.

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List of Acronyms

HCV	Hepatitis C virus
DAA	Direct acting antiviral
LDL	Low density lipoprotein
VLDL	Very low-density lipoprotein
SR	Scavenger receptor
IRES	Internal ribosomal entry site
NS5A	Nonstructural protein 5A
NS4B	Nonstructural protein 4B
DGAT1	Diacylglycerol O- acyltransferase 1
YB-1	Y box binding protein 1
APO	Apolipoproteins
SiRNA	Small interfering RNA
PEG - IFN	Pegylated interferon
PI	Protease inhibitor
SVR	Standard variable rate
RAV	Risk assessment value
GT	Genotype

RCM	Ring closing metathesis
Tf2NPh	N-Phenyl-bis(trifluoromethanesulfonimide)
HTS	High throughput screening
RdRp	RNA dependent RNA polymerase

Chapter 1

Introduction to viral Hepatitis C

1.1 Introduction

Viral hepatitis C is a constricted virus of RNA that is membraned and located in a Hepacivirus named genus that stays inside the Flaviridae family (Thomson & Finch, 2005). The duplication of HCV occurs in the cytoplasm of the liver where main parenchymal tissue exists. However, it does not have the capacity to give structural changes in the cell of host through the invasion of virus. (Timothy R. Morgan, 2006). The viral infection can be acute/chronic, but it affects liver by chronic infection. Chronic HCV occurs by the elevated aminotransferases in the serum which results in a liver fibrosis/inflammation that may further lead to cirrhosis because of more liver inflammation which at the final stage leads to hepatocellular carcinoma (Westbrook & Dusheiko, 2014). This infectious disease turns into cirrhosis especially to some high-risk groups such as: drug abusers who uses shared needles or syringes, persistent dialysis patient, alcohol abusers, hemophiliacs and people who are HIV infected (Heintges, T & Wands, J. R n.d.).

So, it can be said that one of the leading reasons behind chronic disease of liver and liver cirrhosis is viral hepatitis C which is known as the most occurring and persistent bloodborne disease (Timothy R. Morgan, 2006). Every year 500,000 liver cancer cases were found and among them 22% of cases occur due to HCV infection. Studies found that acute hepatitis C turns into chronic infection in 80% cases and 10–20% leads to liver cirrhosis because of chronic liver disease. 1–5% turns into liver cancer within the onset of two to three decades. HCV liver disease or fibrosis occurs or progresses rapidly due to alcohol consumption on a daily basis and females are mostly affected as well. (D. Lavanchy, 2011)

1.2 Hepatitis C: the condition of being prevalent

Infection of HCV is increasing worldwide; persons of all age, gender and races form the globe are affected by HCV. Population-based HCV prevalence can be improved by performing relevant Diagnostic tests and using a representative population sample. Age, gender and ethnicity can be used to stratify the prevalence of HCV infection. However, the published evidence for the prevalence of HCV stays insufficient due to few studies and due to staying limited to only a specific segment of people. (D. Lavanchy, 2011)

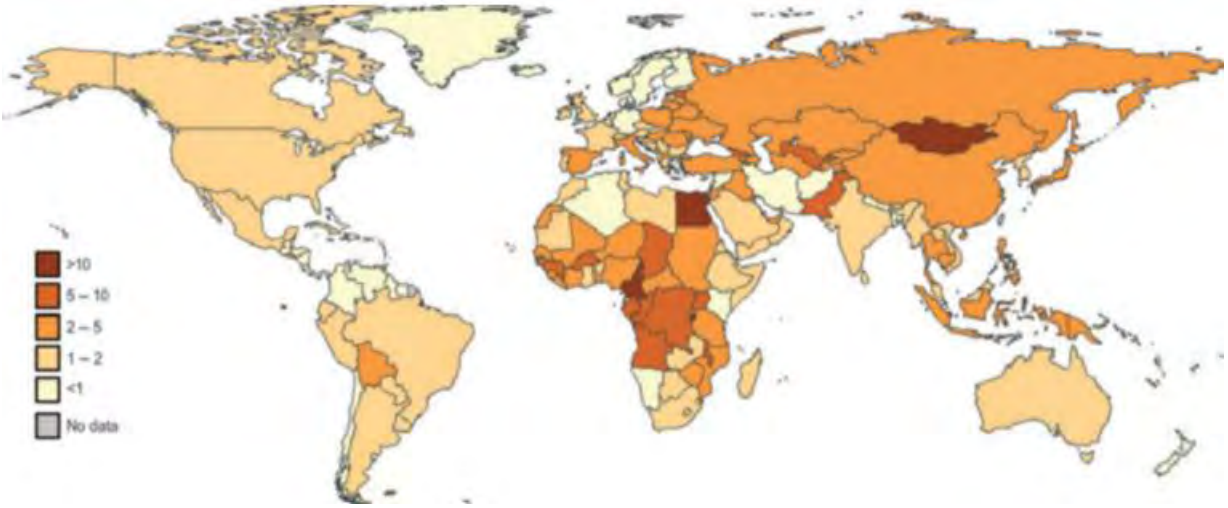


Figure 1: HCV prevalence worldwide in 2010 (D. Lavanchy, 2011)

A World Health Organization report shows that the victims of this persistent HCV stand at 170 million. Number of affected children is not known yet but the number of adult infection rates differs significantly. The infection rate is low in Canada which is 0.1% whereas Egypt has a very high percentage, 18.1%. WHO reports show that people who are exposed to HCV, test positive, indicate they have HCV antibodies**.** (Bostan & Mahmood, 2010)

The infection with HCV poses a worldwide medical problem with the prevalence between 0.6 to 10%. The average incidence across western European countries falls between (1.5 to 3.5) %, while in the nation of England 0.5% is the rate. (Modin et al., 2018)

Worldwide prevalence of HCV stands at 2.8% in the year of 2005 and 185,000,000 individuals reported Hepatitis C antibodies positive, which was calculated by Mohammed Hanafiah along

with his associates. (S. Lanini et al., 2016). Here is a prevalence table of a study that is done in 2010 that shows percentage of overall prevalence across different countries also prevalence in specific regions of those countries to give an idea of how alarming is HCV becoming day by day.

Table 1: Prevalence of Hepatitis C country data table (Bostan & Mahmood, 2010)

Country	**Regions**	**Prevalence % in region**	**Country of overall prevalence**	**Overall prevalence %**
Africa	Rwanda Burundi Guinea Zimbabwe Ghana Ethiopia	17% 11.1% 10.7% 7.7% 2.8% 0.8%	Africa	5.3%
South and central America and Mexico	Republic of Suriname Republic of Trinidad & Tobago República Dominicana Peru republic Mexico	5.5% 4.9% 2.4% 1.6% 0.7%	Bolivia	11.2%
Middle east and southern Asia	Egypt Kuwait Saudi Arabia Morocco	18.1% 3.3% 1.8% 1.1%	Eastern Mediterranean region	4.6%
Europe	Romania Russia Turkey, Greece UK, Finland & Denmark Germany France Italy	4.5% 2% 1.5% 0.02% 0.6% 1.1% 2.2%	Europe	1.03%
Asia and south pacific:	Thailand Vietnam Cambodia Nepal Mongolia	5.6% 6.1% 4.1% 0.6% 10.7%	South East Asia	2.15%

	Japan China India Pakistan	2.3% 3.2–4% 1.8%. 2.4% - 6.5%		
Australia and New Zealand:				1.1% & 0.3%
North America:	Canada	0.1–0.8%	United states	1.8%

After some years, a study of WHO in 2015 provides a clear picture about mortality rate, 1.34 million people found dead from HCV and this death rate has increased more with 22% from the beginning of 2000. If we look into prevalence in 2015, 71 million people are infected with persistent HCV. If the patients with HCV do not go through treatment it can even lead to HCC from which 470,000 people face death. In 2015 the HCV prevalence around the world stood at 1.0%. But the place of highest prevalence has been held by the Eastern Mediterranean Region that is 2.3%. ("Global Hepatitis Report 2017," 2017)

1.3 Percentages of risk factors based on genotypes

As, the earlier section was based on HCV prevalence, now it's become significant to identify the risk factors for which HCV is becoming more prevalent now a days. Viral hepatitis C is a molecule with high variety and it is classified into six genotypes GT 1 to GT 6 with seventy subtypes that were characterized as a, b, c, d. 90 % of the infections took place from GT 1 to 3 in EU, North as well as South USA along with Japan. Topographical site and the transmission mode are joined with genotypes of hepatitis C virus with the occurrences, circulation and individualities (Roman et al., 2008). Luxembourg study of risk factors related to medicine transformation, intimate relations, non-injectable drug, workplace exposure, tattoos, transmission through mothers unto a kid including piercings on the body constitute indicators

of risk. In the country of Australia, the most frequent possibly lethal spread of infection is HCV, involving around thirteen thousand infection cases throughout twenty years preceding this one. Over the course of twenty years there are ten to twenty percent people who are infected will develop a liver condition called cirrhosis. Whereas, the remaining 80 to 85% are going to develop persistent liver disease. There are subtypes of HCV among them 1b, 3a, 1a were very frequent in Salvador, Bahia state and the reported rate is even greater within male. IV users of drugs within France have been mainly connected with HCV-3a, whilst HCV- 1b is related to individuals who have gone through blood transfusions. The critical relationship between virus and host plays the important role in the development of the disease whereas the consumption of alcohol and age of individuals serves as the important determinants. In Iran, the average rate of transmission of HCV is under 1% because of the IV use of drugs or sharing of needles within injectors. Across this Islamic republic the transmission of HCV is one of the most prevalent roots for prolonged liver fibrosis and hepatitis within individuals suffering from a condition called hemophilia. Roman's report shows that the proportion of their gender ratio of Hepatitis C positive were mostly men who were under the age of below forty and the percentage of them is 49.6%. Females are the carriers of genotype 2 and 5 whereas males belong to genotype 3. Transfer method that was noticed was 61.45% because of the repeated usage of syringes or needles. Surgery or dental processes shows 10.62%. Receiving transfusions of blood stands at 4.26%. And 3.9% occurs owing to exchanging blades while grooming. (Bostan & Mahmood, 2010). A summary of groups that were under risks because of modes of transmission that make them vulnerable towards HCV is listed below:

Table 2: List of risk factors in HCV indicating risk groups (Heintges, T & Wands, J. R n.d.)

