


## Benzothiazoles as potential antiviral agents

Yahya I. Asiri<sup>a</sup>, Abdulrhman Alsayari<sup>b</sup>, Abdullatif B. Muhsinah<sup>b</sup>, Yahia N. Mabkhot<sup>c</sup> and Mohd. Z. Hassan<sup>c</sup> 

<sup>a</sup>Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Saudi Arabia, <sup>b</sup>Department of Pharmacognosy, College of Pharmacy, King Khalid University, Abha, Saudi Arabia and <sup>c</sup>Department of Pharmaceutical Chemistry, College of Pharmacy, King Khalid University, Abha, Saudi Arabia

### Keywords

antiviral; benzothiazole; COVID-19; dengue; HIV

### Correspondence

Dr. Mohd. Z. Hassan, Department of Pharmaceutical Chemistry, College of Pharmacy, King Khalid University, Abha 61441, Saudi Arabia.  
E-mails: zaheen@kku.edu.sa; drzahin@gmail.com

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### Abstract

**Objectives** The recent viral pandemic poses a unique challenge for healthcare providers. Despite the remarkable progress, the number of novel antiviral agents in the pipeline is woefully inadequate against the evolving virulence and drug resistance of current viruses. This highlights the urgent need for new and improved vaccines, diagnostics and therapeutic agents to obviate the viral pandemic.

**Key findings** Benzothiazole plays a pivotal role in the design and development of antiviral drugs. This is evident from the fact that it comprises many clinically useful agents. The current review is aimed to provide an insight into the recent development of benzothiazole-based antiviral agents, with a special focus on their structure-activity relationships and lead optimisation. One hundred and five articles were initially identified, and from these studies, 64 potential novel lead molecules and main findings were highlighted in this review.

**Summary** We hope this review will provide a logical perspective on the importance of improving the future designs of novel broad-spectrum benzothiazole-based antiviral agents to be used against emerging viral diseases.

### Introduction

The current global scenario indicates that new microbial threats will continue to emerge at an accelerating pace, mainly due to globalisation and unprecedented climate change.<sup>[1,2]</sup> As compared to the only 260 known human pathogenic viruses, the unknown varieties of viruses represent 99.9% of potential zoonoses, which cannot be diagnosed until symptoms are noticed.<sup>[3]</sup> Moreover, the recent unusual worldwide outbreaks of Nigerian Lassa virus in 2018,<sup>[4]</sup> Indian Nipah virus in 2018,<sup>[5]</sup> Brazilian yellow fever in 2017,<sup>[6]</sup> West African Ebola in 2017,<sup>[7]</sup> Brazilian Zika virus in 2015<sup>[8]</sup> and worldwide coronavirus disease 2019 (COVID-19)<sup>[9]</sup> took terrible tolls, both on human life and on the global economy.

Viruses are obligate intracellular pathogens consisting of a DNA or RNA genome enclosed within a proteinaceous capsid.<sup>[10]</sup> Due to its rapid replications, mutations and adaptations in host cells, the treatment of viral infections with vaccines or small molecules is an arduous task facing healthcare providers.<sup>[11]</sup> However, due to the advent of

newer techniques such as virome, robust cell culture, cytopathic effect, immunofluorescence, high-throughput screening (HTS) and other drug discovery tools, antiviral chemotherapy has witnessed a revolution in controlling HIV-1, HBV, HCV and herpes viruses (HSV and CMV).<sup>[12,13]</sup> In the last three decades approximately 88 new antiviral drugs have been approved by the US Food and Drug Administration (FDA), which includes a diverse array of small molecules, interferons, monoclonal antibodies, peptides and oligonucleotides.<sup>[14]</sup> All these drugs are either acting as host targeting agents such as immunomodulating agents, toll-like receptor (TLR)7 agonists, human inosine-5'-monophosphate dehydrogenase (IMPDH) inhibitors, C-C chemokine receptor type 5 (CCR5) modulators or viral targeting agents such as polymerase, protease, integrase, non-structural protein 5A (NS5A) and neuraminidase inhibitor.<sup>[15-19]</sup> Gene therapies are also now popular in the field of medicine, and they define a new-age viral treatment where genes are delivered into infected cells to interfere with viral replications.<sup>[20]</sup> Despite this remarkable progress, the number of novel antiviral agents in the

pipeline is woefully inadequate against the evolving virulence and drug resistance of current viruses.<sup>[21]</sup> This highlights the urgent need for new and improved vaccines, diagnostics and therapeutic agents to obviate the viral pandemic. The antiviral potential of benzothiazole derivatives has recently gained momentum due to its robust *in-silico* virtual and HTS, which result in higher hit-to-lead discoveries (Figure 1).<sup>[22]</sup> To date, a fair number of related articles have been published but not a single review on this topic has been reported. Thus, there appears to be a real need for a review article summarising the benzothiazolyl antiviral agents. This mini-review makes no attempt to be comprehensive but highlights the potential of benzothiazole analogues against various viral diseases with a focus on structure–activity relationships (SAR), as well as their molecular targets. This review will surely help in generating significant multiple pharmacophore hypotheses and draw novel insights which contribute to the development of benzothiazole-based antiviral agents.

## Anti-dengue agents

Dengue is a mosquito-borne haemorrhagic fever caused by a single positive-stranded RNA virus of family *Flaviviridae*. According to WHO, an estimated 50 million dengue infections occur every year which accounts for thousands of deaths from dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue genotypes, *viz.* DEN1, DEN2, DEN3 and DEN4, are very closely related serotypes; thus their cross-reactive antibodies aggravate the risk of

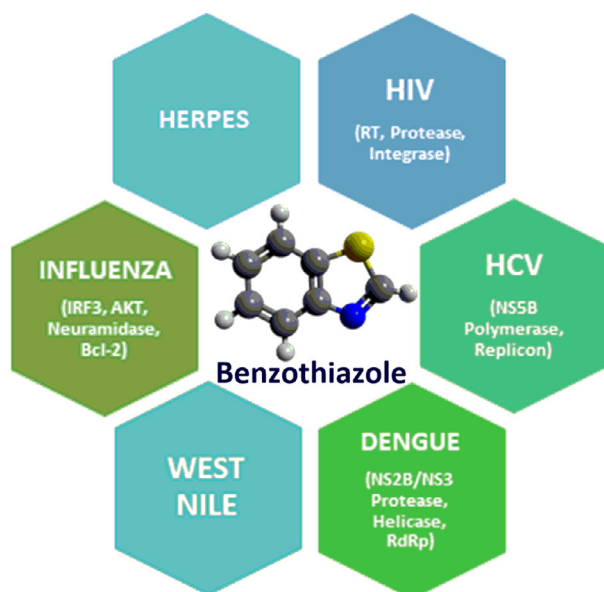
severe dengue shock in people experiencing repeated dengue virus (DENV) infections.<sup>[23,24]</sup> Currently, no effective treatments are available for dengue. Most investigational new anti-dengue agents directly target the viral structural proteins (capsid [C], prM and E) or nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) by cellular proteases and viral serine protease, composed of NS2B and NS3.<sup>[25]</sup>

## DENV NS2B/NS3 protease inhibitors

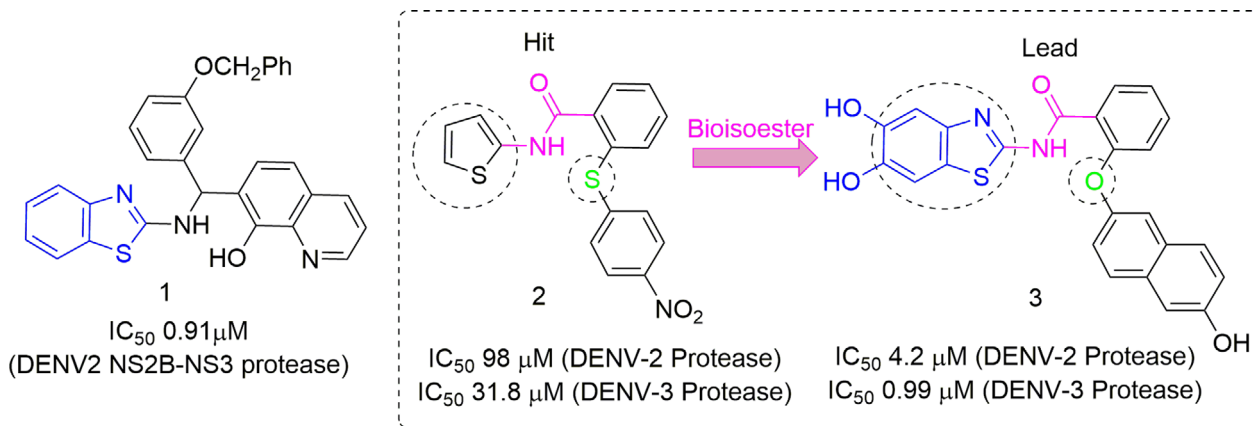
The NS2B/NS3 protease (PR) represents an important molecular target for anti-dengue drug design. Currently, no clinically available PR inhibitors (PIs) exist for the treatment of dengue. Recently, a novel compound library of hybrid benzothiazole-quinoline derivatives was developed as PIs by Lai *et al.* This hybrid pharmacophore demonstrated interesting anti-dengue activity and became a potential lead in the further development of various hybrid PIs. One of the potent compounds identified was compound **1**, which was found to be a competitive inhibitor of DENV2 NS2B/NS3 PR with an  $IC_{50}$  value of  $0.91 \pm 0.05 \mu M$  ( $K_i$   $2.36 \pm 0.13 \mu M$ ).<sup>[26]</sup> Furthermore, a group of scientists led by Lai *et al.* performed HTS research of 250 compounds with the aim of developing nonpeptidic PIs. They identified benzothiazole containing naphthalenoxybenzamide as a potential candidate for a novel anti-dengue drug targeting DENV PR. Initially, a thiophene derivative (**2**) was discovered to inhibit the DENV-2 PR which was optimised by replacing it with the benzothiazole ring system (**3**), and the thioether linkage was bioisosterically replaced by an ether function (Figure 2).<sup>[27]</sup>

