

**SCREENING AND VALIDATION OF SMALL MOLECULES
TARGETING HEPATITIS B VIRAL PROTEINS USING A CELL
CULTURE MODEL**

S. KIRUTHIKA



**KUSUMA SCHOOL OF BIOLOGICAL SCIENCES
INDIAN INSTITUTE OF TECHNOLOGY DELHI
MARCH 2022**

**SCREENING AND VALIDATION OF SMALL MOLECULES
TARGETING HEPATITIS B VIRAL PROTEINS USING A CELL
CULTURE MODEL**

by

S. KIRUTHIKA

Kusuma School of Biological Sciences

Submitted

***in fulfilment of the requirements of the degree of Doctor of Philosophy
to the***

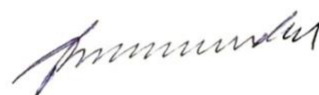


INDIAN INSTITUTE OF TECHNOLOGY DELHI

MARCH 2022

CERTIFICATE

This is to certify that the thesis titled “Screening and validation of small molecules targeting hepatitis B viral proteins using a cell culture model”, being submitted by Ms. S. Kiruthika at the Indian Institute of Technology Delhi for the award of the degree of “Doctor of Philosophy” is a record of the bonafide research carried out by her under my supervision and guidance and conforms to the rules and regulations of Indian Institute of Technology Delhi, India. The results prescribed in this thesis have not been submitted in part or full to any other university or institute for the award of any degree/diploma.



Prof. Vivekanandan Perumal

Professor

Kusuma School of Biological Sciences

Indian Institute of Technology Delhi

New Delhi-110016, India

ACKNOWLEDGEMENTS

I want to express my immense gratitude to my PhD supervisor, Prof. Vivekanandan Perumal, for providing me support, freedom and opportunity to pursue this study. Ever since I started working with him, he has encouraged me to do my best. His invaluable suggestions and insightful discussions made me enjoy my research. With his guidance, I have become a better researcher and a critical thinker. His scientific rigour, dedication, and passion for research have always been a source of inspiration.

During my PhD, I got the opportunity to collaborate with two great scientists, Prof. B. Jayaram and Prof. Anurag Rathore. Prof. B. Jayaram has been an incredible and inspirational collaborator for this project by offering much advice and insight throughout this thesis work. I appreciate and thank Prof. Anurag Rathore for his help with the SPR experiment, which was critical in drawing some key conclusions.

I am also grateful to my research committee, Prof. Manidipa Banerjee, Dr. Ashok Patel and Prof. Ritu Kulshreshtha, for their support and feedback essential to my project's progress. Many of the most exciting experiment and analysis ideas came from discussions with my SRC members. I owe a special thanks to my research committee's chairperson, Prof. Manidipa Banerjee, for her constant support, valuable suggestions and encouragement throughout. I thank all faculty of KSBS for their help and giving me access to their laboratory resources and instruments.

I am grateful to my seniors Dr. Jasmine Samal, Dr. Manish Kandpal and Dr. Banhi Biswas, for optimising many of the protocols giving me the best possible platform for success. I am incredibly thankful to Dr. Jasmine for teaching and training me during the initial stages of PhD and her continued support throughout my PhD. I appreciate and thank my excellent collaborators Dr. Ruchika Bhat and Dr. Rozaleen Dash for their valuable contribution. Working

in the lab was fun due to the cheerful and friendly group of fellow lab mates- Dr. Shivani, Dr. Nandhini, Shivaksh, Ashish, Uzma, Dipanita and Dr. Shruti. I thank Akanksha, Dr. Sukanya, Dr. Mishi, Aquib, Shantanu, Anjali and Dr. Arti for providing great technical assistance and advice. I am also thankful for the countless discussions with Bakul, Dr. Kimi, Dr. Priyanka, Dr. Debajit, Dr. Ramamurthy, Dr. Kritika, Pankhuri, Dibyakanti and Ramesh.

I would also like to thank the staff of KSBS, especially Ankit, for their help. I acknowledge the financial support from the Ministry of Education (erstwhile MHRD), Government of India during my PhD.