Risk Factors (modes of transmission)	Risk groups
Transmitted by parenteral exposure, Shared use of syringe	Drug addicts are highly risk groups because they mostly use the same syringe and needle which is contaminated, sometimes they share that syringe with others.
Shared use of dialysis machine, not changing gloves	Long term hemodialysis patients
Alcohol disrupts the immune response of host to HCV	Patients with alcohol abuse
Sexual transmission by failure to use protection	Prostitutes who have high number of partners
Percutaneous routes to inoculation	People who use razor, toothbrushes of another person
Perinatal transmission	At delivery period or time of labor

1.4 Risks for chronic hepatitis C virus infection

It depends on age, gender, race, jaundice symptoms, HIV infection history and immunosuppression. People who are more than 25 years old and male are prone to HCV infection. (Timothy R. Morgan, 2006)

- **Age at the moment of infestation:** The degree of persistence of HCV tends to be lower in youths. NHANES study revealed that the incidence of chronicity among patients who are under 20, the percentage stands for them is 30%. Whereas, it shows 76% for who are older than 20. Continuous monitoring indicates (55 - 60%) little ones stay the transmission of HCV optimistic within becoming an adult. (Timothy R. Morgan, 2006)
- **Differences in gender for HCV disease illness:** Young individuals significantly possess reduced chronic condition percentage for Hepatitis C disease. Several substantial amounts of breakouts recently arose within the maternal stage who got stained Rh immunological. 17 years

and 20 years follow up study reveals respectively 704 Irish and 917 German women both showed 55% prolonged frequency. (Timothy R. Morgan, 2006)

- **Difference between races with infection caused by HCV:** In case of persistence infection with HCV, African Americans had higher prevalence (86%) than Hispanic whites and Caucasians (68%). 95% users of drugs reported an ongoing infection with HCV in Baltimore. African Americans have much more risk than Caucasians of getting hepatocellular carcinoma. (Timothy R. Morgan, 2006,)
- **Immune response and jaundice**: One study showed that jaundice symptoms are more prone to clearance of viruses. Germany found 43% individuals having chronicity after longitudinal investigation study infected women who have contaminating Rh immune globulin contrast to 60% individuals that stayed anicteric. Strong immune mediated Th1 lymphocytes and mediator reaction against hepatitis C jaundice were observed. The reaction of the immune system adds to the formation of both persistent HCV as well as the fibrosis in the liver. (Timothy R. Morgan, 2006)

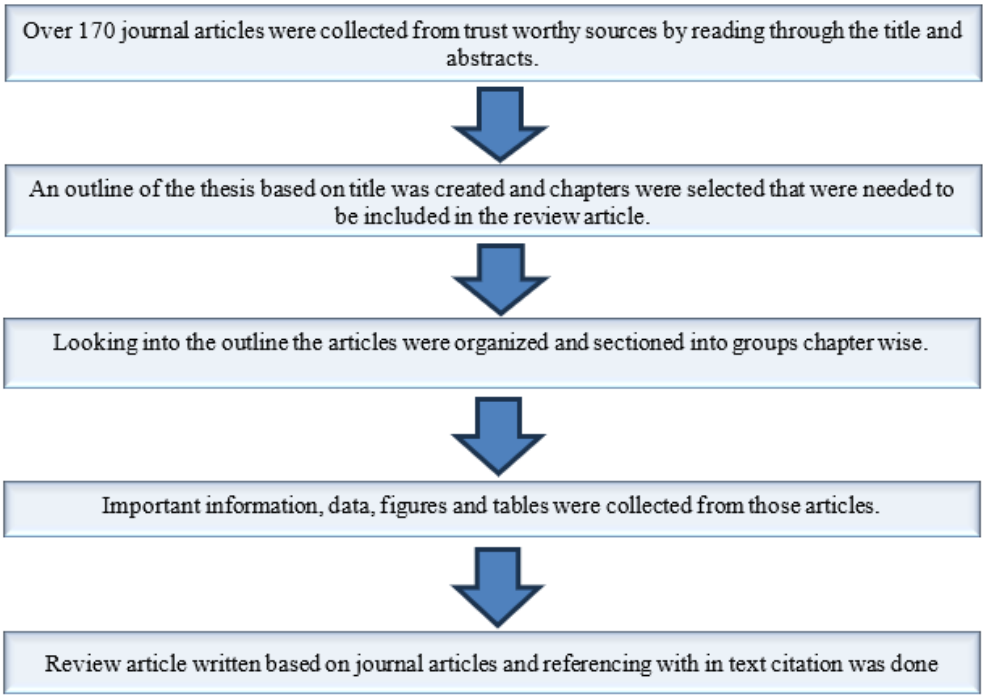
1.5 Objective of the review article

The objective of this review article is to examine therapeutic approaches for Hepatitis C virus infection and the synthetic methods of direct acting antivirals in the existing scientific literature. It provides the overview of HCV impact in human health and also studies approach that are up to date in improvements of the synthesis of DAA, including their prospects to enhance synthetic method that will further result in better efficacy by lowering resistance which shows positive outcome in patients. The article involves the finding the mechanism of HCV its lifecycle, pathogenesis and virulence, classification, total synthesis procedures of antiviral drugs and their structure activity relationships. It shows the development and discovery of DAAs in recent time. Also, it states their clinical impacts and challenges developed in methods of synthetic process of DAAs. This review opens up the stage for future investigation in this area by identifying the knowledge gap in this area of research.

Chapter 2

Materials & Methodology

This review article is written from various journal articles that were found on medical research-based search engines. At first, all the relevant research articles were collected together by reading the title of the article and by going through the abstract based on the topic of the review article. Some renowned search engines like PubMed, google scholar, web of science was used for literature search. Journals like Journal of Hepatology, International Journal of Medical Sciences, the journal of organic chemistry, Bioorganic & Medicinal Chemistry were analyzed. Keywords are useful for this process. From those gathered articles duplicate ones were cut out. Then depending on the relevancy those selected articles were fully reviewed to see if those articles met the criteria of inclusion or not. An outline was made for an easy and accurate writing for each chapter. Then important data and variables were collected as per need. Lastly, referencing was done carefully. And all the information was compiled that was gathered from the studies. In text citation was also done for better understanding and to achieve more comprehension. All the process of methodology is outlined below using a flow diagram:



Chapter 3

The mechanism of (HCV) infection, its life cycle, and virulence:

3.1 The lifecycle of hepatitis C

Previously, the risk factors indicated the entry of viral hepatitis C among risk groups that triggers the life cycle of HCV, which involves glycosaminoglycans where the particles of HCV are moved to the receptor of the cell surface. When the uncoating is done then the translation of viral RNA occurs. Here viral RNA converts and results in a forerunner polyprotein which is turned into one and all viral proteins with the help of cellular proteases. Replication of viruses employs a complex of viral polymerase which takes place on the membrane of endoplasmic reticulum. Reports show that a number of molecules are responsible for receptors for the entry of viruses. When cell culture process is done outside a living organism made the unfavorable determination of receptor/co-receptor requirements complex for Hepatitis C. E2 protein of HCV plays an important part in the binding of receptors, compared to other flaviviruses.

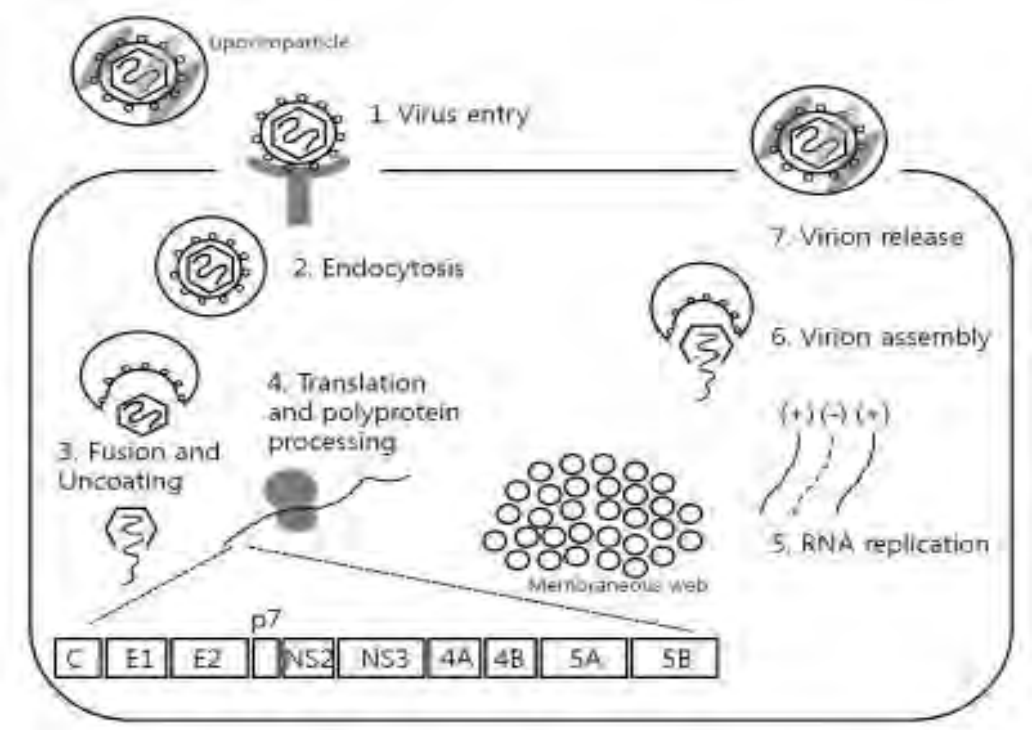


Figure 2: Schematic figure of HCV life cycle (Kim, C. W., & Chang, K. M. 2013)

Human CD81: It is recognized as a nominee for the receptor of hepatitis C. Domain of outside the cell which is E2 that binds to hCD81, 25-kDa that is a protein of membrane which is tetra span. EC2 belongs to the 2nd extracellular domain of Human CD81 which binds to E2 protein. hepG2 which is known as the uppermost layer of the hepatoma cell line that develops bond with viral hepatitis C E2 as well as a pseudotyped VSV conveying chimeric hepatitis C virus proteins of E1, E2. hCD1 which is a soluble recombinant has the ability to neutralize the interconnection of the pseudotyped retrovirus with the cell line of hepatoma Huh-7. For inhibiting the interlinkage in the middle of ability to dissolve truncated human CD81 and E2 neutralization binding assay has been developed.

SR-B type I:

HCV E2 has a possible partner in binding that is a SR-B type I. Type 1a strain of E2 H77 ties up to human a SR-B type I, yet not in mouse SR-R1 via belonging to area 1 that is hyper changing. Human SR-B type I is incorporated into HCV binding upon cells into a Human CD81 through a freeway. Where HVR1 isn't important in HCV. But HVR1 with cDNA clone deficient can pass infection to chimpanzees. The range of HCV positive cells in the liver tissue that is infected is <5% - 100%. Virions produce a rate of fifty particles for each hepatocyte each day. (Bostan & Mahmood, 2010)

The cycle if viral replication involves:

- Attachment & entry to the cell: Requires a contact with the virus who receives the outer side of the cell receptor as well as attachment of protein relating onto the outer side of that molecule.
- Translation of polyprotein along with processing: The genomic RNA is translated by a direct route to the inner part of the cytoplasm. Hepatitis C virus IRES activity is affected by some aspects.
- Replication of RNA: In the synthesis of "– "and "+" strand ribonucleic acid which generates elongation of 3′ end. Also, NS5B is capable of duplicating viral hepatitis C genome which is in full length.

- Assembly of virion and release: core proteins that interact with the genome of RNA, the formation particle begins from that.
- Immunopathogenesis of HCV: Helper T cells that are hepatitis C virus specific helps in activation as well as the distinction of B cells. Also, the stimulating activity of cytotoxic T cells that are virus specific. That experience causes the virus infected cell lysis. Hepatitis C virus can cause long term infection in such cases where mutation in hyper variable regions is not present.