## DENV helicase inhibitors

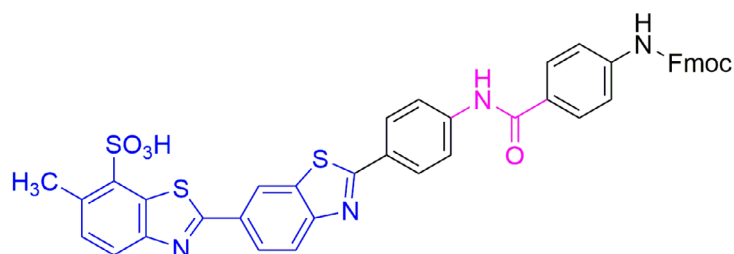
RNA helicase is a common key enzyme in various viruses, such as HIV, HCV, influenza A and swine fever viruses. This enzyme unwinds both DNA and RNA, as well as affecting many DNA and RNA metabolic processes. Thus, RNA helicase represents a key target for new therapeutics against the aforementioned viruses.<sup>[28]</sup> Moreover, drugs targeting one viral variant could be useful for treating another virus. Keeping this in mind, Sweeney *et al.* evaluated potential anti-HCV agents against the DENV using robust DENV NS3 ATPase assay and DENV helicase-catalysed RNA unwinding assays. Surprisingly, NIH molecular probe ML283, a previously reported benzothiazole oligomer (**14**) derived from the dye primuline (**12**), emerged as a novel lead template inhibiting DENV NS3 with an  $IC_{50}$  value of 500 nM, and DENV RNA helicase with an  $IC_{50}$  value of  $3.5 \mu M$ .<sup>[29]</sup> Encouraged by the persuasive results of this benzothiazolyl oligomer, a ligand-based pharmacophore model was also generated to screen 1 201 474



**Figure 1** Antiviral potential of benzothiazole. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

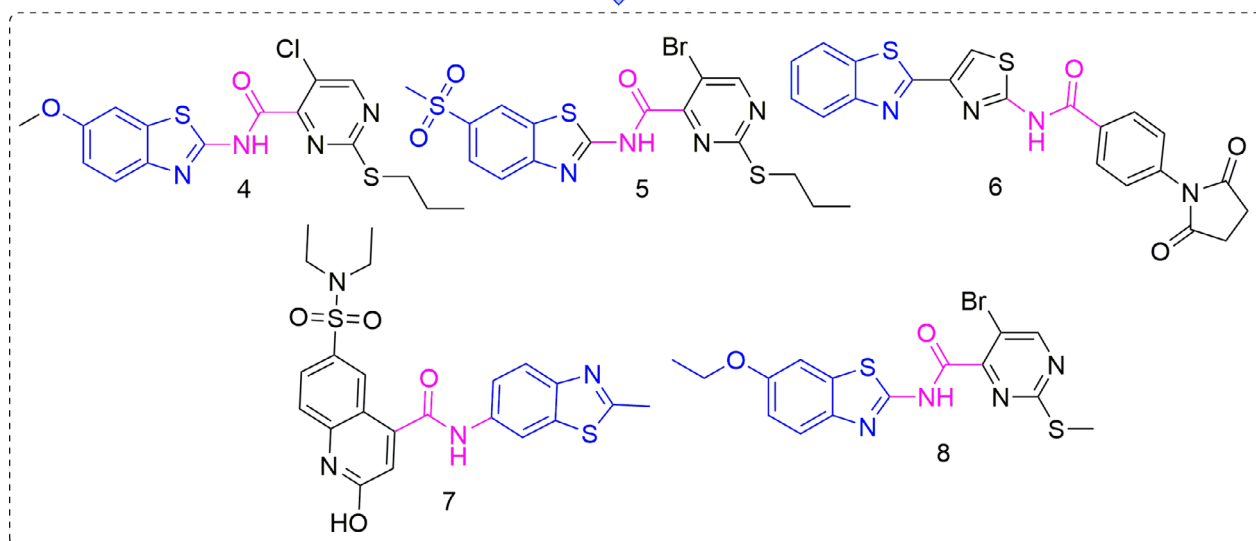


**Figure 2** Benzothiazole as DENV NS2B/NS3 Pls. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



IC<sub>50</sub> 3.5 ± 1.7 μM (DENV RNA Helicase)  
IC<sub>50</sub> 1.3 ± 0.3 μM (DENV RNA ATPase)

1. Pharmacophore search  
2. Docking score



**Figure 3** Development of DENV helicase inhibitors by pharmacophore-based ligand search. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

compounds of the ZINC Database. Filtered compounds were then docked at the active site of NS3 helicase which yielded five potential benzothiazole hits (4–8) with satisfactory ADMET properties. However, the results of *in silico* studies are yet to be validated in a wet lab (Figure 3).<sup>[30]</sup>

### DENV RNA dependent RNA polymerase (RdRp) inhibitors

The RdRp is responsible for viral replication and represents a selective flavivirus molecular target as its homologue is absent in humans. Recently, several new chemotypes targeting DENV RdRp have been discovered by re-evaluating the previously reported compounds that are effective against the HCV NS5B RdRp. In this context, Tarantino *et al.* reported the biochemical and crystallographic characterisation of a pyridobenzothiazole lead (9) as an inhibitor of flavivirus RdRp in micromolar range possessing very high selectivity against DENV2.<sup>[31]</sup> Subsequently, optimisation of a pyridobenzothiazole scaffold by a substituted phenyl ring provided a broad-spectrum antiviral agent 10 (Figure 4).<sup>[32]</sup>

### Anti-hepatitis C virus

Hepatitis C virus (HCV) infection is a major global health concern. It is known as the chief cause of liver failure that often leads to hepatocellular carcinoma. Globally, approximately 150 million people are living with HCV infections.<sup>[33]</sup> HCV is a single-stranded RNA virus of the *Flaviviridae* family with seven major genotypes (HCV1–7).<sup>[34]</sup> High-throughput antiviral drug discovery screens have been extensively performed to identify inhibitors of

viral protease (serine protease for HCV) and inhibitors of the RNA-dependent RNA polymerase (RNA replicase).<sup>[35]</sup> Vaccines for preventing hepatitis A and B are now clinically available. Although available directly acting antiviral agents have been successful in managing HCV, emerging resistance, unfavourable pharmacokinetic properties and high treatment costs continue to present challenges.<sup>[36]</sup> Thus, there is a pressing demand to discover novel anti-HCV therapeutic agents. A significant number of benzothiazole analogues have demonstrated promising anti-HCV activity, as noted in the following.

### HCV NS3 helicase inhibitors

Replication of HCV in human cells requires the action of the HCV non-structural protein 3 (NS3), which exhibits both protease and helicase activities. The HCV polyprotein is comprised of 3000 amino acids that can be divided into a structural region (C–p7 proteins) and a non-structural (NS) region (NS2–NS5B proteins). Only the NS3–NS5B region of the polyprotein is required for genome replication in cell cultures. Thus, HCV NS3 is an imperative drug target due to its main role in viral replication.<sup>[37,38]</sup>

Using the HTS of 827 compounds in the National Cancer Institute (NCI, US) mechanistic set, Li *et al.* discovered commercial dyes thioflavin S (11) and primuline (12) as the most potent inhibitors of NS3 catalysed DNA and RNA unwinding at the micromolar range. Further resolutions into their pure components resulted in potent benzothiazole tetramer (13). Subsequent insight into the contribution of the carboxamide group (14) as a more specific agent helped to obtain a potent helicase inhibitor with an  $IC_{50}$  value of  $2.6 \pm 1 \mu\text{M}$  (Figure 5).<sup>[39]</sup>

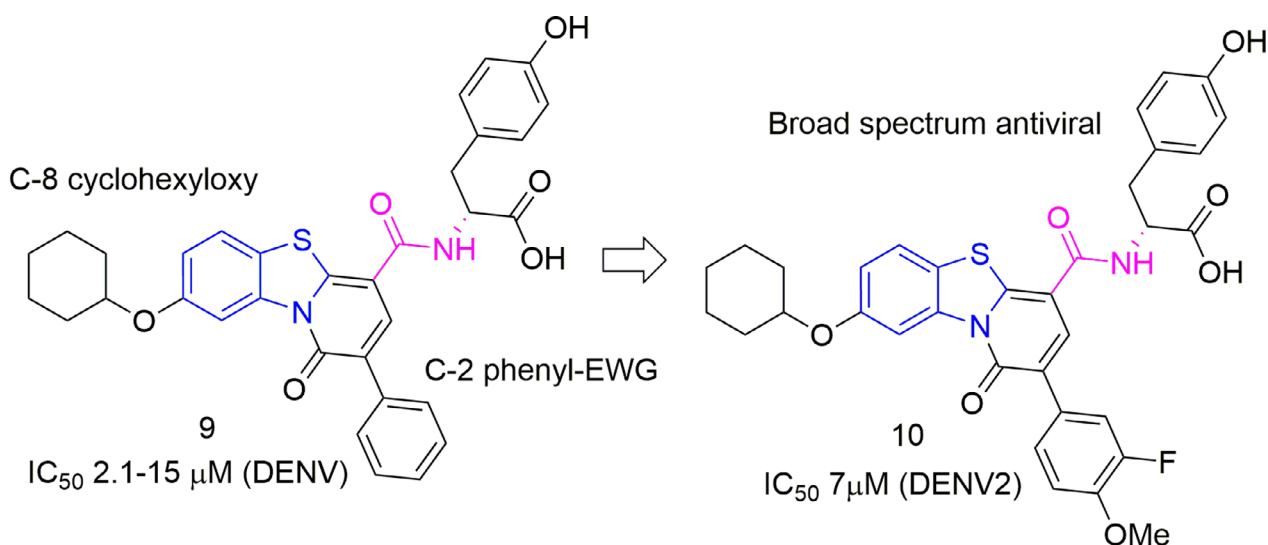
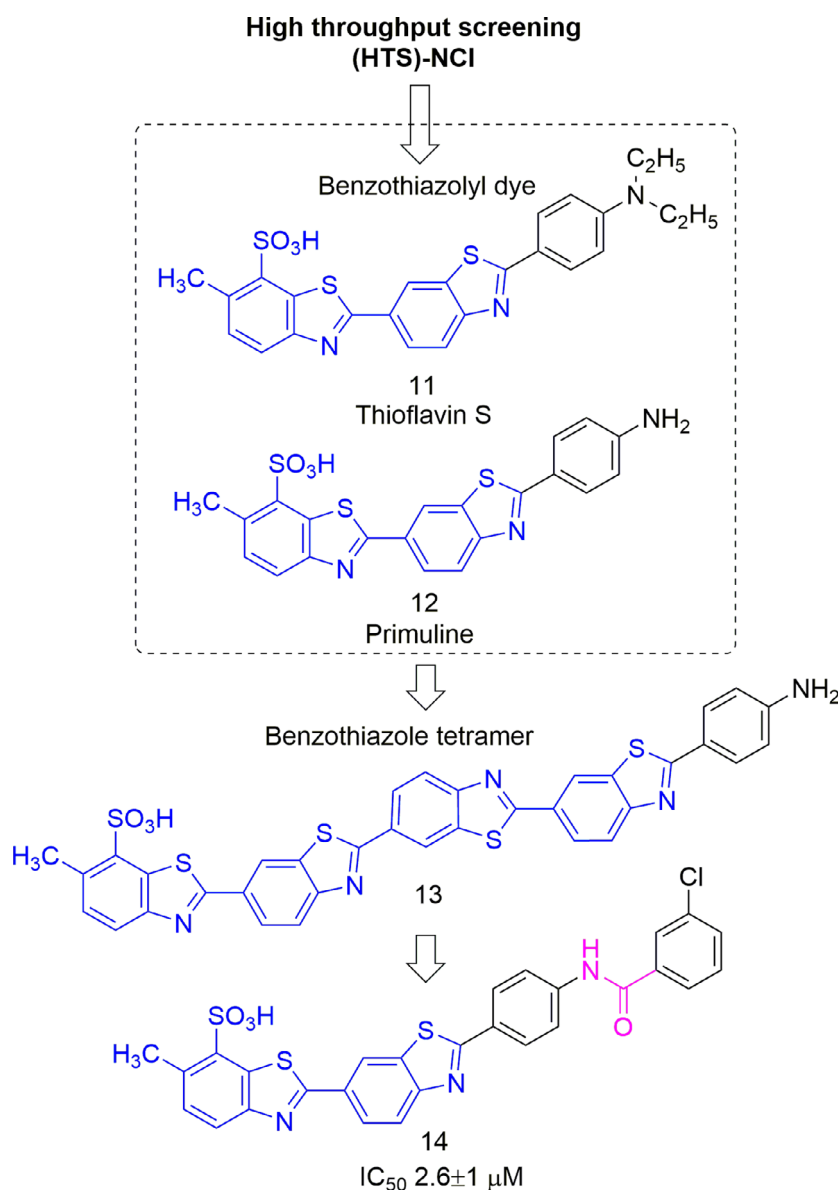