Outside research, my friends are amazing, enthusiastic, funny, and a great source of energy. I would like to thank my dearest friends Dr. Oviya and Sangeet for their immense support and encouragement through all these years. Next, I'd like to thank my family for supporting me in my pursuit of a PhD, with an incredibly huge thanks to my Mom. She has always supported my academic endeavours and first encouraged me many years ago to pursue an engineering degree. I am also grateful to my lovely sister for encouraging me to do my best.

S. Kiruthika

ABSTRACT

Chronic hepatitis B (CHB) infection is a major global problem, with over 290 million CHB carriers. Currently approved antivirals target the reverse transcriptase (RT) activity of the hepatitis B viral polymerase. Emergence of drug-resistance has been reported in a small proportion of CHB patients on prolonged treatment with antivirals. Hepatitis B virus (HBV) produces two secreted antigens, Hepatitis B surface antigen (HBsAg) and HBV 'e' antigen (HBeAg) and seropositivity for either antigens is associated with an increased risk of hepatocellular carcinoma (HCC). Loss of secreted antigens or seroconversion to anti-HBs and anti-HBe are signs of recovery. Therefore, inhibitors targeting HBV antigens may have potential therapeutic applications. In this study, two small molecules (molecules 6 and 7) targeting HBeAg and HBsAg identified using computational methods were experimentally tested in a cell culture model for HBV. Both molecules exhibit low cytotoxicity even at high micromolar concentrations. As confirmed by the dual luciferase assay, both molecules do not interfere with normal cellular transcription or translation. Both molecules led to a reduction in levels of HBsAg, HBeAg, HBcAg, HBV pregenomic RNA, HBV precore RNA, and HBV virion secretion at low micromolar concentrations in wild type and lamivudine-resistant rtM204I mutant HBV.

Tenofovir and entecavir are the most widely used antivirals for treating CHB because of their high efficacy and a relatively high genetic barrier for resistance compared to other antivirals. With increasing usage of tenofovir worldwide for the treatment of CHB, the resistance to tenofovir is expected to rise rapidly. Therefore, the urgent need for new therapies for the tenofovir-resistant HBV is increasingly recognised. Here, the efficacy of molecule 7 on clinically relevant drug-resistant HBV mutants was evaluated using over-length HBV replicons. Molecule 7 inhibits secreted HBsAg and HBeAg encoded by twelve clinically

relevant drug-resistant HBV mutants tested in this study in a dose-dependent manner despite the presence of overlapping mutations in the HBV surface protein. Furthermore, molecule 7 significantly reduced pregenomic RNA, and precore RNA in most of the mutants tested. In addition, molecule 7 inhibits virion secretion from most clinically relevant drug-resistant mutants except for those harbouring the (rtL180M + rtM204V) mutation. Notably, the tenofovir-resistant CYEI mutant and lamivudine-resistant rtM204I mutant were susceptible to molecule 7.

In a recent report ciclopirox, a US-FDA-approved anti-fungal drug, was repurposed as an effective anti-HBV agent against wild-type HBV. Here, the efficiency of ciclopirox against clinically relevant drug-resistant HBV mutants was evaluated using over-length HBV replicons in cell culture. Ciclopirox inhibited HBV core antigen, HBeAg, and hepatitis B virion secretion in multiple clinically relevant drug-resistant HBV mutants, including the tenofovir-resistant CYEI mutant.

Molecule 7 and ciclopirox exhibit promising anti-HBV activity against several drug-resistant mutants and merits further investigation.