3.2 Translation & replication of viral hepatitis C ribonucleic acid includes

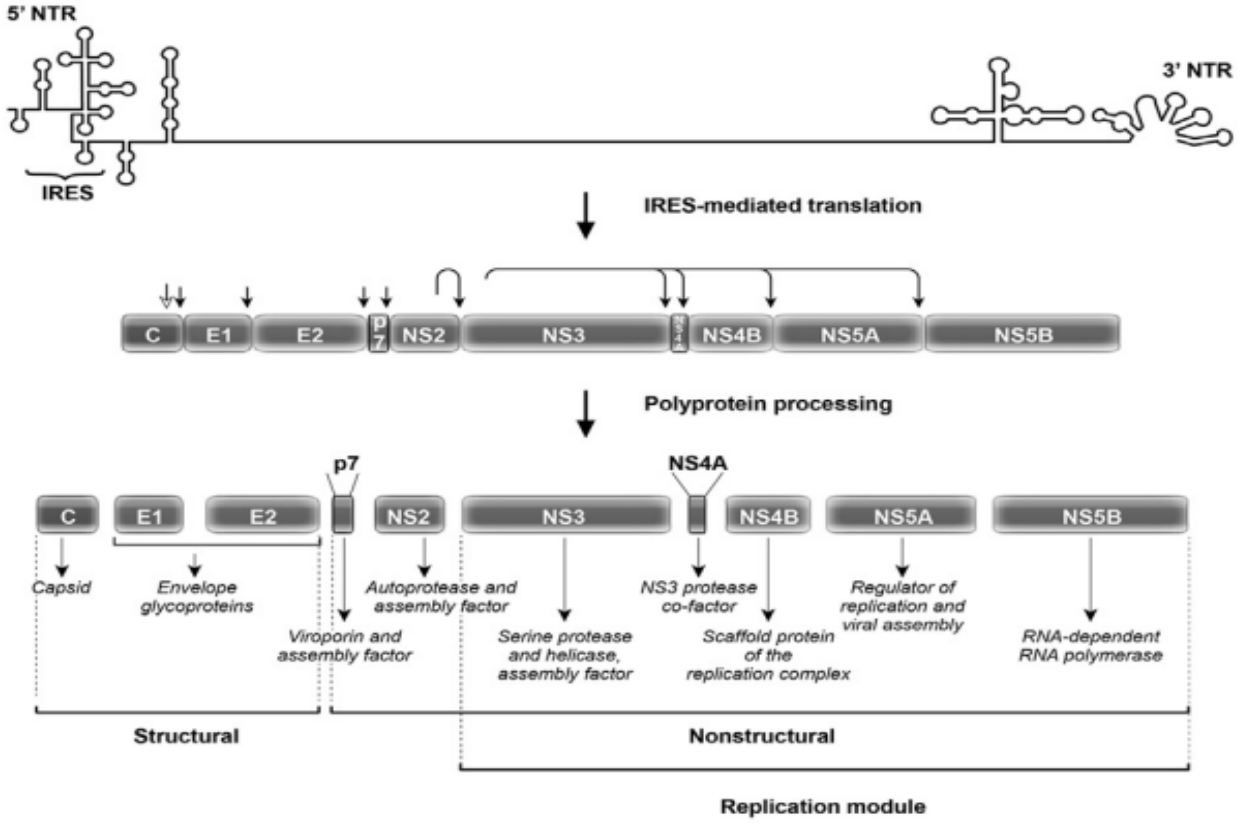


Figure 3: Structure of genome and proteins of HCV (Dubuisson & Cosset, 2014)

The genome of hepatitis C virus involve ORF that is known as single open reading frame also contains untranslated regions fifty and thirty. Which is important for translation of genome and RNA replication of hepatitis C virus. The untranslated 50 region involves IRES (internal ribosomal entry site) where the single polypeptide translation begins. Ten mature proteins were

created from viral polyproteins and the viral and host encoded proteases process that. The main enzyme of RNA synthesis is the viral proteins with membranes from ER. Micro RNA helps in hepatitis C virus replication which is a liver specified (miR-122) which enlists argonaute 2 at the 50 edges of the genome of virus. NS4B membrane proteins are made from a structure using scaffolding of vesicles of membranes that have the function to oligomerize. The rearrangements of global membranes are noticed in the infectious cells of viral hepatitis C that require the collaborative steps in all replicase proteins.

Assembly and morphogenesis of HCV: Release and assembly of the HCV requires structural proteins as well as complete set of genes in ribonucleic acid accumulation. The gathering of HCV particles is connected with the metabolism of lipids. The central region of the protein interconnects with genomic RNA which leads to the formation of nucleocapsids. That is moved to LDSs of cytosol. In the assembly of virion, the central region of the protein builds interconnection with LDs that is important for the recruitment of other components of the virus. To inhibit assembly of viruses the mutations that prevent core interaction with LDs is important. For, budding of virus central part of the protein essentially be gotten back in on or after the LD exterior. NS5A protein shows a key role in the procedure of viral meeting. The C terminal domain of NS5A is needed for the interplay with LDs. NS5A has a C-terminal domain that involves the short time association with the complex of p7-NS2. Mutations in NS3/4A lead to the defect in the assembly of viruses. Helicase area of NS3 and the central protein interaction stands important for the assembly of viruses. The steadiness amid viral hepatitis C ribonucleic acid imitation besides the making of transmittable elements is regulated by (YB-1) which is Y-box-binding protein 1. The remaining NS4B and NS5B, are involved in the viral assembly process. (Dubuisson & Cosset, 2014)

3.3 Hepatitis C virus infection can be chronic

Chimpanzees can be the most occurring example of hepatitis C virus infection that develops long term infection as well as evolves hepatocellular carcinoma after being infected with HCV. Strong intrahepatic CTL - were identified who were having poor antibody responses but solves acute hepatitis C virus infection were identified in two chimpanzees. CTL recognizes the change in dominant epitopes that arose in 3 continuously infected chimpanzees at the short time infection phase.

Immunomodulatory function of HCV: Through the action of holding together of core protein with the receptor of gC1q that aids in the proliferation of T cells as well as phosphorylation of ERK/MEK. HCV can change or modify the innate immune system. Where envelope protein E2 builds a connection with PKR that suppresses eIF2 alpha phosphorylation. NK cells inhibit cytokines production which changes innate immunity that leads to long term infection. Phosphorylation of IRF-3 inhibits NS3/4A complexes that leads to the IFN.

type I transcription. (Kohji Moriishi & Yoshiharu Matsuura, 2003)

To limit the spread: Adaptive immunity is stimulated by innate immunity that works as a first line defense resistant to HCV contagion. RNA of HCV connects with retinoic acid-inducible gene I that activates MAVS protein as well as TIR domain that holds prompt IFN-b (TRIF). Kappa light chain relish of operated cell B as well as IRF3 movement towards nucleus. MAVS and TRIF are cleaved with the help of NS3-4A protease of HCV that moisten the induction of IFN. For limiting HCV repetition then spread of virus innate antiviral responses plays a role in this case. Yet seldom liaise with infection elimination with no action of adaptive immune response. IFN-g is produced by T-lymphocytes which is a hallmark Hepatitis C virus control. The infection that stays more than 6 years is called chronic infection. (L.B. Dustin et al., 2016)

3.4 Antiviral Therapy for long term HCV Contagion

RNAi in HCV is a post transcriptional rule of expression of protein. RNAi involves 2 steps in lower organisms one of them is the process of turning double stranded RNA into (siRNA) as

well as embracing it into the RISC that is called RNA-induced silencing complex. RNA Interference have possible therapeutic uses such as: HIV, HBV and poliovirus. RNA Interference subdue expression of genes and the replication of ribonucleic acid of HCV in the scheme of replicon. Forty percent of patients with long term viral hepatitis C show remission but with side effects that are notable. Number of hepatitis C infected cells can be reduced by a modified pro-apoptotic protein Bid. Recombinant Bid that encodes adenovirus stops replication of HCV that takes place in test tube system. Besides if human livers are inserted into mice, they will be infected through HCV. Presenilin known for core protein that is needed for antiviral therapy. The activity of SPP can be inhibited by some compounds that are evolved for presenilin. (Kohji Moriishi & Yoshiharu Matsuura, 2003)

Chapter 4

Classification of drugs based on current treatments: some standard antiviral drugs

Interferon based therapy was a foundation to treat HCV for more than ten years in the past. PEG-IFN and ribavirin was used as a combination and it shows SVR less than 50%. The study of viral genome as well as getting to know protein structures provide guidance to development of DAAs. Further development in DAAs provides higher efficacy with fewer side effects and fewer interactions between drugs that in result provides good SVR that is more than 90% or it can be more than 95% equally in patients who have failed DAA treatment previously. (Gutierrez et al., 2015). Life cycle of virus becomes unsystematic because of DAAs which targets specific proteins that is encoded with HCV. When PEG interferon is used in combination with DAAs, the efficacy of treatment is increased which causes undesirable effects. The combination therapy of sofosbuvir and ribavirin for around 2 to 3 weeks involves the initially accepted regimens for genotype 2 and 3 which are free from interferon. Peginterferon and ribavirin are used as a combination and attain an advanced SVR amount. Studies of phase 2 and 3 were detected variants that enriches the hepatitis C virus chains in individuals who do not achieve SVR along with telaprevir.

4.1 Studies of SVR based on clinical trials

Hepatitis C virus genotype 1 with polymorphism Q80K have decreased SVR rates is found from Phase 2 and 3 clinical trials. Baseline resistance testing is suggested for genotype 1a patients, if polymorphism Q80K /NS5A is present, simeprevir treatment is avoided. Some examples of NS5A inhibitors are: daclatasvir, ombitasvir and ledipasvir.

In phase 3 clinical trials Elbasvir and Veltapasvir are being studied with grazoprevir which is a NS3 inhibitor and sofosbuvir that is a NS5B inhibitor. NS5A domain I have been targeted by

Daclatasvir. Simeprevir shows RAV that is treatment emergent which gets approval with pegylated interferon and ribavirin. Gln80Lys is a variant for baseline testing,

Asunaprevir and daclatasvir that is an NS5A inhibitor 11 patients were under trial for 24 weeks and attained SVR. Japan's SVR rate is 90% so the combination shows effectiveness. Ombitasvir and daclatasvir are combined with paritaprevir. That is coformulated with CYP4503A4 which is a potent inhibitor named ritonavir. Plasma concentrations and half-life are increased with the metabolism of parataprevir which allows one daily dosing. That is co-formulated with potent inhibitors ritonavir, dasabuvir and ombitasvir which shows high effectiveness against infection G1b and have SVR rate 95 to 100% after 12 weeks with or without ribavirin.

4.2 Combination therapy with inhibitor and DAAs

Potent direct acting antivirals like NS5A inhibitors most clinical regimens used in this. This inhibitor is successfully acted as mixture by means of supplementary DAAs that show good safety and efficacy but resistance is the key obstacle. Along with NS5A RAVs is a frequent baseline earlier to subjection. Genotype 1a and 1b have the lower resistance barrier.