Figure 4 DENV RdRp inhibitors. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



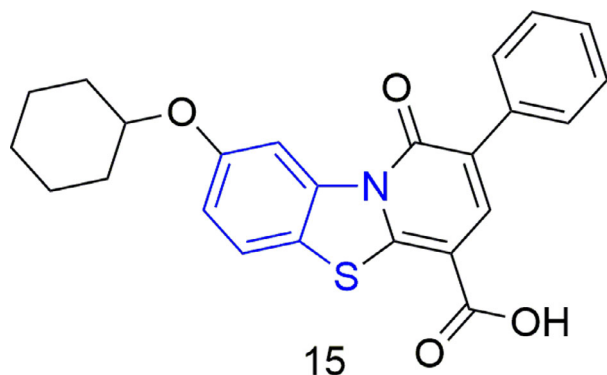
**Figure 5** Benzothiazolyl dyes as potent NS3 helicase inhibitors. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

### HCV NS5B polymerase inhibitors

HCV NS5B polymerase is an attractive target for HCV therapeutic intervention as it is responsible for the replication of positive-strand genomic RNA. HCV NS5B polymerase selectively utilises the viral RNA template which the host mammalian cells are lacking.<sup>[40]</sup> Manfroni *et al.* reported novel pyridobenzothiazole derivatives (**15**) as HCV NS5B polymerase inhibitors through scaffold hopping strategy. These compounds were shown to have interesting polymerase inhibitory activity. However, *in-vitro* studies failed to predict its anti-HCV activity due to poor permeability. Detailed biochemical studies confirmed the allosteric

modulation of pyridobenzothiazoles through non-competitive inhibitions of the ribonucleotide substrate and competitive inhibitions of the RNA template (Figure 6).<sup>[41]</sup>

In the pursuit of identifying novel small molecule inhibitors of hepatitis C virus replication, scientists at the Merck Research laboratory, USA, identified a new library of carbanucleoside derivatives (**16**) as lead molecules.<sup>[42]</sup> SAR studies were conducted around the pyrimidine core to improve the potency and pharmacokinetic profile of these inhibitors. A benzothiazole moiety was found to be the optimal substituent at the pyrimidine 5-position (compounds **17** and **18**). The 4-methyl derivative emerged with enhanced rat *in vivo* profile demonstrating a very good



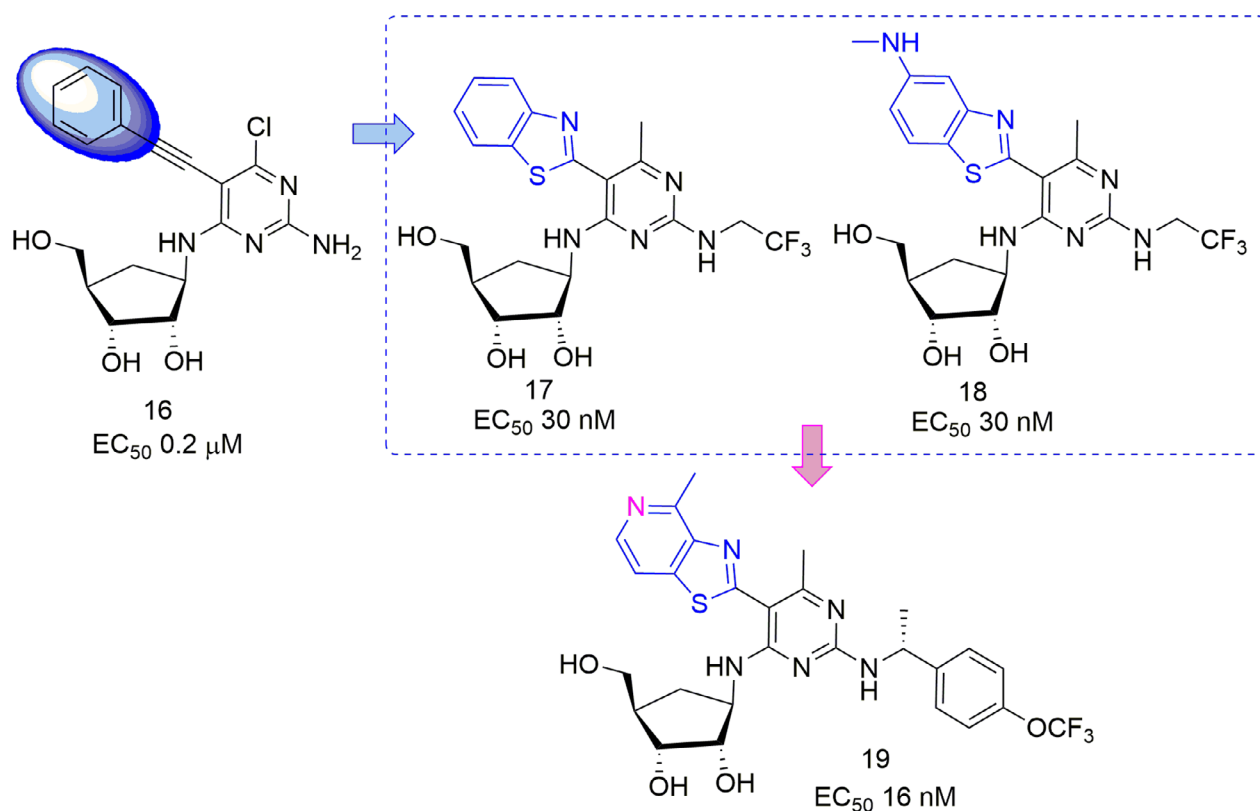
$IC_{50}$  11  $\mu$ M (NS5B polymerase)

**Figure 6** Pyridobenzothiazoles as NS5B polymerase inhibitors. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

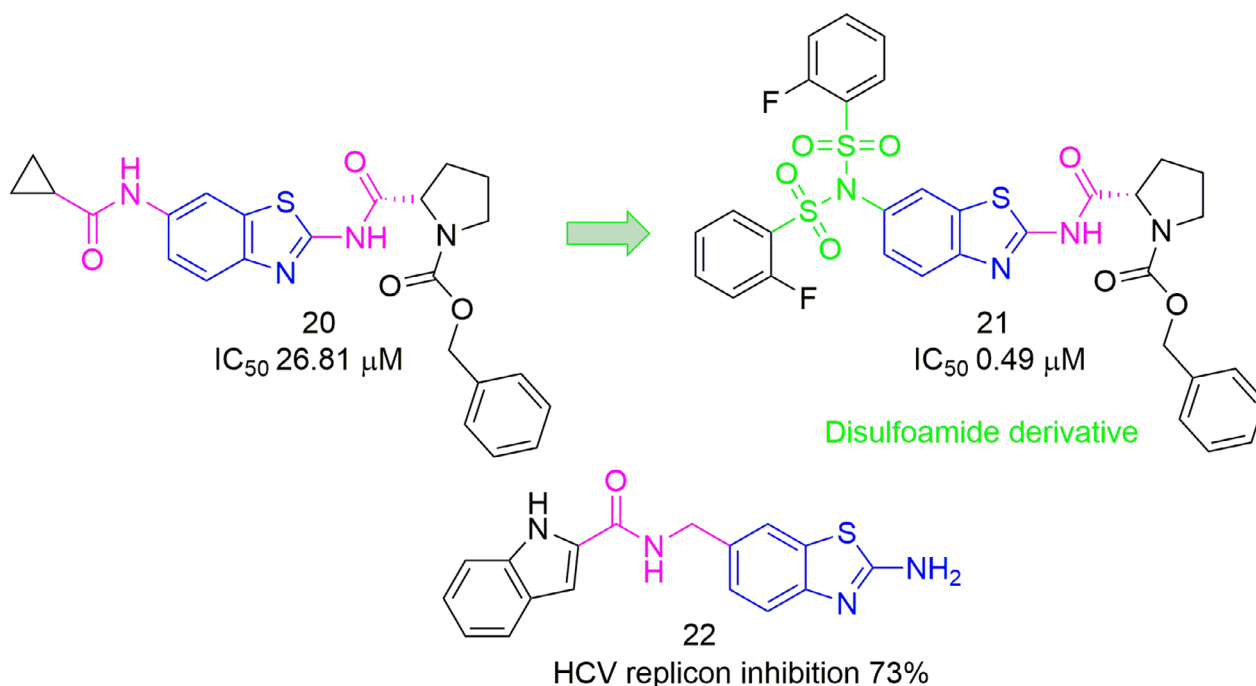
replicon potency, selectivity and rodent plasma/target organ concentration.<sup>[43]</sup> Further, introduction of a nitrogen atom into the benzene ring of a previously identified HCV replication (replicase) benzothiazole inhibitor, resulted in the discovery of more potent pyridothiazole analogues (19) (Figure 7).<sup>[44]</sup>