सारांश

क्रोनिक हेपेटाइटिस बी (सीएचबी) संक्रमण एक प्रमुख वैश्विक समस्या है, जिसमें 290 मिलियन से अधिक सीएचबी रोगी हैं। वर्तमान में स्वीकृत एंटीवायरल हेपेटाइटिस बी वायरल पोलीमरेज़ की रिवर्स ट्रांसक्रिप्टेस (आरटी) गतिविधि को लक्षित करते हैं। एंटीवायरल के साथ दीर्घकालिक उपचार पर सीएचबी रोगियों के एक छोटे से अनुपात में दवा-प्रतिरोध के उद्भव की सूचना मिली है। हेपेटाइटिस बी वायरस (एचबीवी) दो स्रावित प्रतिजन पैदा करता है, हेपेटाइटिस बी सरफेस एंटीजन (एचबीएसएजी) और एचबीवी 'ई' एंटीजन (एचबीईएजी) और दोनों में से किसी भी प्रतिजन के लिए सेरोपोसिटिविटी हेपेटोसेलुलर कार्सिनोमा (एचसीसी) के बढ़ते जोखिम से जुड़ी है। प्रतिजनों की हानि या सेरोकोनवर्जन ठीक होने के संकेत हैं। इसलिए, HBV प्रतिजनों को लक्षित करने वाले अवरोधकों के संभावित चिकित्सीय अनुप्रयोग हो सकते हैं।

HBeAg और HBsAg को लक्षित करने वाले संभावित छोटे अणु अवरोधकों की पहचान कम्प्यूटेशनल वर्चुअल स्क्रीनिंग, डॉकिंग और आणविक गतिकी सिमुलेशन का उपयोग करके की गई थी। ZINC डेटाबेस से दस लाख अणुओं की जांच के बाद, आठ छोटे अणुओं को प्रायोगिक परीक्षण के लिए चुना गया था (प्रो. बी. जयराम, रसायन विज्ञान विभाग, आईआईटी दिल्ली के सहयोग से)। आठ छोटे अणुओं में से, दो छोटे अणुओं (अणु 6 और 7) ने HBsAg, HBeAg, HBcAg, HBV प्रीजेनोमिक RNA, HBV प्रीकोर RNA, और HBV वायरियन स्राव को जंगली प्रकार और लैमीवुडीन-प्रतिरोधी rtM204I उत्परिवर्ती HBV में कम माइक्रोमोलर सांद्रता में प्रभावी ढंग से रोक दिया। . दोनों अणु उच्च

माइक्रोमोलर सांद्रता पर भी कम साइटोटोक्सिसिटी प्रदर्शित करते हैं और सामान्य सेलुलर ट्रांसक्रिप्शन या अनुवाद में हस्तक्षेप नहीं करते हैं।

टेनोफोविर और एंटेकाविर सीएचबी के इलाज के लिए सबसे व्यापक रूप से इस्तेमाल किए जाने वाले एंटीवायरल हैं क्योंकि उनकी उच्च प्रभावकारिता और अन्य एंटीवायरल की तुलना में प्रतिरोध के लिए अपेक्षाकृत उच्च आनुवंशिक बाधा है। सीएचबी के उपचार के लिए दुनिया भर में टेनोफोविर के बढ़ते उपयोग के साथ, टेनोफोविर का प्रतिरोध तेजी से बढ़ने की उम्मीद है। इसलिए, टेनोफोविर-प्रतिरोधी एचबीवी के लिए नए उपचारों की तत्काल आवश्यकता को तेजी से पहचाना जा रहा है। यहां, चिकित्सकीय रूप से प्रासंगिक दवा प्रतिरोधी एचबीवी म्यूटेंट पर अणु 7 की प्रभावकारिता का मूल्यांकन अधिक लंबाई वाले एचबीवी प्रतिकृतियों का उपयोग करके किया गया था। अणु 7 एचबीवी सतह प्रोटीन में अतिव्यापी उत्परिवर्तन की उपस्थिति के बावजूद एक खुराक पर निर्भर तरीके से इस अध्ययन में परीक्षण किए गए बारह नैदानिक रूप से प्रासंगिक दवा प्रतिरोधी एचबीवी म्यूटेंट द्वारा एन्कोड किए गए एचबीएसएजी और एचबीईएजी को रोकता है। इसके अलावा, अणु 7 ने प्रीकोर और प्रीजेनोमिक आरएनए को अधिकांश म्यूटेंट में काफी कम कर दिया। इसके अलावा, अणु 7 (rtL180M + rtM204V) उत्परिवर्तन को छोड़कर अधिकांश चिकित्सकीय रूप से प्रासंगिक दवा प्रतिरोधी म्यूटेंट से वायरियन साव को रोकता है। विशेष रूप से, टेनोफोविर-प्रतिरोधी C4E1 उत्परिवर्ती और लैमिवुडिन-प्रतिरोधी rtM204I उत्परिवर्ती अणु 7 के लिए अतिसंवेदनशील थे।