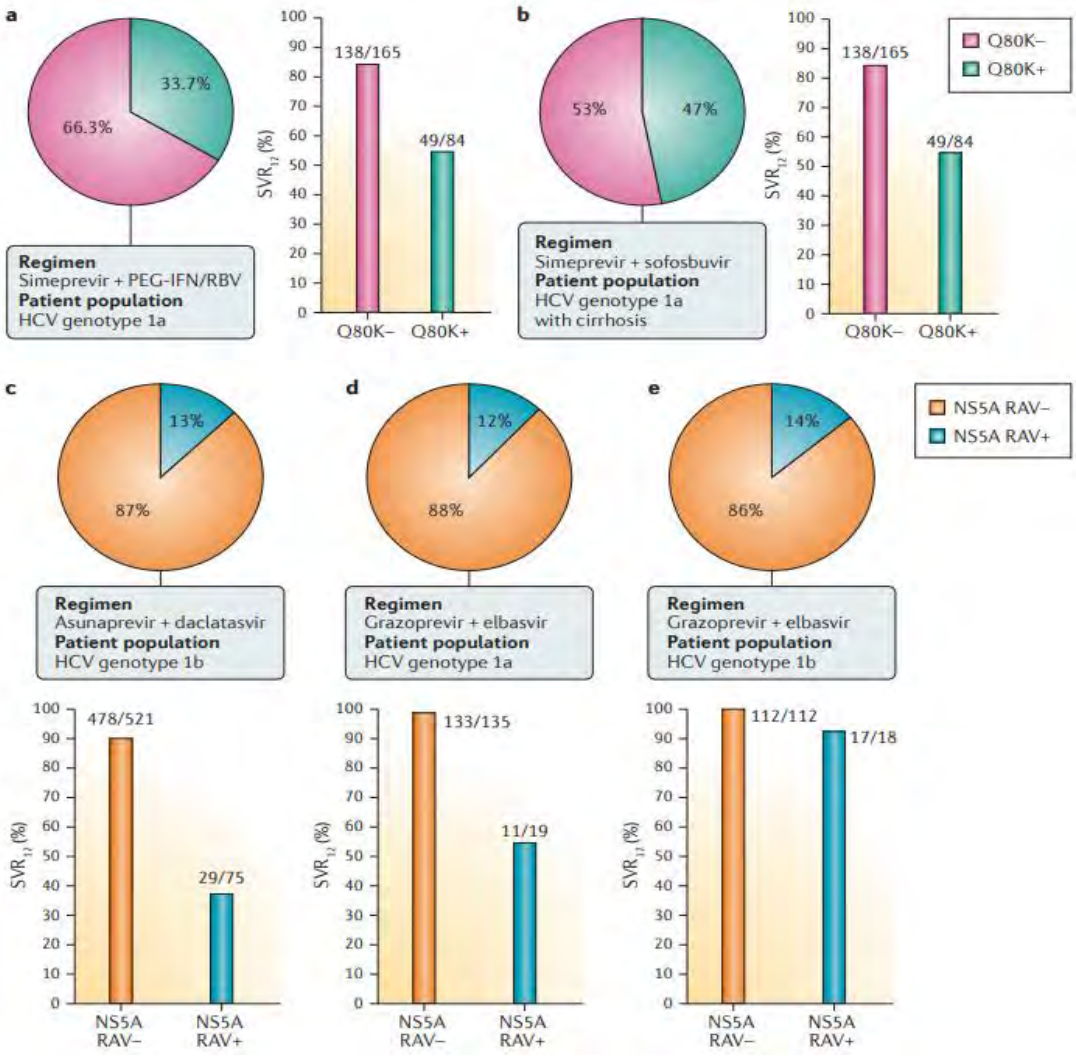


Figure 4: On various DAAs regimen the baseline effect of RAVs (Götte & Feld, 2016)

Genotype 2 and 3 is usual baseline variants.in NS5A inhibitors with PIs is the key determinant outcome of baseline NS5A resistance. 12% patients have G1b infection with NS5A baseline resistance. With resistance the SVR rate in these patients stands at 40% whereas, without resistance the SVR rate stands at 89%. Before using this combination, rules of Japan commend starting point test of RAV. 2nd cohort protease inhibitor Grazoprevir besides NS5A inhibitor Elbasvir, baseline RAVs endure a difficulty. 58% of genotype 1a patients with RAVs attain SVR, in the phase 3 trial. 14% patients were present in baseline RAVs but SVR is present in 94% of patients in the G1b population. When RAVs constitute the variants that were identified have greater clinical significance. Is greater than 15% of the population. Affected role through

genetic constitution 1a who have RAVs more than 15% the amount of SVR stands at 89%. For the detection of relatively abundant variants DNA sequencing shows more effectiveness.

Testing the baseline of RAV would be advantageous for regimens that contain NS5A inhibitors in the particular inhabitants. Different therapeutic options if detected the expenditure as well as the complication of testing RAV might be rationalized.

4.3 Accomplishments in clinical areas of DAAs

Direct acting antivirals have exceptional accomplishments in clinical areas which shows good outcomes in the actual world work as well as in clinical trials. The period of clinical evolution factor will mark the space of efficacy accompanied by the outcome for hepatitis C virus genotype 3 which have the potential to have infection with liver cirrhosis, co-infection of human immunodeficiency virus and patients with specified simultaneous presence of two or more diseases. The challenge that is last became distended by the uptake of treatment to secure the health benefit of the public. (Götte & Feld, 2016)

DAAs have shown recent therapy of hepatitis C virus has limitations forty to fifty percent of hepatitis C virus G1 and G4 patients timely achieve SVR. peginterferon and ribavirin possess serious side effects that involve RBC deficiency, deficiency of platelets in the blood which leads to the bleeding into the tissue, cytopenia's and neutropenia. Different types of alpha interferon fall under learning of possible suitability because of the monthly dosage. Taribavirin drugs are freshly introduced and have low efficacy yet achievable charge of anemia. Also, have much side effects such as: similar to influenza symptoms. (Saira Munir et al., 2010)

4.4 Challenges in the development of vaccine

There are challenges remaining for patients with HCV. The key challenge is the evolution of vaccines that restores the response of T cells and creates antibodies that show neutralizing activity in contrast to viral lipo-particles. Also, there is no proper regimen for the patients who have DAA treatment failure previously and they are in need of new treatment now that remains in research. Another difficult task is to identify asymptomatic patients. To eliminate HCV

traditional vaccine development should be the goal now. Prophylactic vaccine usage is the major clinical need that is not achieved now. So, the aim should be developing a vaccine of HCV that will be effective in the treatment. (Stanciu et al., 2021)

Chapter 5 Total synthesis of five antiviral drugs

5.1 Daclatasvir: NS5A inhibitor

Daclatasvir is a NS5A protein which works as an inhibitor of viral hepatitis C. It is known as DAA which has powerful action that is pan genotypic (McCormack, 2015). It stops synthesis process of hepatitis C RNA that is intracellular and also it inhibits the assembly of virion with emission (Keating, 2016). Lead 12 here is the prototype molecule, if lead 12 cut short by trimming the phenyl group which further results in molecule 13 that reduces the potency of the molecule**.** Capsizing the molecule 13 cap chirality results in a number 1 molecule that shows very high inhibitory potency in opposition to all genotypes of viral hepatitis C replicons and hybrid replicons. Also, species that undergoes preclinical trial for them molecule 1 revealed pk properties parameters in which bioavailability in oral exhibits range from (38-100) % as well as safety parameters which shows support in clinical trial 1 improvements. (Belema et al., 2015)

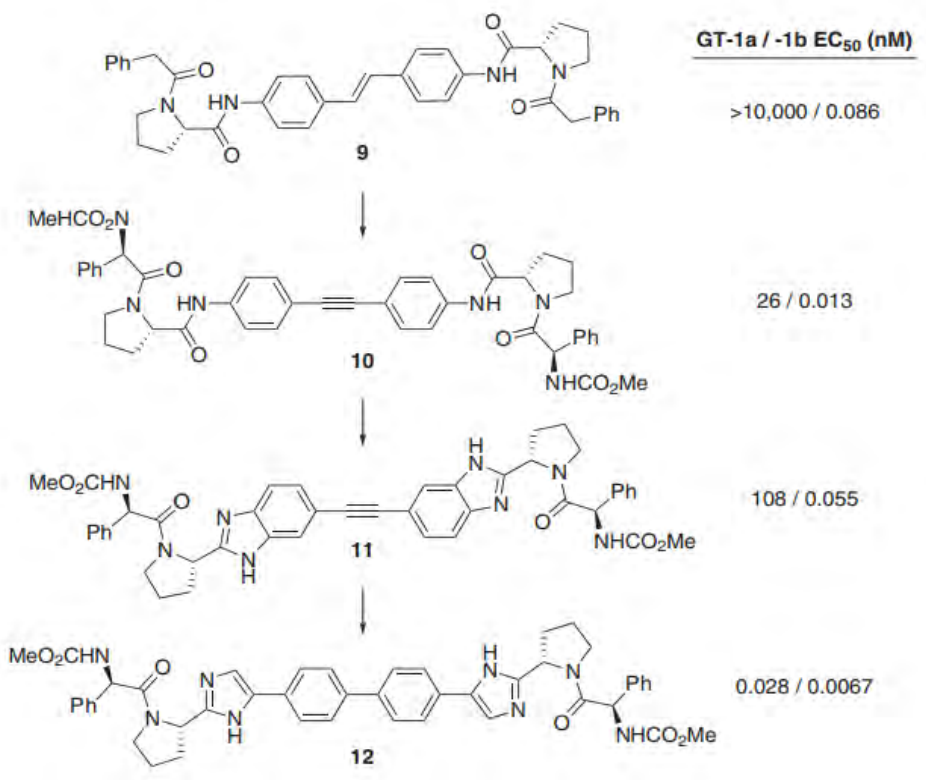


Figure 5: Daclatasvir formation of lead 12 (Belema et al.,2015)

Chemotypes associated Daclatasvir antiviral which is a NS5A protein recognized as likely target for Daclatasvir. As a result of refusal to accept as well as to bestow on N terminal mutation and the identification of domain-1 occurs. In the cells of replicon, NS5A proteins are colocalized with inhibitor NS5A that contains aside. Daclatasvir when combined with NS5A cell free protein, binds directly and provides sensitivity to RNA and mutants that are resistant conferring. Daclatasvir works as a reducer of affinity of NS5A for RNA. (Belema et al., 2015)

5.1.1 Route for Suzuki Coupling and palladium catalyzed coupling

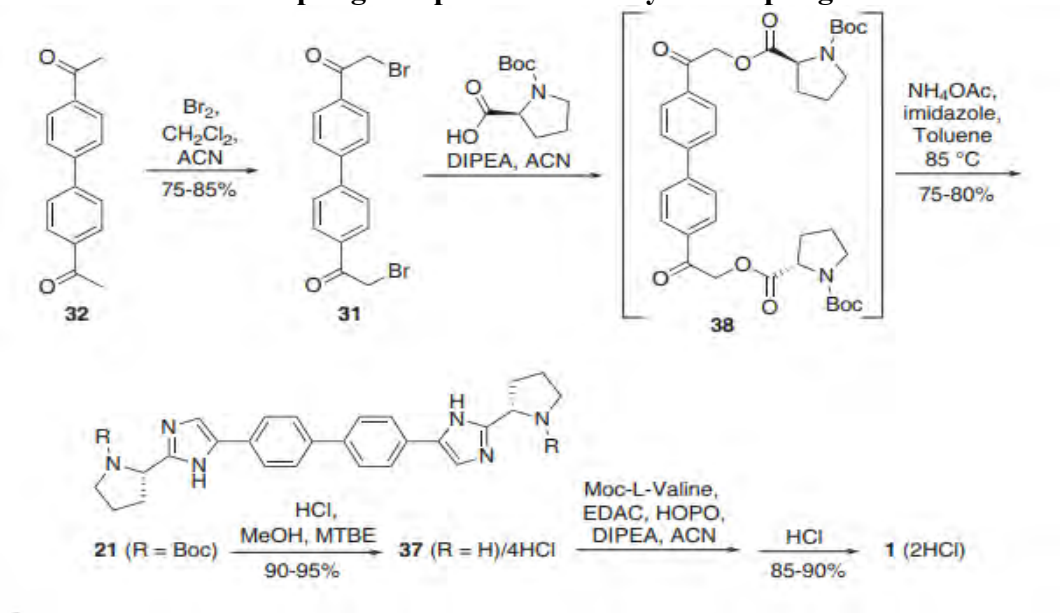


Figure 6: Route for Suzuki couplings (Belema et al., 2015)

At first, Bisacetophenone 32 was used as a starting material and after bromination it produces dibromide 31 with purity ranges from (50 to 75) %. In reaction it must be observed that the extra amount of bromine should be removed at a time that is the major reason behind gaining bis-bromination as product (Easter et al., 2016). Then acetonitrile is introduced which retains HBr into the solution mixture. Imidazole is used as a buffering agent, so that less structural changes occur. Categorization reaction happening through t α-acyloxy ketone that forms α-bromoacetophenone and the carboxylic acid generation. 1H-4-substituted imidazole compression with ammonium acetate was achieved in a great temperature coil reactor that is made of stainless steel. Temperature more than or close to 150 °C was vital for the formation

of the anticipated imidazole that have great percentage purity (Carneiro et al., 2015). Methanol helped to isolate crystalline Boc-protected hexacyclic core where chemical purity is more than 99% and chiral purity is slightly more than that >99.5% and yield is 75 to 80%. HPLC purification method was employed (Easter et al., 2016). Enhancement of the radiochemical purity was done by recrystallization to fit wants. Outcome from this method was methanol also acetone at 50 °C that produce 78% yield and 99.9% was the chemical purity whereas 98.9% is radiochemical purity (Easter et al., 2016). The Boc groups were removed via HCl. Lastly, coupling of peptides using coupling agents like EDAC/HOPO with Moc-valine. Daclatasvir was isolated using anhydrous HCl which forms di-hydrochloride salt. The DCV is separated from acetone or methanol. Batch synthesis is the manufacturing process of drug of anti-viral that requires 3 to 15 days production time, needs noteworthy personnel as well as organization. Construction of the completely united manufacturing schemes that were based on flow proposes an alternate tactic that can be much economical by greener substitute (A. Rana et al., 2020). Where bis-acetophenone 32 plays a role in the 43 to 58% of the total collection (Belema et al., 2015).