### HCV replicon inhibitors

HCV replication in cell lines was practically impossible before the development of subgenomic replicons which replicate freely in the human hepatoma cell line Huh-7. Significant progress has been achieved with regard to the replicon system, allowing for the validated protocols of replication assays for HCV genotypes 1a, 1b, and 2a. The HCV replicon system has opened new venues for detailed molecular studies of RNA replication and HCV-host interactions as well as for the discovery of novel inhibitors of HCV replication.<sup>[45]</sup> Considering the uses of the HCV replicon assay, researchers at Second Military Medical University, China performed a cell-based anti-HCV screen of an intramural compound against the HCV genotype 2a variant. Their finding demonstrated that benzothiazolamide derivative (20) possesses significant HCV inhibitory activity ( $IC_{50}$  = 26.81  $\mu$ M) with little cytotoxicity ( $CC_{50}$  = 155  $\mu$ M). The compound was found to be effective against other genotypes such as the 1b HCV replicon ( $IC_{50}$  9.3  $\mu$ M). Further optimisation has resulted in a disulphonamide analogue of benzothiazole (21) as potent anti-HCV agent with good selectivity towards the target NS5A.<sup>[46]</sup> Similarly, another scientist, Montalva et al., similarly



**Figure 7** Benzothiazolyl-carbanucleoside as NS5B polymerase inhibitors. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**Figure 8** Benzothiazolyl-carboxamide as HCV replicon inhibitors. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

reported some benzothiazole carboxamide derivatives as HCV replicon inhibitors. Indole containing benzothiazole derivative (**22**) demonstrated 70% suppression of the HCV replicon and 35% cytotoxicity against the host cells (Figure 8).<sup>[47]</sup>

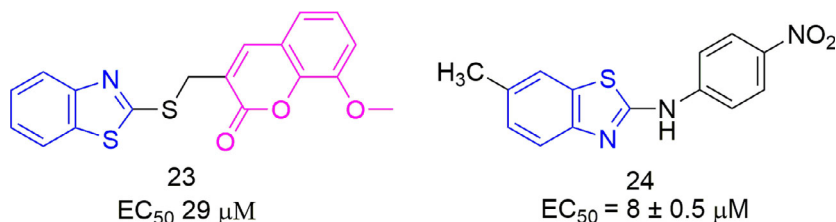
### 3.4 Miscellaneous anti-HCV agents

Some of the reported anti-HCV agents do not have any selectivity against the specific molecular targets and act through multiple or unknown mechanisms. Neyts *et al.* reported the benzothiazolyl-coumarin conjugated compounds linked through -SCH<sub>2</sub>- as potential antiviral agents inhibiting HCV replications. A methoxy-substituted coumarin analogue (**23**) exhibited low micromolar EC<sub>50</sub> at 29 μM against HCV.<sup>[48]</sup> Peng *et al.* also demonstrated the significant inhibitory anti-HCV activity of 2-(4-nitroanilino)-6-methylbenzothiazole (**24**). The benzothiazole derivative **24** was found to inhibit HCV RNA-dependent

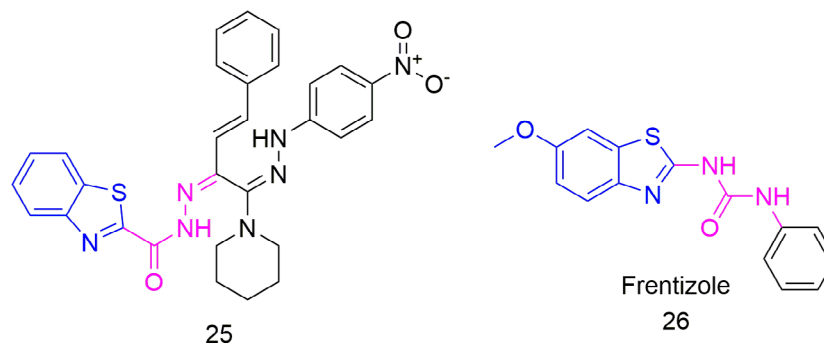
RNA polymerase (RdRp) and HCV RNA replication in a dose-dependent manner (EC<sub>50</sub> 8 ± 0.5 μM), consistent with a non-competitive model of inhibition (kinetic constant *K*; 7.76 μM) (Figure 9).<sup>[49]</sup>

### Anti-herpes agents

Herpes simplex virus (HSV) 1 (cold sores, fever and blisters) and 2 (genital herpes) are highly contagious human viruses of the Herpesviridae family. Many of the common antiviral drugs are in clinical use for the treatment of HSV, e.g. idoxuridine, trifluridine, acyclovir, famciclovir and foscarnet. Nevertheless, research is still ongoing in the development of newer agents with enhanced activity.<sup>[50]</sup> El-Sherbeny *et al.* evaluated some pyrimido[2,1-b]benzothiazole and benzothiazolo[2,3-b]quinazoline derivatives for anti-HSV activity. These compounds showed potential antiviral effects against HSV1 with a 50–61% reduction in the viral plaques.<sup>[51]</sup> Abdel-Aziza *et al.* also synthesised



**Figure 9** Miscellaneous anti-HCV benzothiazoles. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**Figure 10** Benzothiazoles as anti-herpes agents. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

some benzothiazolyl-aryldiazones. Evaluation against HSV1 revealed that piperidinyl amidrazones (**25**) possessed significant antiviral activity (Figure 10).<sup>[52]</sup>

A non-toxic benzothiazolyl urea drug, frentizole (**26**) which was approved by the FDA for the treatment of rheumatoid arthritis and systemic lupus erythematosus has been studied for the immunosuppressive and super-immunosuppressive dose levels on the resistance of the mice to viral infections. The mean survival time of specific pathogen-free male mice pre-treated with frentizole or azathioprine at 100, 50 or 25 mg/kg and infected with herpes simplex and influenza virus was reduced.<sup>[53]</sup>

## Anti-HIV agents

Acquired immunodeficiency syndrome (AIDS) is mainly caused by human immunodeficiency virus type 1 (HIV-1) and continues to be a major contributor to the global burden of disease as it has claimed more than 32 million lives so far.<sup>[54]</sup> Recently, outstanding progress in the discovery of novel anti-HIV agents has led to implementing better therapeutic regimes for this devastating virus.<sup>[55]</sup> The development of a combination antiretroviral therapy has completely transformed HIV from a progressive illness with a fatal outcome into a chronic manageable disease.<sup>[56]</sup> The majority of the currently utilised anti-HIV drugs exert their effects through validated HIV molecular targets which include transcriptase, integrase and protease enzymes.<sup>[57]</sup> Hence, developing potential anti-HIV agents targeting these enzymes remains a main research interest. Towards this end, numerous benzothiazolyl derivatives have been reported to exhibit potent anti-HIV activity both *in vitro* and *in vivo*; however, the clinical efficacy of benzothiazole has not yet been demonstrated.

## Reverse transcriptase inhibitors

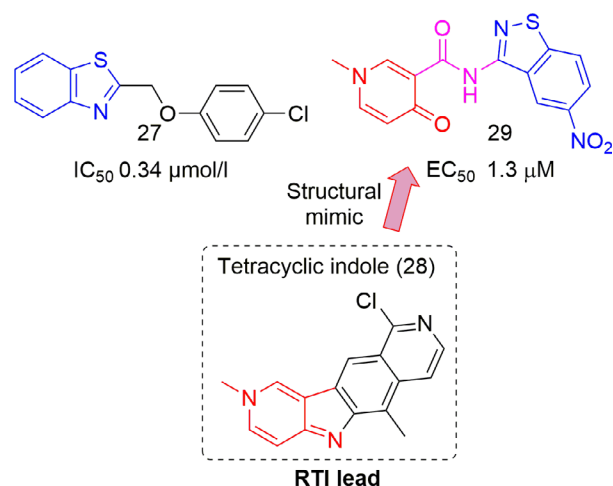
HIV-1 reverse transcriptase (RT) is one of the vital targets of retroviruses. It catalyses the conversion of HIV-RNA

into a double-stranded DNA which then integrates into the human genome.<sup>[58]</sup> In recent years, there has been increasing evidence of the importance of non-nucleoside RT inhibitors (NNRTIs) for HIV therapeutic intervention. Encouraged by this response, Akbay *et al.* synthesised some benzothiazole derivatives as potential RT inhibitors determined through the scintillation proximity assay using bromodeoxyuridine (BrdUMP). Its antiviral potency was indicated by the *in vitro* binding inhibition of thymidine with the RT enzyme. Compound 2-(p-chlorophenoxymethyl)benzothiazole (**27**) was found to be more active than the other compounds, having an IC<sub>50</sub> value of 0.34 μmol/l (Figure 2).<sup>[59]</sup> More recently, Cheung *et al.* also performed a screening of a library of 256-compounds to identify a structural “mimic” of a lead fused tetracyclic indole derivative (**28**).<sup>[60]</sup> Diheteroarylamide-type compounds, containing a common 5-nitroisobenzothiazole derivative (**29**), emerged as potent anti-HIV agent. This compound was evaluated against the isolate (E00443), which greatly reduced susceptibility to NNRTI's and remained active, with an EC<sub>50</sub> of 1.3 μM (Figure 11).<sup>[61]</sup>