हाल ही की एक रिपोर्ट में, यूएस-एफडीए-अनुमोदित कवकरोधी दवा, सिक्लोपिरॉक्स को जंगली-प्रकार के एचबीवी के खिलाफ एक प्रभावी एंटी-एचबीवी एजेंट के रूप में पुनर्निर्मित किया गया था। यहां, चिकित्सकीय रूप से प्रासंगिक दवा प्रतिरोधी एचबीवी म्यूटेंट के खिलाफ सिक्लोपिरॉक्स की दक्षता का मूल्यांकन कोशिका संवर्धन में अधिक लंबाई वाले एचबीवी प्रतिकृतियों का उपयोग करके किया गया था। सिक्लोपिरॉक्स ने HBcAg, HBeAg, और हेपेटाइटिस बी वायरियन स्राव को कई चिकित्सकीय रूप से प्रासंगिक दवा-प्रतिरोधी HBV म्यूटेंट में रोक दिया, जिसमें टेनोफोविर-प्रतिरोधी CYEI म्यूटेंट भी शामिल है।

अणु 7 और सिक्लोपिरॉक्स कई दवा प्रतिरोधी म्यूटेंट के खिलाफ एंटी-एचबीवी गतिविधि का वादा करते हैं और आगे की जांच का गुण रखते हैं।

Table of Contents

<i>Aknowledgements</i>	<i>i</i>
<i>Abstract</i>	<i>iii</i>
<i>List of figures</i>	<i>xiii</i>
<i>List of Tables</i>	<i>xvi</i>
<i>Abbreviations</i>	<i>xvii</i>
<i>Thesis outline</i>	<i>xviii</i>
1. Review of literature	2
1.1 Introduction	2
1.2 HBV morphology and Genome organisation	3
1.2.1 P ORF	6
1.2.2 S ORF.....	7
1.2.3 C ORF	9
1.2.4 X ORF	11
1.3 HBV Lifecycle	11
1.3.1 Viral entry	11
1.3.2 HBV cccDNA formation and transcription.....	12
1.3.3 Encapsidation.....	13
1.3.4 Maturation	13
1.3.5 Virus assembly and Intracellular amplification	14
1.3.6 HBV Integration	14
1.4 Epidemiology of HBV	15
1.4.1 Global distribution of HBV Genotypes	16
1.5 HBV mutations	18
1.5.1 Precore/core mutations	18
1.5.2 Surface mutations	19
1.5.3 Polymerase mutations	20
1.5.4 HBx mutations	21
1.6 Chronic Hepatitis B	21
1.6.1 Phases of CHB.....	21

1.6.2	Age at infection and chronicity.....	22
1.6.3	HBV-induced Hepatocellular carcinoma	23
1.7	HBV vaccine.....	23
1.8	Treatment.....	24
1.8.1	Interferon	24
1.8.2	Nucleoside/nucleotide analogues	25
1.8.3	Direct acting antivirals in development.....	28
1.9	Cell culture models for studying HBV	33
1.10	References	35
2	<i>Experimental screening of potential small molecule inhibitors targeting HBV proteins</i> 55	
2.1	Introduction	55
2.2	Materials and methods.....	59
2.2.1	Molecules	59
2.2.2	Cytotoxicity testing	59
2.2.3	Plasmids	60
2.2.4	Transfection	60
2.2.5	Estimation of secreted and intracellular HBV proteins.....	60
2.2.6	Dual luciferase Assay.....	61
2.3	Results	62
2.3.1	Cytotoxicity studies.....	64
2.3.2	Initial experimental screening of computationally predicted molecules for inhibition of HBsAg, HBeAg and HBcAg.....	66
2.3.3	Molecules 6 and 7 do not interfere with cellular transcription and translation machinery	68
2.4	Discussion.....	69
2.5	References	71
3	<i>Understanding the effect of selected small molecule inhibitors on various stages of HBV replication</i>	79
3.1	Introduction	79