5.1.2 Development series in discovery of drug which targets viral hepatitis C

The simplification of a molecule that consists of two identical molecules connected with each other (lead) in addition to thiazolidine moieties occurs by removing which leads to the discovery of compound 9 that is known as stilbene chemotype. This compound 9 is very potent towards the replication of HCV GT-1b and also shows less potency towards GT-1a. For the optimization of compound 12 antiviral spectrums should be expanded in the time of constructional accountability. For this modification is needed, addition of cap of pyrrolidine, change in structure to include phenyl carbamate moiety in the peripheral part. Thus, chemotype binarylimidazole is discovered. Which shows potency in high level towards both replicons which are GT-1a and GT-1b. In Spite of having greater potency this compound showed oral

bioavailability that is poor in rats. The ultimate phase aimed at increasing properties of PK. (Belema et al., 2015).

Daclatasvir is a DAA agent that is approved in the EU for use in combination with other medicinal products for the treatment of chronic HCV genotype 1, 3 or 4 infections [3], and in the USA for use in combination with sofosbuvir, with or without ribavirin. (Keating, 2016)

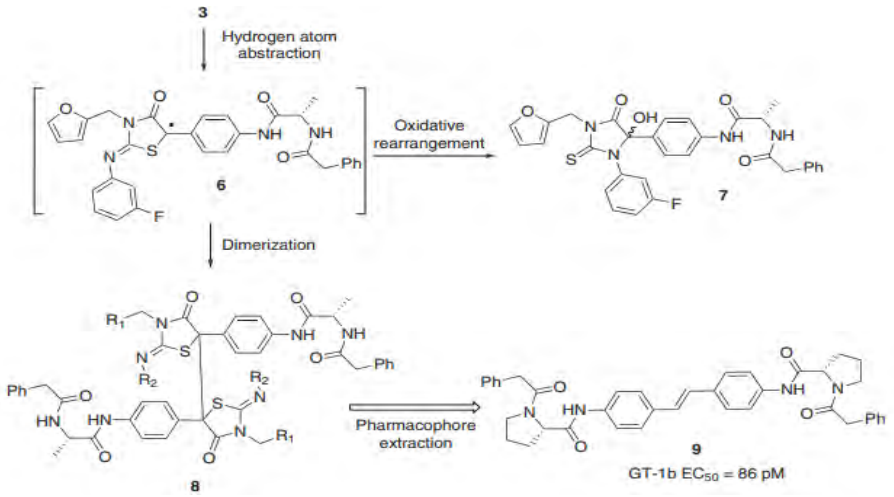


Figure 7: Formation of stilbene chemotype 9 (Belema et al., 2015)

5.2 Simeprevir

Simeprevir is known as a NS34A protease inhibitor of viral hepatitis C. It has a core unit of cyclopentane as well as a macrocycle that is 14 membered. It has synthetic procedures such as hydrogenation of trans-Cyclopentanone-3,4-dicarboxylic acid, lactone cyclization, cinchonidine salt crystallization separation. The intermediate of diene cyclization occurs by ring closing metathesis which is a macrocycle of 14 membered rings.

Ring closing metathesis (RCM) optimization: For this optimization GH1 was used as a catalyst. (8 to 25) % issues of epimerization occurred because of remaining bases from steps that are upstream. To solve this problem 10 mol% methanesulfonic acid is needed to reduce that problem of epimerization. (Horva´ th et al., 2019)

Formation of polymer issue and concentration issue: concentration at 0.01 M the outcome of the reaction is molecular polymers that possess high mass (10 to 18) % and GPC is the

analyzation technique. Chromatography, treatment of charcoal, Nanofiltration of organic solvent is used to maintain purity. (Horva´ th et al., 2019)

Toluene at reflux testing: G1, GH2, M2 are tested. And for screening methods of solvents DCE and toluene were picked on the basis of results of screening. (Horva´ th et al., 2019, #) Literature methods include CSTR, SHD, templating, distillation, and agents for encapsulation that enhance efficiency of macro crystallization. (Horva´ th et al., 2019)

Modification of substrate: Enhanced macro crystallization efficiency is shown by N-Boc-modified substrate 8 along with catalysts of 2nd generation in comparison with diene 7. The time of reaction is 0.5h for the cyclization of 8 with catalyst M2 in reflux toluene testing. Meanwhile, 7 along with GH1 catalyst in DCE reflux testing took 6h. Diene 8 showed no epimerization. (Horva´ th et al., 2019)

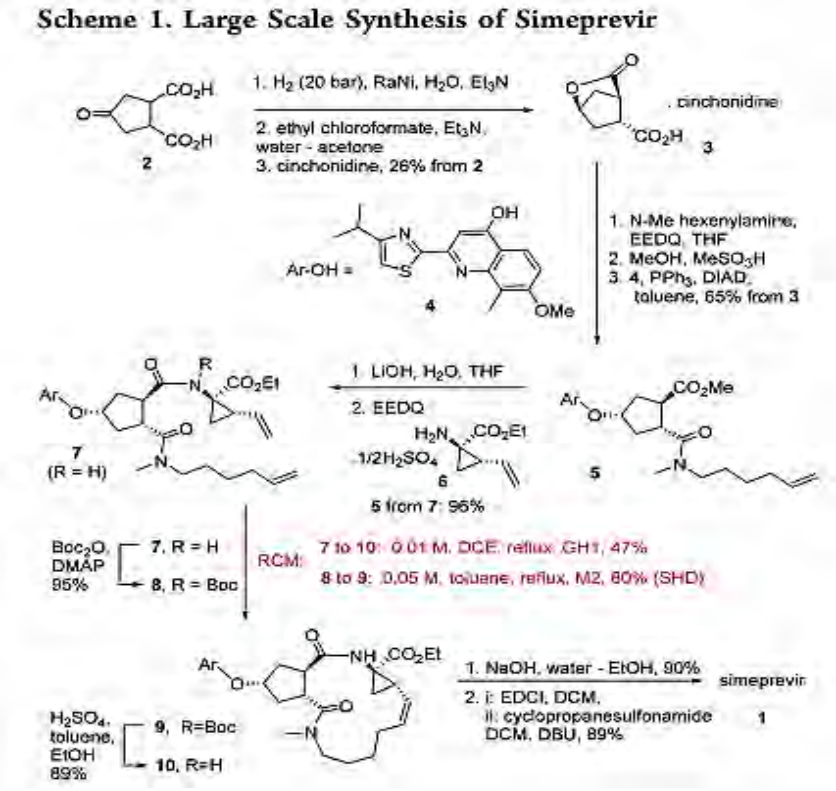


Figure 8: Synthesis of Simeprevir in large scale (Horva´ th et al., 2019)

Ring closing metathesis of 8 along with catalyst M2 falls under kinetic control; on the other hand, RCM of 7 with catalyst GH1 is a part of thermodynamic control. (Horva´ th et al., 2019)

5.3 Grazoprevir

The antiviral drug grazoprevir is known as a potent NS3/4a protease inhibitor. There are some challenges in the synthesis process such as: coupling of lithium acetylide as well as enzymatic aspiration based on this starting synthesis procedure that is ineffective as well as unsafe. 2 substituted cyclopropanols have practical synthesis that is asymmetrical which throws a mentionable challenge. (Xu et al., 2017)

For the formation of chlorohydrin, 3-butenyl magnesium bromide was used with epichlorohydrin. For the step of elimination, the base of LiNH(CH2)2NEt2 was evolved. That ensures homogeneousness as well as a good strong condition when the production occurs on a large scale. (Xu et al., 2017)

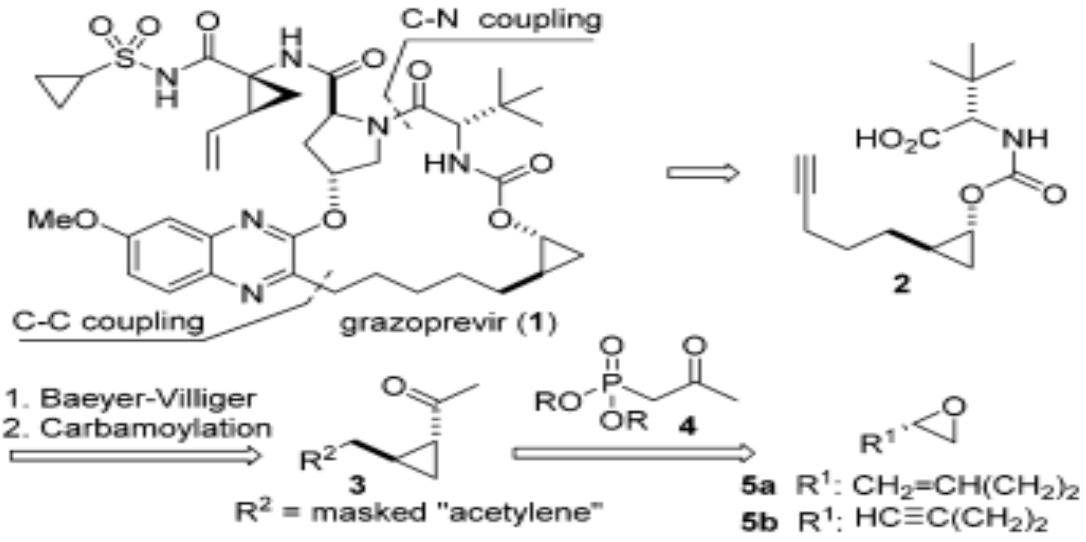


Figure 9: Retrosynthetic analysis of Grazoprevir (Xu et al., 2017)

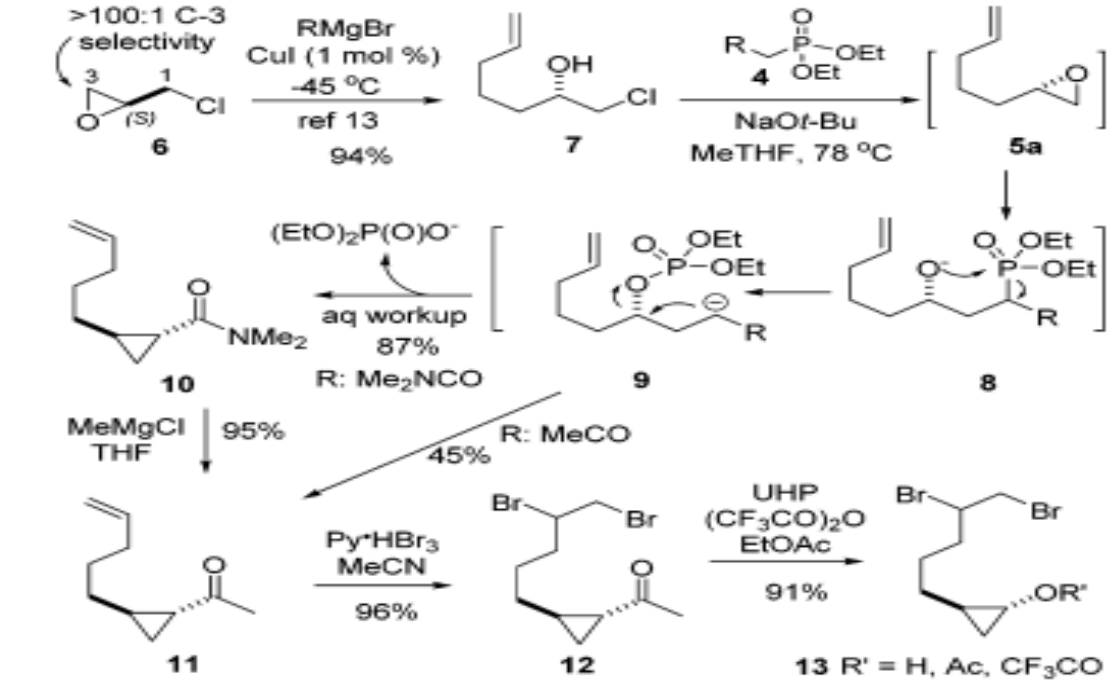


Figure 10: dibromide 13 process of preparation (Xu et al., 2017)