## Protease inhibitors

HIV-1 protease (HP) is essential for the life-cycle of HIV and the maturation of infective HIV virion by catalysing the hydrolysis of Gag and Gag-pol polyproteins resulting in the production of structural proteins, such as viral envelope glycoproteins and the enzymes reverse transcriptase, integrase, and protease.<sup>[62]</sup> Thus, it is an important target for HIV treatments. Research efforts aimed at developing a protease inhibitor (PI) led to the approval of many PI drugs in the mid-90s which are now being used in combination with RTIs in highly active antiretroviral therapy (HAART).<sup>[63]</sup> Previously, the structure-based drug design (SBDD) and X-ray crystallographic analysis of HP has resulted in exciting peptide-based PIs as preclinical drug candidates, however, the majority of them failed in clinical trials due to their poor physicochemical properties. This





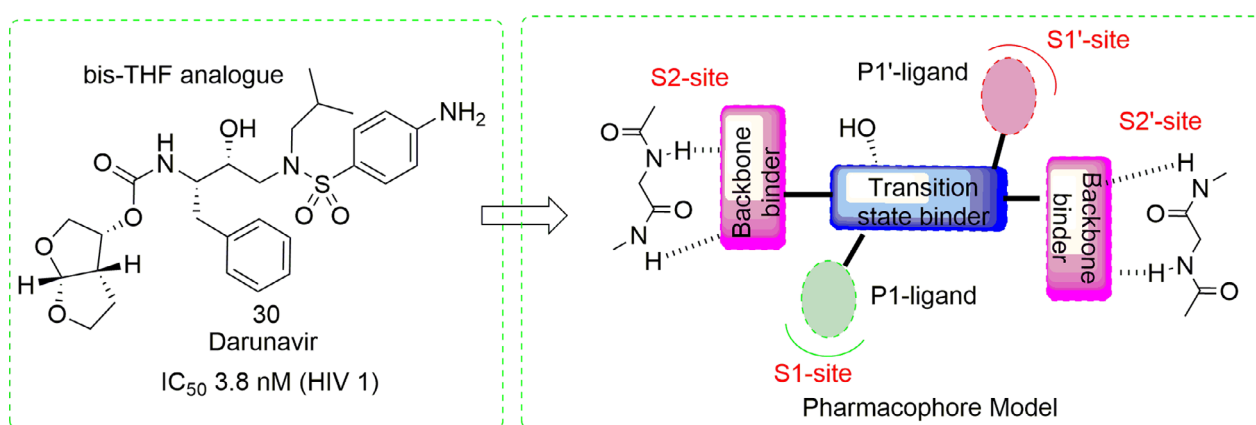
**Figure 11** Development of novel benzothiazolyl reverse transcriptase Inhibitors (RTIs). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

inspired the development of several PIs containing non-peptidic fragments with superior biopharmaceutical properties.<sup>[64]</sup> In pursuit of this goal, the research laboratory of Prof. A. K. Ghosh in collaboration with Tibotec Inc., Belgium, also extensively studied on the SBDD strategies based upon promoting extensive interactions in the active site of HP, particularly with the backbone atoms. They were the pioneers in discovering a range of exceptionally potent non-peptide HIV-1 PIs, of which one drug darunavir (DRV) (IC<sub>50</sub> 3.8 nM) (30), was approved in 2006 by the US FDA for the treatment of drug-resistant HIV. DRV, a 3 (*R*),3a(*S*),6a(*R*)-*bis*-tetrahydrofuran-yl-urethane (*bis*-THF) analogue is now utilised as a frontline therapy for HIV/AIDS. Based on the protein crystallography of darunavir bound to HP, they proposed a pharmacophore model for PI effective against drug-resistant HIV. The model suggests

that the hydrophobic, electrostatic and critical hydrogen bonding with the backbone atoms located in the S2 to S2'-subsites of protease is essential. The transition hydroxyl group binds to a catalytic aspartate, while a P1 and P1' ligand occupy the S1 and S1' subsites (Figure 12).<sup>[65]</sup>

To further optimise the *bis*-THF structural template, they introduced an unprecedented 6-5-5 ring-fused crown-like tetrahydropyranofuran as the P2 ligand and an aminobenzothiazole as the P2' ligand with the (*R*)-hydroxyethylsulfonamide isostere. The resultant analogue 31 emerged as a potent PI against drug-resistant viruses with several fold higher potency (IC<sub>50</sub> 0.39 nM) than the drug DRV (Ghosh *et al.*,<sup>[66]</sup>). Further modification was carried out by introducing a 6-5-5 ring-fused octahydrocyclopentylpyranofuran as the P2 ligand and difluorophenylmethyl as the P1 ligand. The resulting analogue 32 showed excellent enzyme inhibitory potency (IC<sub>50</sub> 27 pM) with a strong antiviral activity against a panel of highly multidrug-resistant HIV 1 variants.<sup>[67]</sup> Extensive lead optimisation at the P2' region was aimed at improving the broad-spectrum activity as well as the overall ADME profile of these derivatives.

Surleraux, *et al.*, 2005, at Tibotec BVBA, Belgium, did parallel research exploring the P2' region to identify new classes of broad-spectrum PIs with improved pharmacokinetic properties. They developed an aminobenzothiazolyl analogue (33) of DRV with pronounced broad-spectrum activity.<sup>[68]</sup> Although DRV has a high genetic barrier to the development of HIV-1 resistance, many DRV-resistant strains have recently been reported.<sup>[69]</sup> Keeping these in mind Takamatsu *et al.* delved more into the development of PI-resistant antiviral drugs. They reported a new series of compounds produced by introducing P2-amino-substituted-*bis*-tetrahydrofuran-yl-urethane (*bis*-THF) (34). The *bis*-THF derivative compounds emerged as potent agents inhibiting the replication of wild-type HIV-1 (EC<sub>50</sub>



**Figure 12** Pharmacophore model of PI to combat drug resistance. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

0.22–10.4 nM) and multi-PI-resistant HIV-1 variant, including highly DRV-resistant HIV<sub>DRV</sub>RP51. This compound had an EC<sub>50</sub> ranging from 1.6 nM to 30.7 nM.<sup>[70]</sup> Ali and collaborators, at the University of Massachusetts Medical School, developed a series of novel PIs having phenylloxazolidinone P2 ligands (35). SAR studies showed that the introduction of polar substituents, such as methylsulfonyl group at the 3-position of the phenyl ring as P2 ligand significantly increased *in-vitro* antiviral activity. A benzothiazole ring as P2 ligand was found to be optimal for enzymatic and antiviral activity against wild-type viruses and MDR viruses.<sup>[71]</sup> Another group of scientists at Janssen Infectious Diseases BVBA, Belgium, discovered a new class of benzothiazole amide derivatives as novel pharmacokinetic enhancers of PIs inhibiting the CYP3A4 enzyme by replacing the sulphonamide with an amide linkage. The *in vitro* antiviral activity of amide derivatives against the wild type HIV-1 demonstrated that the replacement of sulphonamide group was not a good choice, as none of the compounds were active. However, an *in vitro* human liver microsomes (HLM)-based CYP3A4 inhibition assay showed a promising result. The amide analogue 36 demonstrated excellent 'boosting' properties when tested in dogs (Figure 13).<sup>[72]</sup>

In an effort to identify novel agents effective against the multidrug-resistant mutant HP, Ung, et al., 2014, performed a virtual screening of the Center of Chemical Genomics (CCG) library against the eye site pharmacophore model of HP. Further evaluation of the computational hits resulted into the identification of quinolinyl analogue (37) as a promising protease proteolytic agent. A subsequent ligand-based lead-hopping method yielded a novel potent 4-nitro-2-(2-thioxo-2,3-dihydrobenzo[d]thiazol-6-yl)isindoline-1,3-dione (38) as an allosteric modulator of HP proteolytic activity (Figure 14).<sup>[73]</sup>