3.2	Materials and methods.....	81
3.2.1	Plasmid constructs.....	81
3.2.2	Transfection of HBV plasmid into Huh7 cells	81
3.2.3	Estimation of secreted and intracellular HBV proteins.....	81
3.2.4	Transfection of sub-genomic expression constructs.....	82
3.2.5	Estimation of HBV precore and pregenomic RNA	82
3.2.6	Quantification of secreted HBV	83
3.2.7	Data Analysis.....	84
3.3	Results	85
3.3.1	Molecules 6 and 7 inhibit HBsAg and HBeAg in a dose-dependent manner.....	85
3.3.2	Molecules 6 and 7 inhibit intracellular HBsAg and HBcAg.....	86
3.3.3	Inhibition of HBsAg and HBeAg by molecules 6 and 7 is independent of other HBV-encoded proteins	87
3.3.4	Molecules 6 and 7 led to significant reduction of HBV precore and pregenomic RNA.....	89
3.3.5	Molecules 6 and 7 inhibit hepatitis B virion secretion	90
3.4	Discussion.....	91
3.5	References	94
4	<i>To assess the efficiency of selected small molecule inhibitors on clinically relevant drug-resistant HBV.....</i>	100
4.1	Introduction	100
4.2	Materials	104
4.2.1	Plasmid constructs.....	104
4.2.2	Transfection of HBV plasmid into Huh7 cells	104
4.2.3	Estimation of secreted HBV proteins.....	104
4.2.4	Estimation of HBV precore and pregenomic RNA	105
4.2.5	Quantification of secreted HBV	105
4.3	Results	106
4.3.1	Molecules 6 and 7 inhibit HBsAg & HBeAg encoded by lamivudine-resistant HBV in a dose-dependent manner	106
4.3.2	Molecules 6 and 7 led to significant reduction of precore RNA and pregenomic RNA encoded by lamivudine-resistant HBV.....	107

4.3.3	Molecules 6 and 7 inhibits HBsAg & HBeAg encoded by clinically relevant drug-resistant HBV mutants in a dose-dependent manner	112
4.3.4	Molecules 6 and 7 led to reduction of precore RNA and pregenomic RNA encoded by clinically relevant drug-resistant HBV mutants.....	117
4.3.5	Molecules 6 and 7 inhibit hepatitis B virion secretion in clinically relevant drug-resistant HBV mutants	118
4.4	Discussion.....	119
4.5	References	122
5	<i>Experimental screening of FDA approved small molecules for repurposing as anti-HBV agents</i>	<i>126</i>
5.1	Introduction	126
5.2	Materials and methods.....	128
5.2.1	Molecules	128
5.2.2	Cytotoxicity assay	128
5.2.3	Plasmids.....	128
5.2.4	Transfection of HBV plasmid into Huh7 cells	129
5.2.5	Estimation of HBV proteins.....	129
5.2.6	Transfection of sub-genomic expression constructs	129
5.2.7	Quantification of secreted hepatitis B virion	130
5.3	Results	131
5.3.1	Cytotoxicity testing	132
5.3.2	Initial experimental screening of FDA-approved drugs for repurposing against HBV proteins	133
5.3.3	Cefixime inhibits HBsAg and HBeAg in nanomolar range at a single dose	136
5.3.4	Cefixime-mediated inhibition of HBsAg and HBeAg is not independent of other HBV proteins.	136
5.3.5	Ciclopirox inhibits HBV core antigen encoded by clinically relevant drug-resistant HBV mutants.....	137
5.3.6	Ciclopirox inhibits HBeAg encoded by clinically relevant drug-resistant HBV mutants	138
5.3.7	Ciclopirox inhibits virion secretion in clinically relevant drug-resistant HBV mutants	139