This synthesis procedure follows some steps. The final building block was attained with no intermediary isolation. This synthesis starts from epichlorohydrin and the ultimate building block 2 was achieved. The final product's total yield stands at 51% and it has purity of more or equal to 97%. (Xu et al., 2017)

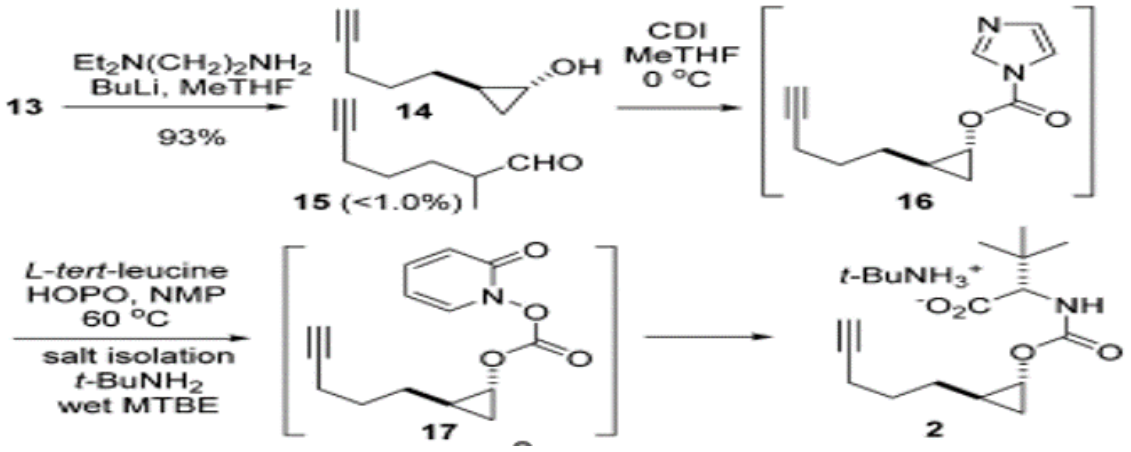


Figure 11: Building block 2 method of synthesis (Xu et al., 2017)

5.4 Emitasvir

Emitasvir works as a potent NS5A inhibitor. It shows effectivity towards G1. wide range of antiviral treatment is needed for this development. Methods of initial synthesis involve chemical resolution that results in a reduced yield and lower purity because of low atom economy. For the synthesis of Emitasvir acylation of 3,6-dihydroxybenzonorbornane take place that attains enantiopure intermediary this synthesis pathway is called retrosynthesis.

Development of the process includes enzymatic reactions that make the most effective use of the situation by utilizing the process of hydrolysis in solvents that is organic in nature. For enhancing the purity and the product outcome particular PH and temperature need to be maintained. (Xie et al., 2021)

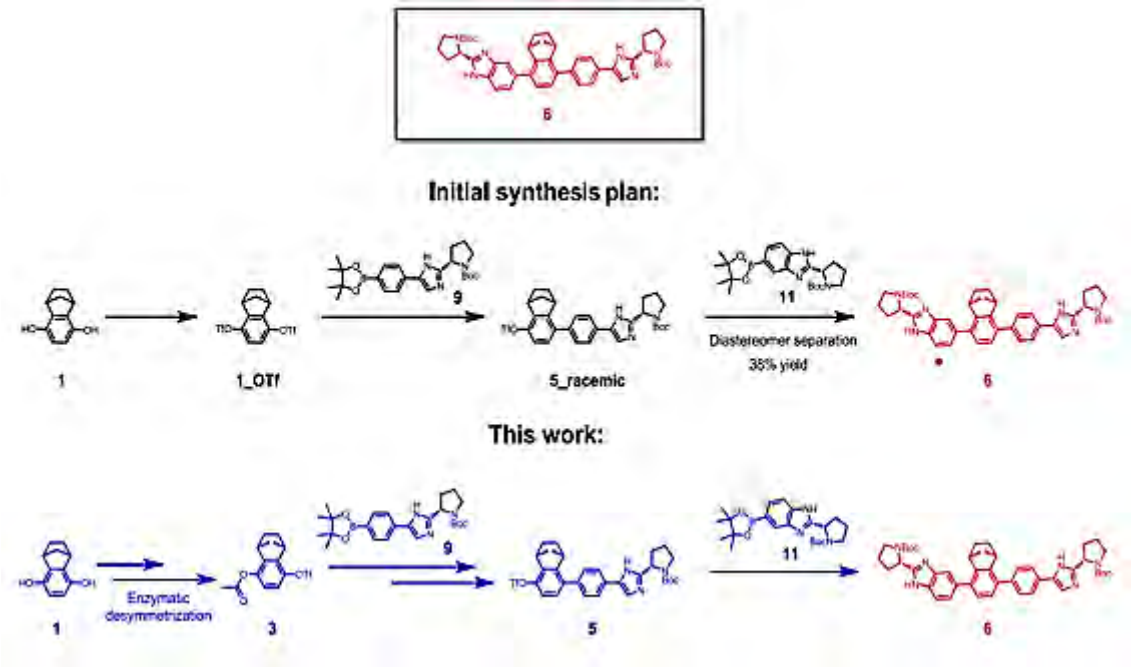


Figure 12: Routes of Synthesis for intermediary compound 6 (Xie et al., 2021)

5.4.1 Necessary Couplings

Triflation: Tf2NPh is a sulfonylating reagent and triethylamine is used as a base for optimizing situations. As a result, enhance selectivity with improved yield.

Suzuki coupling: If more optimization is required then it goes through Suzuki coupling. In this coupling sodium hydrogen carbonate was used as a base as well as a solvent was used with it that results in a greater selectivity and yield.

Amide coupling: Carboxylic acids and amines forms amide bonds that is called amide coupling

Deblocking: Tert-butyloxycarbonyl is removed by using trimethylsilyl reagent or hydrochloric acid in different types of solvent. Protective groups are removed in this method and this is named as deblocking.

(Xie et al., 2021)

5.4.2 Optimization to achieve high yield and efficiency

For optimization of the condition of the reaction that results in a high yield and purity different bases and catalyst screening was done.

- For suppressing side reactions and to improve selectivity Tf2NPh which a triflating reagent optimization is needed.
- To improve efficiency of the reaction temperature and solvent is needed.

(Xie et al., 2021)

5.5 Sofosbuvir

Sofosbuvir is an antiviral drug where protide technology is used, here potential antiviral agents are pyridine and pyrimidine thioglycoside phosphoramidates. Sofosbuvir and tenofovir alafenamide uses protide technology, in this technology the bioavailability of nucleoside increases by masking nucleosides which further enhance absorption and give improved delivery. There are synthetic methods used for making thioglycoside phosphoramidates that

are aimed at nucleophilic substitution reaction and have following deprotection measures. In this method there are heterocyclic bases and moieties of sugar that are linked together by thioether bonds which are assured through X-ray crystallography. (Abu-Zaied, et al., 2020)

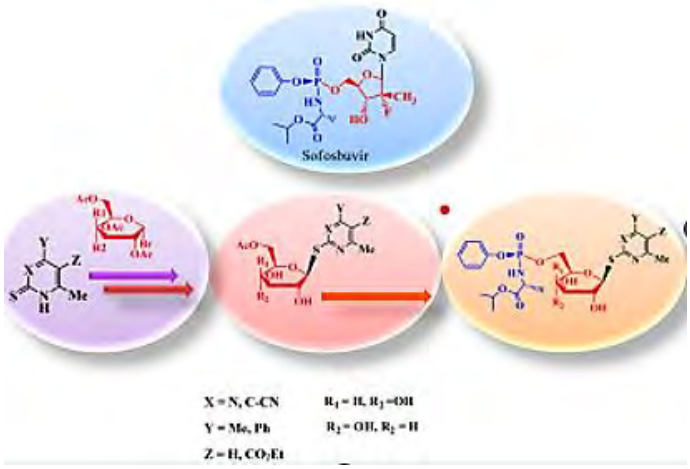


Figure 13: Synthesis of Sofosbuvir (Abu-Zaied, et al., 2020)

5.5.1 Assessment of antiviral activity

The synthesis of thioglycosides provides 8 compounds which undergo specific tests against DNA and RNA. Adenovirus and herpes simplex virus 1 falls under DNA virus and HCV, HAV, Coxsackievirus B4, are RNA viruses. Nontoxic doses are established by cytotoxic assays. Where high safety profiles showing compounds over different cell lines are compounds 11 and compounds 5b. Mentionable antiviral activity has been shown by compound 11 in opposition to Herpes simplex virus -1 and Coxsackievirus B4. Synergistic effect has been observed when it is used in combination with acyclovir averse to herpes simplex 1 virus. Compound 11 shows average activity against viral hepatitis C when it's used with sofosbuvir. (Abu-Zaied, et al., 2020,)

5.5.2 Future improvement areas of DAAs for better therapeutic approach:

There are some therapeutic challenges that need to be addressed by identifying the area of improvement in therapeutics and logistics. Such as there is a problem in genotype 3 HCV for the patients of cirrhosis, sudden drop of SVR identified here. Although DAAs like sofosbuvir

and daclatasvir show significance act towards GT3 and yield high SVR 97 to 98% as well as combination of DAAs like grazoprevir and elbasvir works clinically well if they were combined with nucleotides. Combination of sofosbuvir and ribavirin shows good therapeutic activity for non-cirrhosis patients. Also, this combination is useful for patients with GT2.

For future improvement, short therapy is preferred by patients and contributors. This way is considered as a good way to reduce cost for many but this is an argumentative topic. The access to treatment is not available for low to middle earning countries but has limited availability in high to middle income countries. Healthcare providers must be trained with proper awareness so that the rate of diagnosis increases.

The next generation DAAs have goals to accomplish such as identifying problems like chronic kidney diseases as well as cirrhosis patients with GT 3 infection. Pan genotypic regimens need to be developed because it shows equal effectiveness in every class which eliminates the genotyping needs and monitoring that were done during treatment. Therapy needs to be short and patients who break down treatments like 1st and 2nd generation, for them salvage regimen is needed. This treatment is useful for reducing possible extra use of treatments also, it stays away from viral strains that are multi drug resistant. (Feld & Foster, 2016)

Chapter 6

SARs of select antiviral drugs

HCV treatment options are therapies that are based on interferon is the traditional treatment. Hepatitis C virus RdRp enzymes have allosteric sites in it, and non-nucleoside inhibitors point to that site that offers special benefits compared to other analogues of nucleoside on the basis of profile of resistance and specificity.