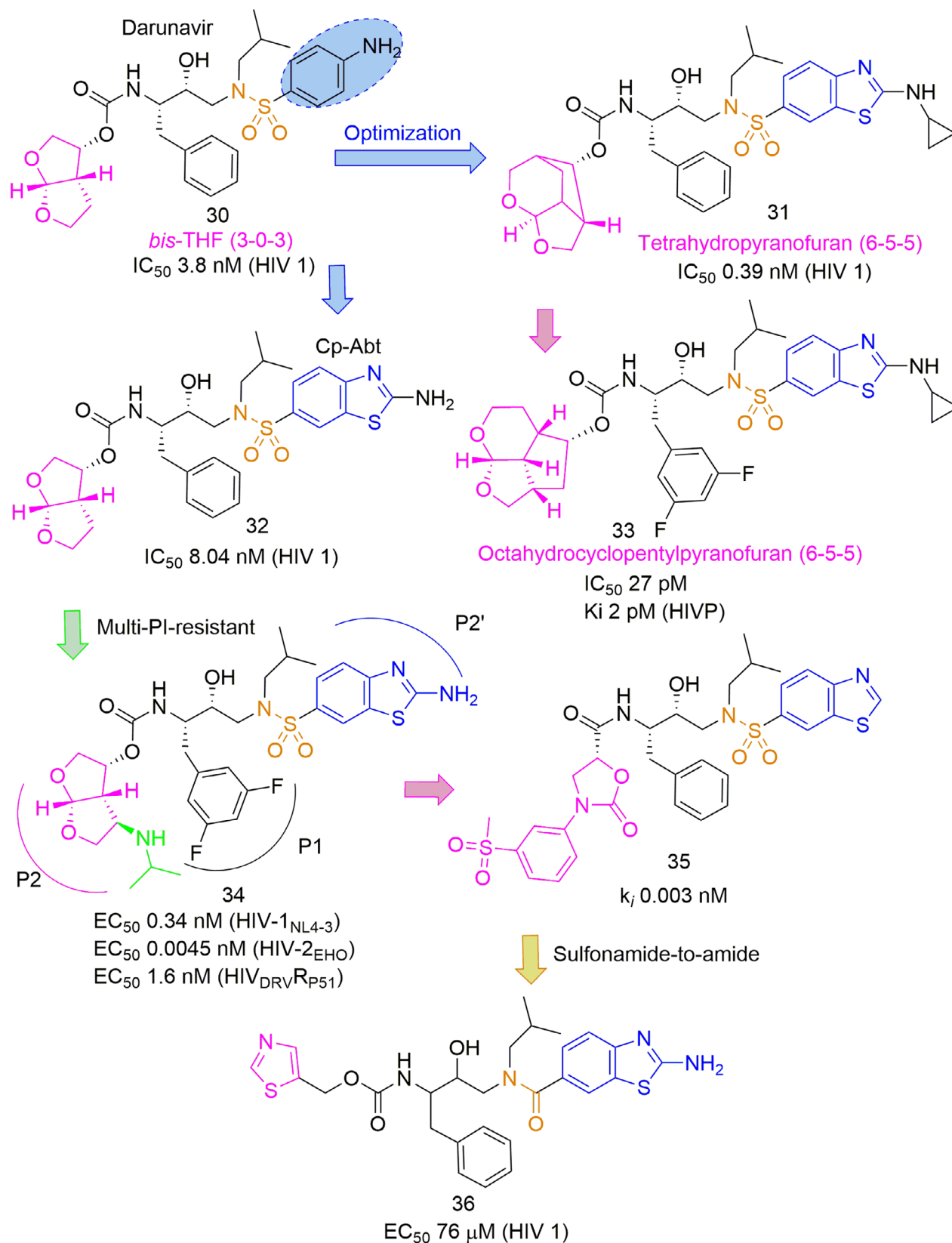
## Integrase inhibitors

The HIV-1 integrase (IN) enzyme is the retroviral enzyme that catalyses the insertion of the virally derived cDNA ends generated by the viral RNA genome through RT into host chromosomes. Since the approval of the IN inhibitor (INI) raltegravir, research on the development of INI has gained huge attention and several derivatives of different classes have been identified so far.<sup>[74,75]</sup> Towards this end, Gu et al. reported the discovery of a novel benzothiazolyl INI. In this study, a total of 80 000 natural compounds were virtually screened for their INI properties and subsequently tested for cell-based antiviral activities. Out of these, 3-(1,3-benzothiazol-2-yl)-8-[[bis(2-hydroxyethyl)amino]methyl]-7-hydroxy-2H-chromen-2-one (D719, 39) inhibited IN nuclear translocation in the immunofluorescence assay at 10 μM (Figure 15). The *in-vitro* anti-HIV activity of

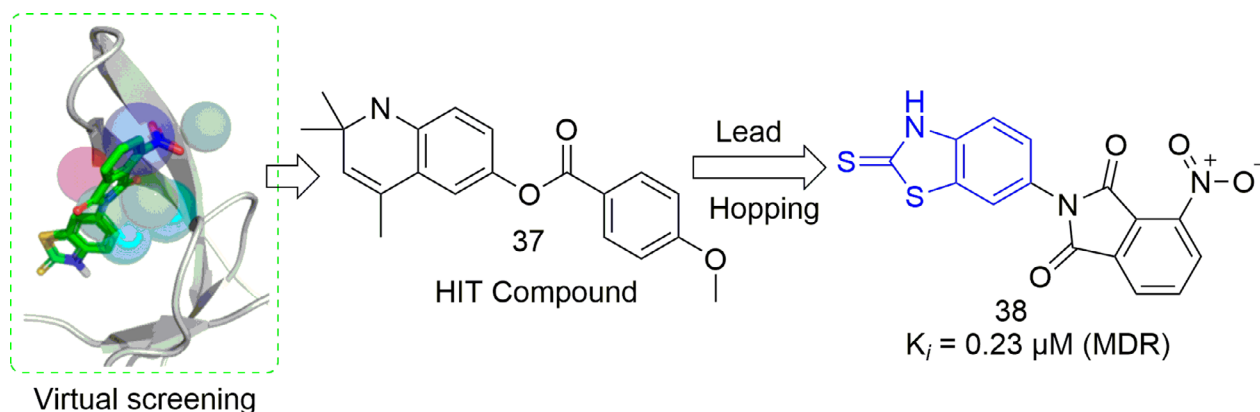
D719 revealed that p24 antigen production in HIV-1<sub>IIIB</sub>-infected human T cells was drastically reduced to half of its maximal effective concentration of 4.33 μM. Thus, D719 provides a novel lead and new insight into the molecular mechanism for future drug development of INI.<sup>[76]</sup>

## Miscellaneous anti-HIV agents

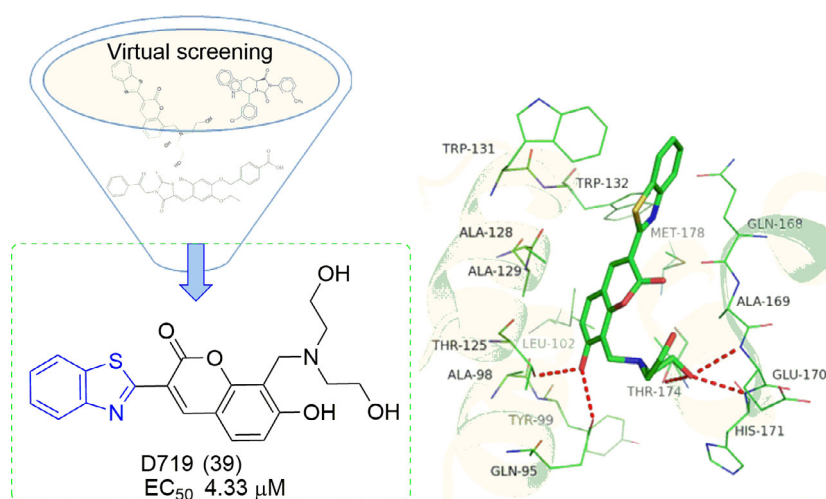
Molecular hybridisation of bioactive pharmacophores has become a major contributor to the development of several synergistic biologically active molecules.<sup>[77]</sup> Based on this approach, different moieties have been clubbed with a benzothiazole derivative to optimise the antiviral potential of benzothiazole leads. Most of these hybrid benzothiazolyl analogues do not have any specific mechanism of HIV inhibitory activity but possess remarkable *in-vitro* anti-HIV potential (Figure 16). Based on these, Vicini *et al.* prepared two series of benzo[d]isothiazole (40) and benzothiazole (41) analogues containing Schiff bases and evaluated *in-vitro* against the HIV-1 (Retrovirus). The EC<sub>50</sub> value of hybrid methyl derivative of benzo[d]isothiazole (40) was found to be > 5 μM.<sup>[78]</sup> Bhavsar *et al.* reported the hybrid benzothiazolyl-coumarins as potential anti-HIV agents. The synthesised compounds were evaluated for their anti-HIV activity using the MTT method and showed potent antiviral activity against the wild type HIV-1 strain. Among these, a 6-chlorobenzothiazole derivative (42) showed a promising anti-HIV effect with an EC<sub>50</sub> < 7 μg/ml. The SAR of these compounds showed that hydroxy substitution at the coumarinyl ring increased its activity, whereas bulky substitutions at the phenyl ring decreased the antiviral potency of these benzothiazole derivatives.<sup>[79]</sup> A series of benzothiazole-containing dioxothiazolines connected through an acetamide linkage (43) was also reported. While these compounds were not that much active against HIV-1 as compared to the reference drug, they provided an important lead for future development.<sup>[80]</sup> Heredia *et al.* screened some in house antimicrobial compounds against the TZM-bl cells with the CCR5-dependent HIV-1 BaL strain *in vitro*. A benzothiazolyl compound containing an indole moiety 5660386 (44) emerged as the most potent antiretroviral agent, having potency in the nanomolar range. This hybrid derivative inhibits HIV-1 entry into cell lines, binds to the HIV-1 envelope protein and inhibits the interaction of GP120 to CD4. This compound had a unique and broad-range activity against primary HIV-1 isolates from different subtypes and geographical areas.<sup>[81]</sup> In 2011, Urano *et al.* discovered a benzothiazolyl-pyrimidine (BMMP, 45) small molecule as an inhibitor of the oligomerisation of HIV-1 Pr55Gag which is a key process in HIV-1 virion production.<sup>[82]</sup> The BMMP template was further optimised by linking with biotin, then evaluated against the presumed target, HIV-1 Pr55Gag or CA, by



**Figure 13** Benzothiazole PIs discovered by Tibotec BVBA. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**Figure 14** Development of PI through ligand-based lead-hopping. [Colour figure can be viewed at wileyonlinelibrary.com]



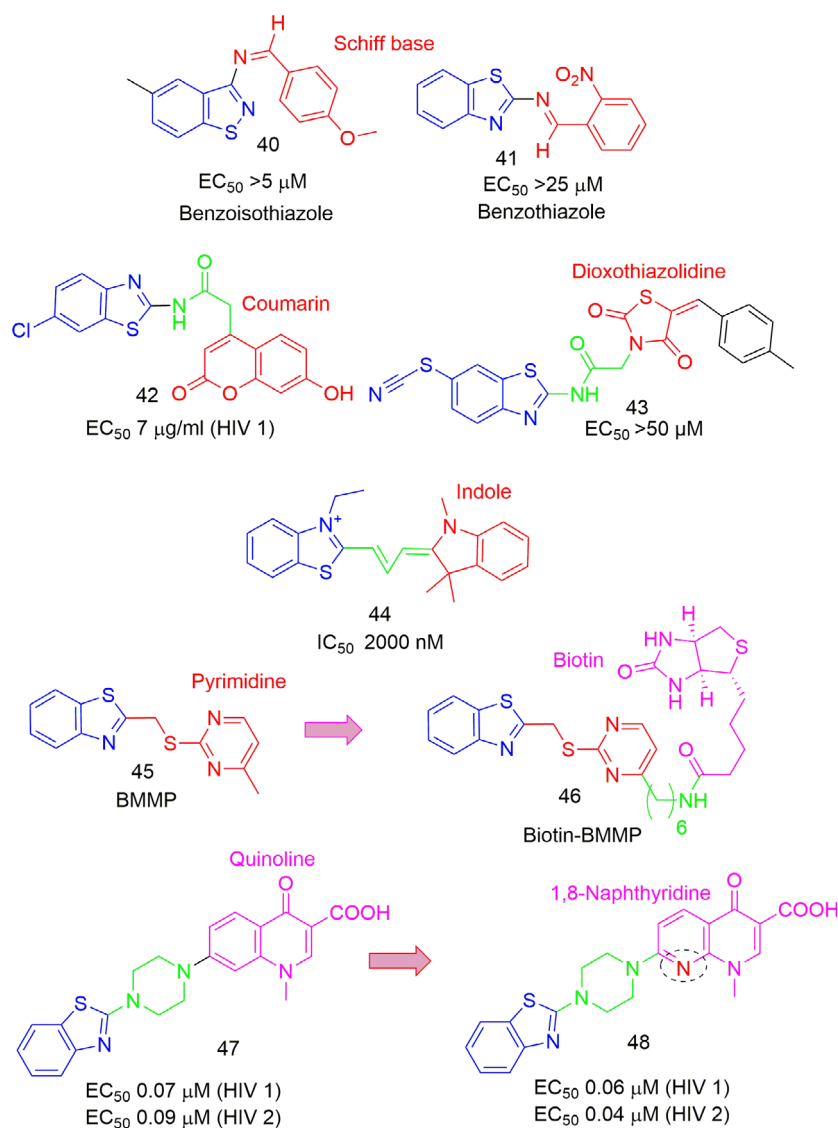
**Figure 15** D719 inhibiting the integrase nuclear translocation.<sup>[76]</sup> [Colour figure can be viewed at wileyonlinelibrary.com]