5.4	Discussion.....	140
5.5	References	142
6	<i>Future directions</i>	146
	<i>Author's Resume</i>	147

List of figures

Figure 1.1 Schematic diagram of the HBV genome.	5
Figure 1.2 Domain organization of HBV polymerase	6
Figure 1.3 The open reading frame encoding hepatitis B surface antigen.	8
Figure 1.4 Schematic illustration of the translation of HBV precore and core proteins	10
Figure 1.5 Schematic overview of Hepatitis B virus life cycle	15
Figure 1.6 Global prevalence of hepatitis B virus infection.	16
Figure 1.7 Distribution of HBV genotypes by country.....	17
Figure 1.8 Structures of approved oral antivirals.....	26
Figure 1.9 Chemical structures of representative capsid assembly modulators (CAMs)	30
Figure 2.1 Molecular scaffolds of selected small molecules (1-8).	57
Figure 2.2 Cell viability in presence of Molecules 1-8.....	65
Figure 2.3 Initial screening of small molecules 4, 6, and 7 for inhibition of HBsAg.....	66
Figure 2.4 Initial screening of small molecules (1-8) for inhibition of HBeAg.	67
Figure 2.5 Initial screening of small molecules (1-8) for inhibition of intracellular HBcAg.	68
Figure 2.6 Relative luciferase expression in Huh7 cells transfected with luciferase reporter and renilla control plasmids and treated with molecule 6 or molecule 7.....	69
Figure 3.1 Dose-dependent inhibition of wild type 1.3×HBV encoded HBsAg and HBeAg by molecule 6 and molecule 7.	85
Figure 3.2 Inhibition of intracellular surface antigen expressed from wild type 1.3×HBV construct by molecule 6 and molecule 7	86
Figure 3.3 Inhibition of intracellular core antigen expressed from wild type 1.3×HBV construct by molecule 6 and molecule 7.....	87
Figure 3.4 Inhibition of secreted surface antigen expressed from sub-genomic PreS2/S construct by molecule 6 and molecule 7.....	88
Figure 3.5 Inhibition of secreted ‘e’ antigen expressed from sub-genomic PreC/C construct by molecule 6 and molecule 7	89
Figure 3.6 Inhibition of HBV precore RNA encoded by wild type 1.3×HBV construct at 10 µM concentration of molecule 6 and molecule 7.....	89
Figure 3.7 Inhibition of HBV pregenomic RNA encoded by wild type 1.3×HBV construct at 10 µM concentration of molecule 6 and molecule 7.....	90
Figure 3.8 Inhibition of secreted hepatitis B virion/ml supernatant at 10 µM concentration of molecule 6 and molecule 7	90

Figure 3.9 Schematic representation of the effect of molecule 6/7 on different steps of hepatitis B virus replication	92
Figure 4.1 Schematic representation of the mutations in HBV polymerase's reverse transcriptase region and overlapping surface ORF of HBV.	101
Figure 4.2 Dose-dependent inhibition of lamivudine-resistant rtM204I mutant HBV encoded HBsAg and HBeAg by molecule 6 and molecule 7	106
Figure 4.3 Inhibition of HBV precore RNA encoded by lamivudine-resistant rtM204I mutant 1.3×HBV construct at 10 µM concentration of molecule 6 and molecule 7	107
Figure 4.4 Inhibition of HBV pregenomic RNA encoded by lamivudine-resistant rtM204I mutant 1.3×HBV construct at 10 µM concentration of molecule 6 and molecule 7	108
Figure 4.5 Molecule 7 mediated dose-dependent inhibition of secreted HBsAg encoded by wild-type, C, E, CE, YE and CY mutant HBV.....	112
Figure 4.6 Molecule 7 mediated dose-dependent inhibition of secreted HBsAg encoded by CYE, CYEI, MV, CYEMV, CYELMV and CYELMVI mutant HBV.....	113
Figure 4.7 Molecule 7 mediated dose-dependent inhibition of secreted HBeAg encoded by wild-type, C, E, CE, YE and CY mutant HBV.....	114
Figure 4.8 Molecule 7 mediated dose-dependent inhibition of secreted HBeAg encoded by CYE, CYEI, MV, CYEMV, CYELMV and CYELMVI mutant HBV.....	115
Figure 4.9 Inhibition of HBV precore RNA encoded by wild-type and mutant 1.2×HBV construct at 10 µM concentration of molecule 7.....	117
Figure 4.10 Inhibition of HBV pregenomic RNA encoded by wild-type and mutant 1.2×HBV constructs at 10 µM concentration of molecule 7	118
Figure 4.11 Hepatitis B virion secretion in wild-type, C, E, CE, YE, CY, CYE, CYEI, MV, CYEMV, CYELMV and CYELMVI mutant HBV after treatment with molecule 7.	119
Figure 5.1 Cell viability of Huh7 cells in presence of pentetic acid, tiludronic acid, cefixime, and ciclopirox.....	133
Figure 5.2 Initial screening of pentetic acid, tiludronic acid, and cefixime for inhibition of HBsAg	134
Figure 5.3 Initial screening of pentetic acid, tiludronic acid, and cefixime for inhibition of HBeAg	135
Figure 5.4 Inhibition of HBsAg and HBeAg by cefixime at nanomolar concentrations.....	136
Figure 5.5 (A) secreted surface antigen expressed from sub-genomic preS2/S construct at 100 nM cefixime (B) secreted 'e' antigen expressed from sub-genomic preC/C construct at 80 nM cefixime.	137