HTS is the method through which benzothiadiazine was recognized which acts as a potent inhibitor of hepatitis C virus RdRp enzyme. At the N1 position of quinolinone ring structural

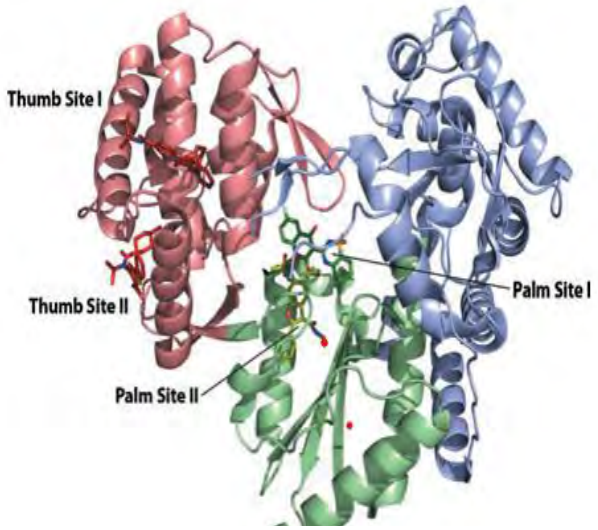


Figure 14: Subdomains of RdRp with its allosteric binding sites (Haudecoeur et al., n.d.)

modifications were done that provided the potency of inhibition. Benzothiadiazine cores have different substitution patterns that work to increase potency. At N1 position, a bulky group of alkyl or it can be cyclic groups can show the activity of inhibition at increased rate. Whereas, 5,6,7,8 position of the ring quinolinone can increase potency if modifications are made. The structural insights is provided by X-ray crystallography about how the inhibitors work with the enzyme of RdRp. Some major binding interactions are at the catalytic site of enzymes and the allosteric site pockets where hydrophobic interactions and hydrogen bonding interactions occur. (Haudecoeur et al., n.d.)

6.1 Derivatives of piperazinone

NS4B is important as a new DAAs that has a target for proteins that are nonstructural Compound 2A was found by high throughput screening which is a derivative of piperazinone that works as a inhibitor of genotype 1 of viral hepatitis C. NS4B is the target of compound 2a that is shown by studies of resistance mapping strategy.

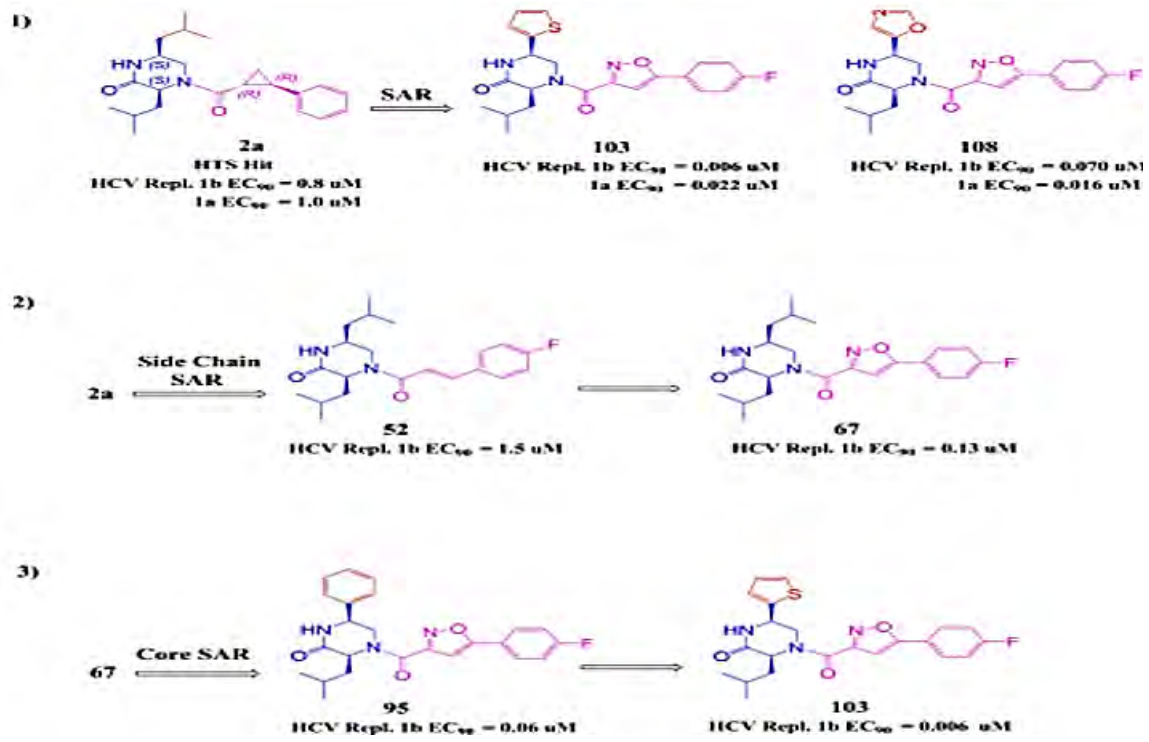


Figure 15: SAR of the piperazinone core (Kakarla et al., 2013)

Key findings from this SAR are:

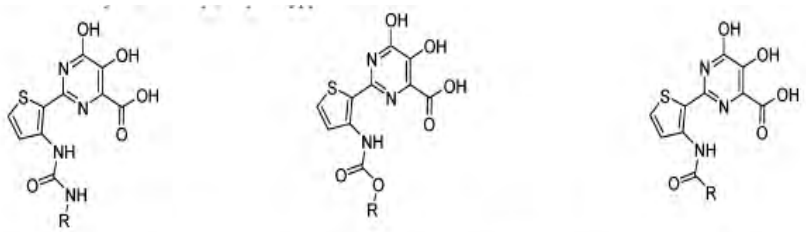
1. 2a Side chain of phenylcyclopropyl amide when replaced with a compound 67 that is p-fluorophenylisoxazole-carbonyl moiety there is tenfold increase in the potency of genotype 1.
2. If carbon 6 is replaced by compound 67 moiety that is hydrophobic in nature with compound 95 that is a phenyl moiety so the reduction in the potency of genotype 1 do not occur.
3. Improved potency has been shown at carbon 6 position against hepatitis C virus genotype -1a as well as genotype-1b if the modification is done with compound 103 and compound 108. They are, respectively, heterocyclic thiophene and isoxazole moiety.

NS4B has residues 90 and 98 that possess mutation in high frequency have shown activity on compounds 2a and 95 that are reducing. Good stability in metabolism was shown by the derivative of phenyl (95) but in the case of the parent compound the potency of genotype 1b does not show any improvement.

(Kakarla et al., 2013)

6.2 NS5B polymerase`

Dihydroxy pyrimidine carboxylates is the reversible inhibitor of the polymerase of NS5B. In case of replication assays that are cell-based hepatitis C virus for this compound 34 which is a dihydroxy pyrimidines with a 2-thienyl group showed upgraded activity. Thiazole like Electron withdrawing group substitution at position 2 of the pyrimidine showed crucial improvement in the potency. Electronic properties of the substituents affect the potency of compounds. Where it's advantageous is if it is an electron withdrawing group Dihydroxy pyrimidines is hydrophilic in nature. At physiological PH dihydroxy pyrimidines many times live as mono or dianions, their ability to cross cell membranes has limited effects that affects the activity that is based on cells. If the lipophilicity is increased it is because of the improved cell penetration effort. Some assays are cell based, potent inhibitors like compound 34 which is a derivative of urea showed activity in the assay of enzymes



R	compd	IC_{50} (μM)[a]	EC_{50} (μM)[a,b]	compd	IC_{50} (μM)[a]	EC_{50} (μM)[a,b]	compd	IC_{50} (μM)[a]	EC_{50} (μM)[a,b]
Ph	**29**	0.58	>50	-			**30**	0.80	>50
$(CH_2)_2Ph$	**31**	0.62	>50	**32**	0.46	>50	**33**	0.58	>50
CH_2(2-Cl-Ph)	**34**	0.15	9.3 ± 2.8	**35**	0.14	31.5 ± 6.4	**36**	0.21	53.3 ± 6.3
CH_2(2-Cl-6-Me-Ph)	**37**	0.09	13.6 ± 3.2	-			-		
CH_2(2,6-Cl_2-Ph)	-			**38**	0.19	18.6 ± 2.3	-		
CH_2-3-indolyl	-			-			**39**	0.20	>50
CH_2-3-benzothienyl	**40**	0.22	30.0 ± 4.1	-			**41**	0.23	>50
CH_2-1-naphthyl	-			**42**	0.14	13.6 ± 2.3	-		
CH_2-(2-Ph-3-indolyl)	-			-			**43**	0.28	>50

Figure 16: Inhibitory action of NS5B RdRp by 2-(2-Thienyl) dihydroxy pyrimidines (Koch et al.,

2006)

The mechanism of action involves an inhibitor binding model which requires an active site Mg ion in the polymerase that demands pyrophosphate-like chelation. Structure activity relationships as well as experiments on mutagenesis supports this theory. So, for the activity pyrimidine ring substitutions such as -COOH and -OH are important.

Lipophilicity of compounds is aimed to enhance the cell-based activity. Compound 34 shows success in this case despite the need for more optimization. (Koch et al., 2006)

6.3 Allosteric inhibitors

Inhibitors in in vitro screening includes applicants of twenty-three that are tested with the help of assay of RNA dependent polymerase inhibition. In this test, important inhibitions are shown by four compounds where compound 3 have IC50 values of 55.2 µM and compound 4 has 60.2 µM.

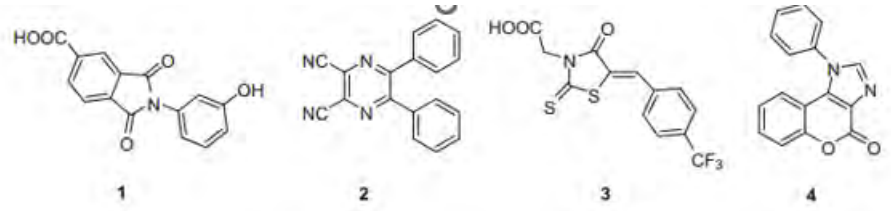


Figure 17: Chemical structures of compounds 1 to 4 (Talele et al., 2010)

Table 3: List of compounds showing Anti- NS5B RdRp activity (Talele et al., 2010)

List of compounds	Molecular weight	C log p^b	Inhibition percentage at 250 µM^c	IC_{50} (µM)^d
1	283	1.73	55 ± 2.8	≈ 200
2	282	3.24	48.6 ± 4.9	≈ 250
3	347	2.31	71 ± 7.0	55.2 ± 1.10
4	262	2.89	62.5 ± 7.7	60.2 ± 1.17

In the study of structure activity relationship rhodamine analogues 5 to 22 compounds were found to be available commercially. And the analogues that are newly synthesized are found to

be compounds from 23 to 28. Point to be noted from major findings is compound 22 showed the most potency where the value of IC50 stands at 10.6 µM when the scaffold of rhodamine was optimized among the analogues that were synthesized. If the modes of binding were analyzed the potential binding modes are the studies of docking of compounds 27 and 28 that are derivatives of optimization.