means of surface plasmon resonance. The newly synthesised Biotin–BMMP hybrid (**46**) inhibited HIV-1 replication but showed no interaction with Pr55Gag or CA, suggesting that antiviral activity occurs through a different mechanism.<sup>[83]</sup>

The 6-aminoquinolones have also emerged as a promising new class of HIV expression inhibitors, interfering with the Tat-mediated transactivation of the HIV-1 LTR promoter. On the basis of these recent findings, Tabarrini *et al.* did extensive research on 6-desfluoroquinolones showing marked anti-HIV activity in acutely, chronically, and latently HIV-1 infected cell cultures.<sup>[84]</sup> Further optimisation of 6-desfluoroquinolones demonstrated that the bioisosteric replacement of the quinolone (**47**) nucleus by an 1,8-naphthyridone (**48**) is beneficial when a suitable arrangement of the functional groups is present at the C-6, N-1, and C-7 positions. The SAR results showed that a substitution with 1-(1,3-benzothiazol-2-yl)piperazine present at the C-7 position was advantageous.<sup>[85–87]</sup>

## Anti-influenza agents

Human influenza also known as ‘flu’ is mainly caused by influenza A or B virus infections. This virus consists of single-stranded RNA enveloped in a glycoprotein surface, along with haemagglutinin and neuraminidase (NA). Infections with influenza A viruses of the subtypes H1N1 and H3N2 are the main causes of respiratory tract disease. Current antiviral drugs target the M2 ion-channel protein (e.g. adamantanes) and NA (e.g. zanamivir and oseltamivir). However, many resistant influenza virus strains have been reported in several countries, highlighting the need for new antiviral drugs.<sup>[88]</sup> The first report on benzothiazole derivatives as anti-influenza came in 1969 from Paget *et al.* They reported the structure naphthothiazolylureas (**49**) effective against the *coxsackievirus* A21 *Coe virus*.<sup>[89]</sup> Encouraged by this, Akerfeldt at KABI group, Sweden performed the *in vivo* preclinical screenings of aminobenzothiazoles



**Figure 16** Hybrid benzothiazoles as anti-HIV agents. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

(ABT) on mice infected with influenza A2 strains. A 4-chloro substituted ABT (**50**) ( $LD_{50}$  180 mg/kg) showed 100% animal survival against the Influenza A2 virus (Figure 17).<sup>[90]</sup>

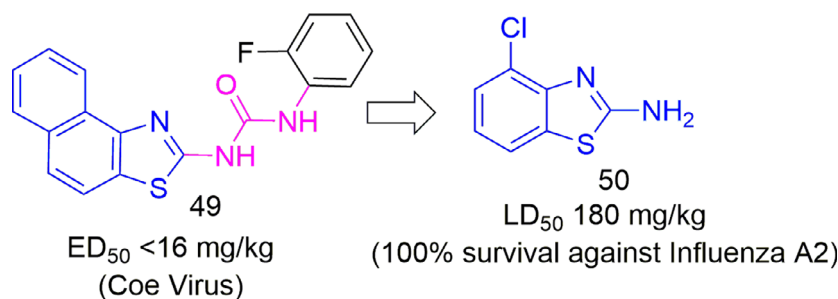
### IRF3 agonists

The RIG-I-like receptors (RLRs) are pathogen recognition receptors which play a major role in the pathogen sensing of RNA virus infections to initiate antiviral immunity.<sup>[91]</sup> Following the AViD HTS platform against a very large library of 47 000 small compounds, a benzobisthiazole hit compound KIN1000 (**51**) was identified as an RLR signal activating agent. This HTS procedure utilised the Huh7 reporter cell lines containing the ISG54, ISG56, or IFN- $\beta$

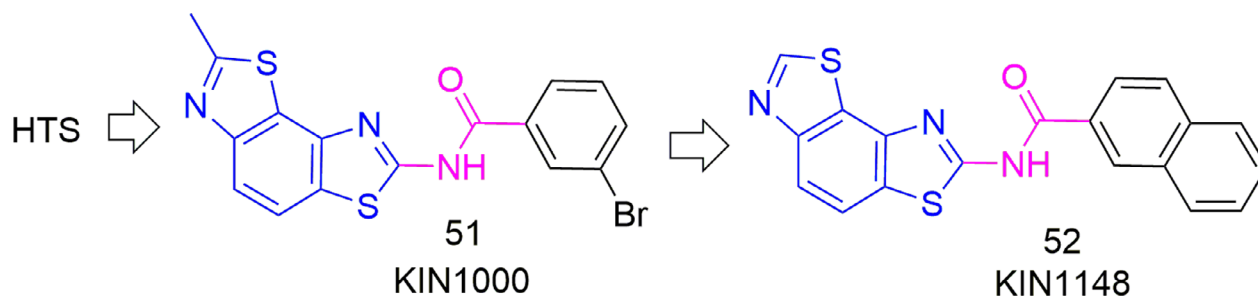
promoters, all IRF3-genes triggered through the RLR pathway. Finally, a new lead analogue KIN1148 (**52**), was designed by structural modifications of the phenyl with naphthyl ring. Compound KIN1148 showed increased protection and reduced viral load when administered along with suboptimal dose of H1N1 vaccine to influenza virus A-infected mice. KIN1148 also induced an influenza virus-specific IL-10 and Th2 response by T cells in the lymph nodes (Figure 18).<sup>[92]</sup>

### AKT Inhibitors

The protein kinase-B (AKT) pathway is an important mechanism in the infection and replication of influenza viruses. Inhibition of AKT kinase activity has been reported

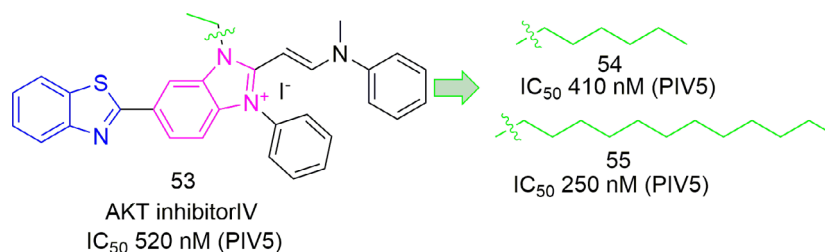


**Figure 17** ABTs as potential anti-influenza agents. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**Figure 18** A benzobisthiazole IRF3 agonist as an influenza vaccine adjuvant. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

to suppresses the entry and replication of influenza virus.<sup>[93]</sup> To probe its significance, Peterson *et al.* at the University of Kansas, USA, focused on the discovery of novel antiviral agents targeting the AKT-IV. They identified a potent benzothiazolyl AKT-IV inhibitor (ChemBridge 5233705) (**53**), having broad-spectrum antiviral and anti-cancer activity. SAR showed that the replacement of the *N*-ethyl substituent with *N*-hexyl (**54**) and *N*-dodecyl (**55**) groups increased the antiviral potencies of AKT-IV inhibitors against the recombinant parainfluenza virus 5 (PIV5), expressing luciferase in HeLa cells.<sup>[94]</sup> Further, detailed biomolecular studies revealed that the cationic benzimidazole moiety exhibited dose dependent paradoxical positive or negative effects on the phosphorylation of AKT. Moreover, compound **53** was found to trigger swelling, disintegration and depolarisation of mitochondria, elevation of ROS, and essentially, the complete inhibition of the cellular consumption of oxygen (Figure 19).<sup>[95]</sup>



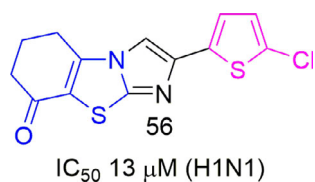
**Figure 19** Benzothiazolyl AKT-IV inhibitors. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

### Neuramidase inhibitors

Galochkina *et al.* reported that a novel tricyclic structure containing 2-substituted 7,8-dihydro-6H-imidazo[2,1-b][1,3]benzothiazol-5-ones (**56**) was a promising anti-influenza A virus. The thiophene substituted derivative demonstrated excellent antiviral activity (CC<sub>50</sub> > 1000 μM, SI = 77). To get insight into the molecular mechanism of its anti-influenza potential, a detailed NA enzyme inhibition assay was also carried out; however, the result was not significant (Figure 20).<sup>[96]</sup>

### Bcl-2 inhibitors

Apoptosis is a host cellular defence mechanism against virus infections generally initiated by the pattern recognition receptors (PRRs). These receptors recognise the attacking viruses and signal to Bcl-2 proteins which leads to mitochondria membrane permeabilisation (MoMP),