Figure 5.6 Ciclopirox inhibits intracellular core antigen in wild-type and clinically relevant drug-resistant HBV mutants	138
Figure 5.7 Ciclopirox inhibits secreted HBV ‘e’ antigen in wild-type and clinically relevant drug-resistant HBV mutants	139
Figure 5.8 Ciclopirox-mediated inhibition of hepatitis B virion secretion	139

List of Tables

Table 2.1 Computationally predicted small molecules proposed for experimental testing. ...	62
Table 3.1 IC ₅₀ values (μM) for wild-type HBV encoded HBsAg and HBeAg.....	86
Table 4.1 Clinical significance of single amino acid substitution in the reverse transcriptase region of HBV.....	102
Table 4.2 IC ₅₀ values (μM) for lamivudine-resistant rtM204I mutant HBV encoded HBsAg and HBeAg.....	107
Table 4.3 Details of the 1.2× HBV replicons used in this study.	109
Table 4.4 IC ₅₀ values (μM) for inhibition of wild-type and mutant HBV encoded HBsAg and HBeAg by molecule 7.....	116
Table 5.1 FDA approved small molecules proposed for repurposing against HBV proteins.	131

Abbreviations

aa	Amino acid
ALT	Alanine aminotransferase
CAM	Capsid assembly modulator
cccDNA	Covalently closed circular DNA
CDC	Centre for Disease Control
CHB	Chronic Hepatitis B
CYEI	rtS106C + rtH126Y + rtD134E + rtL269I
dIDNA	Double stranded linear DNA genome
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunoassay
ER	Endoplasmic reticulum
HBcAg	Hepatitis B core antigen
HBeAg	HBV ‘e’ antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HBx	Hepatitis B x protein
HCC	Hepatocellular carcinoma
HIV	human immunodeficiency virus
HTS	High-throughput screening
MV	rtL180M + rtM204V
NA	Nucleoside/nucleotide analogues
NTCP	Sodium taurocholate co-transporting polypeptide
OBI	Occult hepatitis B infection
ORF	Open reading frame
PEG-IFN	Pegylated interferon- $\alpha 2\alpha$
pgRNA	Pregenomic RNA
RC DNA	Relaxed circular DNA
RNA	Ribonucleic acid
RT	Reverse Transcriptase
US FDA	United States Food and Drug Administration
WHO	World Health Organization
YMDD	Tyrosine-methionine-aspartate- aspartate motif

Screening and validation of small molecules targeting Hepatitis B viral proteins using a cell culture model

Thesis outline:

- Chapter 1: Review of literature
- Chapter 2: Experimental screening of potential small molecule inhibitors targeting HBV proteins
- Chapter 3: Understanding the effect of selected small molecule inhibitors on various stages of HBV replication
- Chapter 4: To assess the efficiency of selected small molecule inhibitors on clinically relevant drug-resistant HBV
- Chapter 5: Experimental screening of FDA approved small molecules for repurposing as anti-HBV agents
- Chapter 6: Future directions

Chapter 1

Review of literature