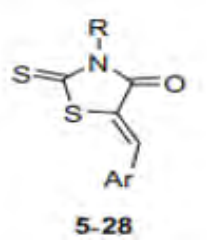


Figure 18: Chemical structure of compound 5-28 (Talele et al., 2010)

Table 4: SAR of compound 3 derivatives (Talele et al., 2010)

List of compounds	R	Ar	Molecular weight	C log p^b	IC_{50} (µM)
5	-CH_2COOH	3-CF_3Ph	347	2.31	50.9 ± 1.4
6	-CH_2COOH	3-BrPh	358	3.02	20.2 ± 1.2
7	-CH_2COOH	2,4-ClPh	348	3.40	17.9 ± 1.2
8	-CH_2CH_2COOH	2,4-ClPh	362	3.96	19.9 ± 1.3
9	-$CH_2CH_2SO_3H$	2,4-ClPh	398	3.20	23.5 ± 1.2
10	-$CH_2CH_2CH_2COOH$	2,4-ClPh	376	4.31	58.0 ± 1.5
11	–$(CH_2)_5COOH$	2,4-ClPh	404	5.10	16.1 ± 1.3
12	-CH_2COOH	Naphthalene-1-yl	329	3.60	61.4 ± 1.3
13	-$CH(COOH)CH_3$	Naphthalene-1-yl	343	3.94	68.1 ± 1.5
14	–$CH(COOH)CH(CH_3)_2$	Naphthalene-1-yl	371	4.64	16.9 ± 1.2
15	–$CH(COOH)CH(CH_3)_2$	4-FPh	339	4.13	42.9 ± 1.3
16	–$CH(COOH)CH(CH_3)_2$	2-ClPh	355	4.18	36.1 ± 1.2
17	–$CH(COOH)CH(CH_3)_2$	2,4-ClPh	390	4.73	14.1 ± 1.2
18	–$CH(COOH)CH_2CH_2CH_3$	2,4-ClPh	390	4.71	28.2 ± 1.1
19	–CH(COOH)CH2CH2SCH3	2,4-ClPh	422	5.26	29.2 ± 1.2
20	–$CH(COOH)CH_2CH(CH_3)_2$	2,4-ClPh	404	5.28	18.9 ± 1.1
21	–$CH(COOH)CH_3$	2,4-ClPh	362	3.94	13.3 ± 1.2
22	–$CH(COOH)CH_2Ph$	2,4-ClPh	438	5.77	10.6 ± 1.5
23	-CH_2COOH	2-NO_2Ph	324	2.10	16.2 ± 1.4
24	-CH_2COOH	4-NO_2Ph	324	2.01	21.9 ± 1.3
25	-CH_2COOH	4-FPh	297	2.65	42.1 ± 1.0
26	-CH_2COOH	3-ClPh	313	2.82	18.4 ± 1.2
27	-CH_2COOH	3,4-ClPh	348	3.39	8.4 ± 1.5
28	-CH_2COOH	3-PhenoxyPh	371	4.26	7.7 ± 1.4

If more improvement in the potency is needed more structure activity relationship studies were needed as well as optimization may help in case of selectivity. (Talele et al., 2010)

6.4 Benzimidazole Derivatives

Vitamin B12 is present on Benzimidazoles that achieved distinction because of their existence in this as well as on evolution as anthelmintics and fungicides. PPI, anthelmintics, antivirals as well as antihypertensives are included in different regions of therapeutics. In the 19th century benzimidazoles were synthesized and there are strategies that are synthetic that involve the oxidation of sulfur, nitro groups reduction as well as reactions that are copper based. Benzimidazole showed antiviral activity such as hepatitis B virus also shows selectivity that is high and reduced cytotoxic activity if it is being compared with lamivudine.

Study of SAR showed that the inhibitory activity of hepatitis B virus has been affected by substitution patterns of benzimidazole rings. The potency of the derivative of benzimidazole which is an antiviral agent is affected by the stereochemical substitutions and configuration (Yadav & Ganguly, 2014)

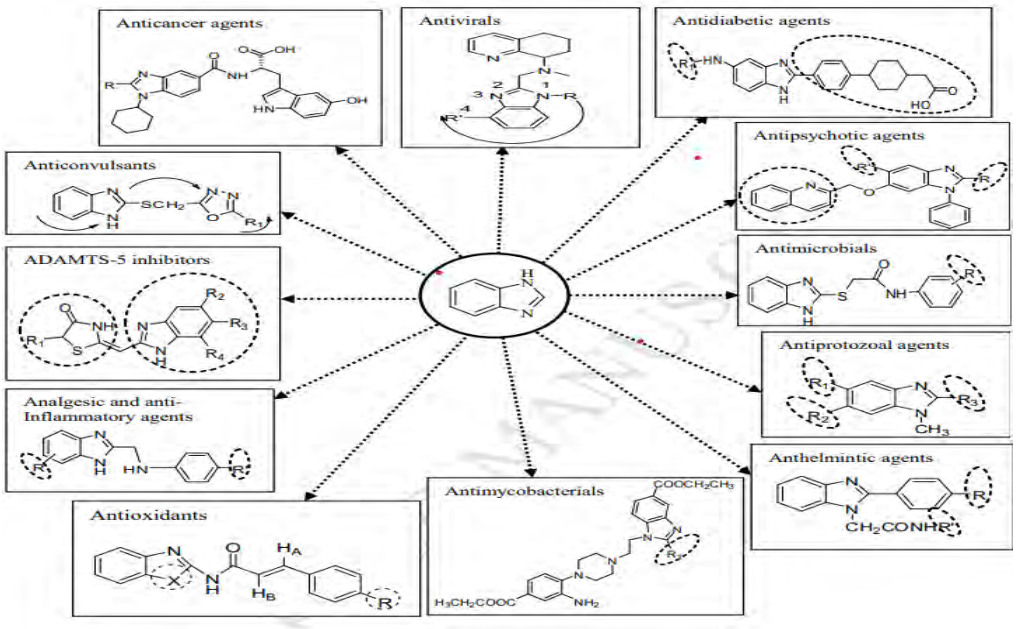


Figure 19: Modifications in structure of benzimidazole (Yadav & Ganguly, 2014)

Chapter 7

Impact of the review article

This review article has positive impact in various fields such as-

In future research and investigation: this review involves synthetic procedures with SARs obtained from literature studies. It will help researchers to get to know that information from this especially individuals who are entering into this area of research. It will keep them up-to-date about relevant information. From this review they will get to know the future challenges that may come, which will help them in the development of novel drugs. It will give them future direction and provide guidance towards therapeutic approaches that is needed to develop more effective medications.

Effect on the enhanced treatment of HCV: If the hepatitis C virus treatment is improved by understanding the current complications and areas that needed to be improved are identified once, so the cost will be less and therapies will become more affordable as well as it will become more patient-friendly

In education programs and trainings: This review will be helpful for pharmacy students or medical students who can use this review paper for their knowledge of the latest HCV therapeutic approaches as well as they can use this as a starting point for their thesis purpose. Also, this review will be useful for health care professionals who are under training to develop their knowledge about the latest treatments approaches in HCV.

Impact on pharmaceuticals: Existing therapeutic approaches can be modified and drug with better efficacy and potency may develop which will have impact on the cost reduction as well as patient compliance. Moreover, data from this review may help in regulatory approvals also.

Chapter 8

Conclusion

Viral hepatitis C now has become a major health threat worldwide. Millions of people have been affected by it and the number of infected people is increasing day by day. So, it becomes important to improve the therapeutic approaches of traditional therapies. Direct acting antivirals have revolutionized the treatment of HCV by reducing the time for onset of action, lowering the side effects, and enhanced rate of cure. In this review article, the key findings of SARs showed the importance of modification of SAR to improve efficacy, potency, and selectivity of antivirals. But future efforts should be given on challenges like reducing the resistance besides increasing the efficacy of the therapy. The synthetic findings from DAAs such as daclatasvir NS5A inhibitor involves imidazole formation which gives enhanced purity and directed to greener substitute that is more economical approach in drug synthesis. In case of grazoprevir that is NS3/4a Protease inhibitor goes through a synthetic step for the formation of chlorohydrin that ensure homogenous condition in large scale production. Whereas NS5B polymerase inhibitor sofosbuvir uses protide technology that masks nucleosides to get improved delivery and absorption. Following the synthesis procedure of antivirals combination therapy of DAAs can give fewer side effects and good clinical outcome with high percentage of SVR. Also, this review showed the path to overcome resistance of DAAs. As, HCV is a curable disease, combination of newly potent drugs also possess good resistance barrier are applicable for the countries where these treatments are not costly for the actual control of this viral infection. Lastly, this review article points out the challenges and improvement areas to eradicate HCV globally by identifying the synthetic methods that were used in HCV therapies.

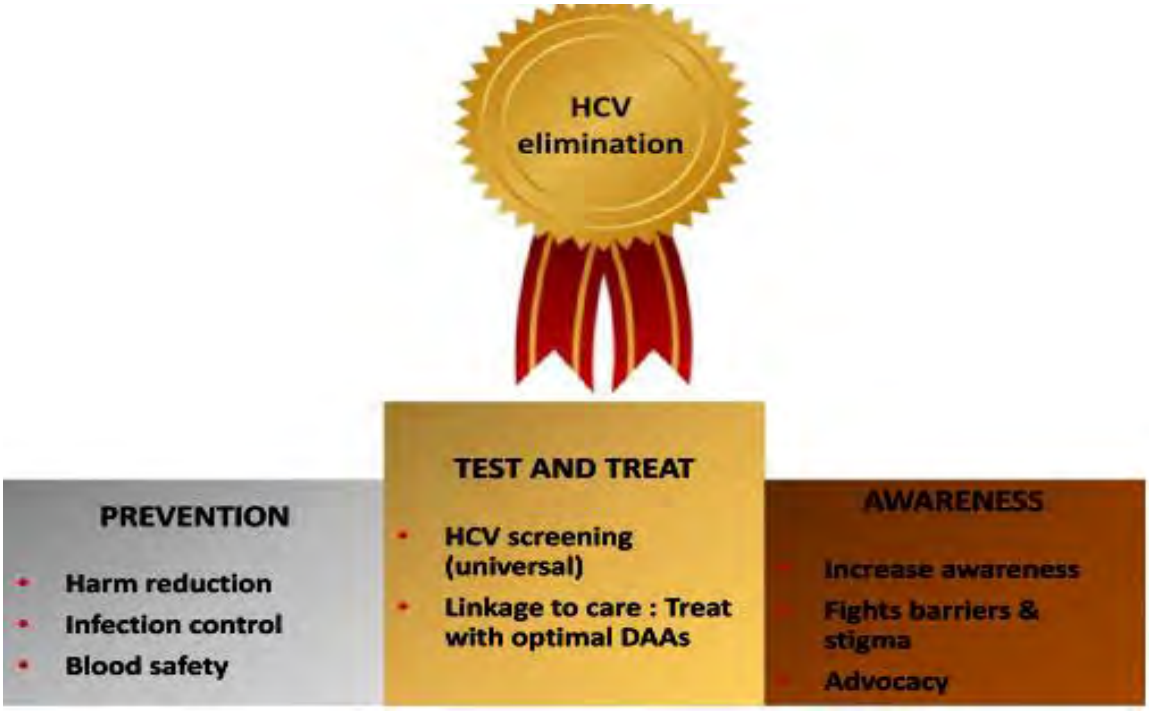


Figure 20: Steps to achieve HCV elimination globally (Asselah, Marcellin, and Schinazi 2018)

If there is a treatment failure, compliance is the first thing to be ensured. After that, testing of resistance need to be taken to test failures that caused by virological issues. For newly permitted combinations, it can be used to treat patients having previous treatment failure and also for those who have gained resistance due to direct acting antivirals variants. To eradicate viral hepatitis C effective screening method must be done. Direct acting antiviral cost is needed to be reduced as well. Data based on real life examples is needed for efficaciousness, tolerance as well as to be adherent. Also to eliminate hepatitis C globally direct acting antivirals that are triple or quadruple have gained enhanced compliance by reducing the treatments DOA such as it goes down to 1-2 months from 3-4 months. Leading a healthy lifestyle by maintaining diet and exercising regularly are recommended highly for effective treatment as well. HCV treatment is gaining huge transformation because of having some regimens that can be provided orally now using the direct acting antiviral combinations. Also, DOA is reduced for treatment. This treatment includes good profile of safety as well as better tolerance. (Asselah, Marcellin, and Schinazi 2018).

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