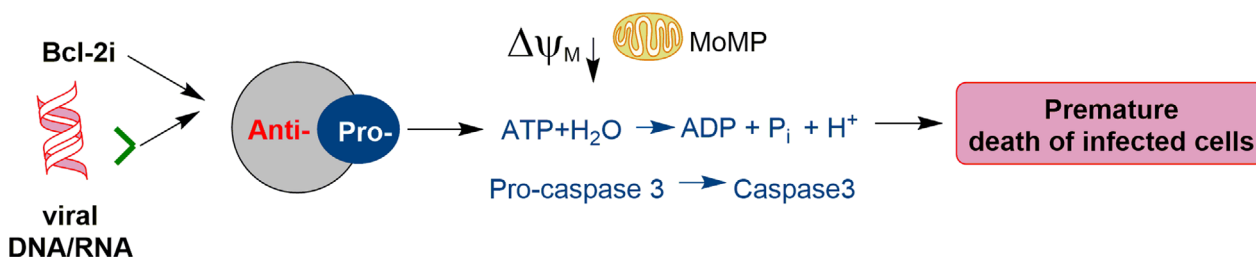
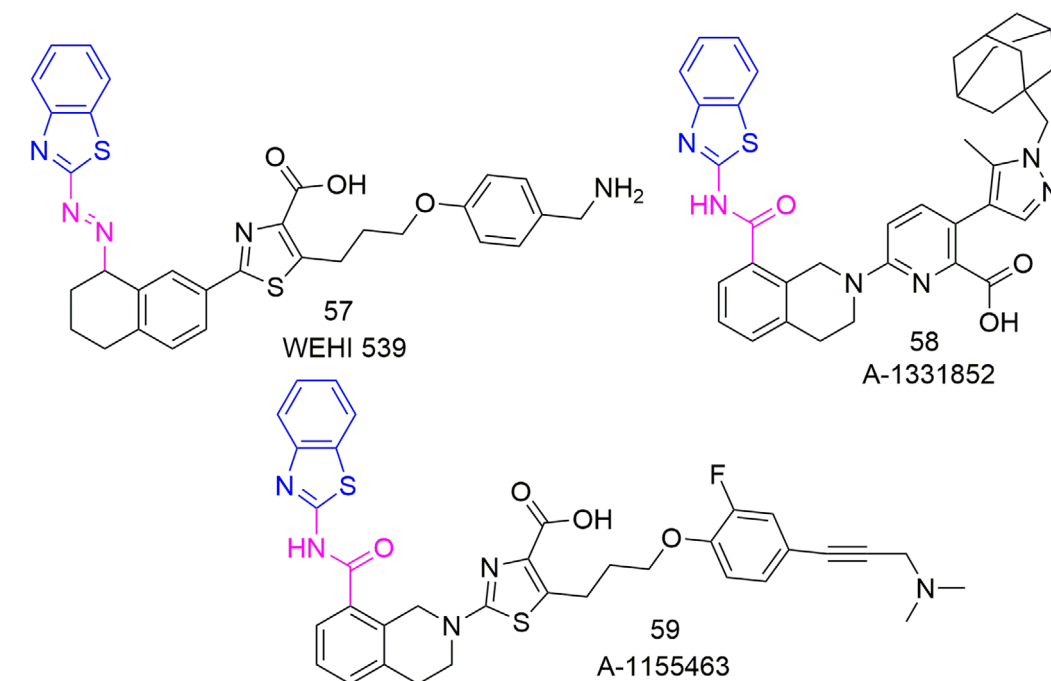


**Figure 20** 7,8-Dihydro-6H-imidazo[2,1-b][1,3]benzothiazol-5-ones as anti-influenza. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

cytochrome c release and ultimately cell death (Shim, *et al.*<sup>[97]</sup>). Bulanova *et al.* identified some safer benzothiazole derivatives (**57**, **58** & **59**) as inhibiting cellular anti-apoptotic Bcl-2 proteins (Bcl-2i), which induced the premature cell deaths of RNA/DNA virus infected cells. The apoptosis assay was performed on the viability and death of influenza A virus infected cells (Figure 21).<sup>[98]</sup>

## Anti-West Nile virus

West Nile virus (WNV) is a single-stranded RNA flavivirus typically spread by mosquitoes. Currently, no drug or vaccine is available for the treatment of WNV. Similar to the other flaviviruses, WNV NS2BNS3 protease is also a promising target for WNV treatment.<sup>[99]</sup> Using robust screening procedures, including *in silico*, HTS, *in vitro* and *in vivo* assessments, on a large array of compounds through hit-to-lead (H2L) optimisation has resulted in many potent WNV PIs. Scientists at Georgetown University Medical Center, USA, also performed an HTS assay for the WNV protease by assessing approximately 32 000 small molecules. Interestingly, they discovered an 8-hydroxyquinoline (8-HQ) chemotype analogous with compound **60** as a promising PI (Mueller, *et al.*,<sup>[100]</sup>). Further insight into the SAR of 8-HQ was also established by making substitutions



**Figure 21** Benzothiazoles inhibiting Bcl-2i. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

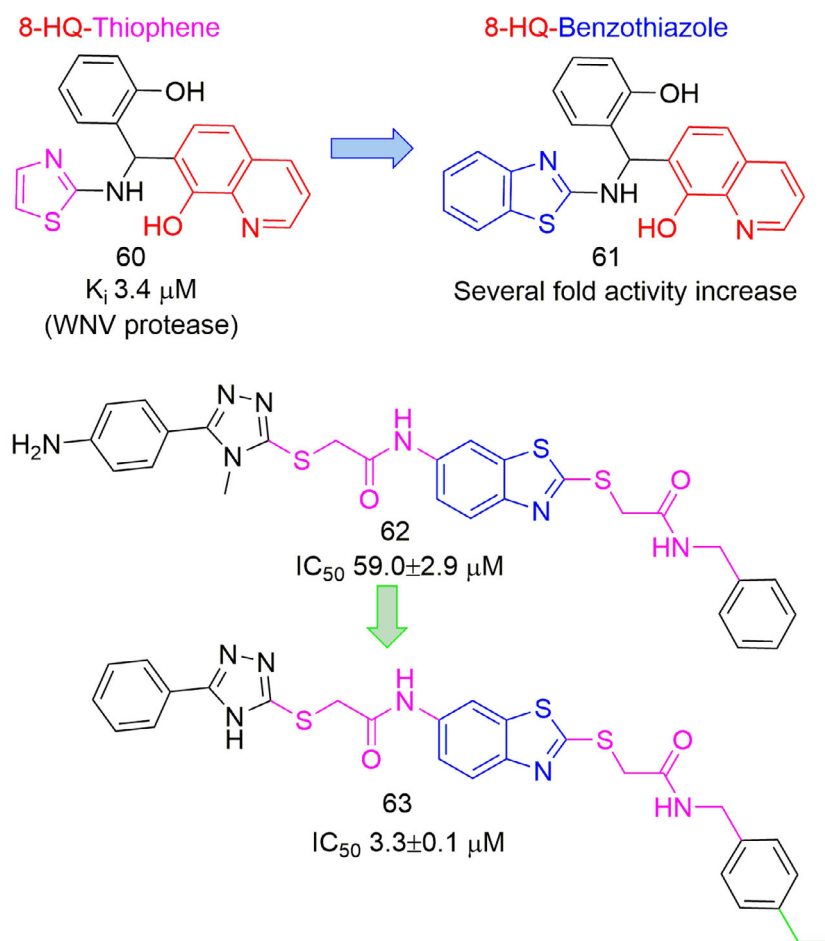
around the core template. Replacement of thiazole (**60**) with a benzothiazole (**61**) moiety at the R3 position provided the analogues with great inhibitory activity that formed strong interactions with the WNV NS2B/NS3pro residues Asp75 at the active site.<sup>[101]</sup> Likewise, NUS-Singapore researchers implemented a preliminary screening of approximately 110 compounds for the WNV NS2BNS3 protease inhibition assay at 100  $\mu\text{M}$  and identified a benzothiazolacetamide derivative (**62**) as a WNV PI. This hit molecule underwent more extensive optimisation in a subsequent step to yield a potent lead compound (**63**) containing an ethyl group at the *para* position of the phenyl ring. The affinity of the lead was found to improve by several orders of magnitude against WNV NS2B NS3 protease than DENV2 NS2B NS3 protease (Figure 22).<sup>[102]</sup>

## Discussion

Benzothiazole ring has gained much attention because of its easy functionalisation at various ring positions, which

makes them attractive synthetic compounds for designing and development of the novel antiviral drugs in future. Various benzothiazole analogues were reported to have promising antiviral properties. We summarised their antiviral potential along with their molecular frameworks in Table 1. Many of these compounds had desired pharmacokinetic properties and broad spectrum of antiviral activity against the wide range of viruses acting through different molecular targets such as protease, helicase, polymerase, reverse transcriptase, integrase and neuramidase.

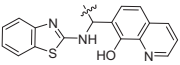
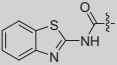
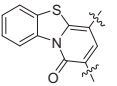
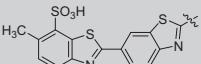
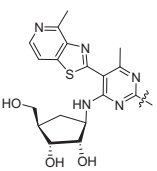
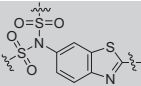
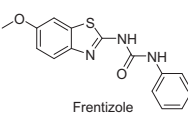
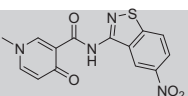
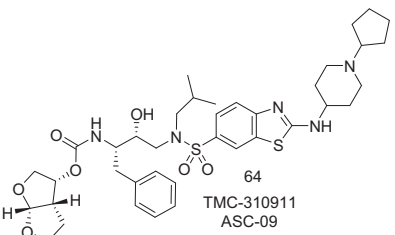
Ongoing research shows that aminobenzothiazoles (ABTs) are a very promising and common pharmacophore for the design and development of inhibitors/agonists against the NS2B/NS3 protease, RNA helicase, Bcl-2i, IRF3 and reverse transcriptase. ABT containing 8-hydroxyquinoline (8-HQ) compounds (**1**) exhibited very promising antiviral activity by DENV2 NS2B-NS3 protease inhibition. These inhibitors bind into the active site of the NS3pro near to the catalytic triad residues: His51, Asp75 and Ser135 like the peptide-based inhibitors. The observed



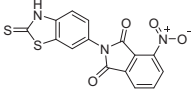
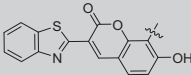
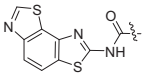
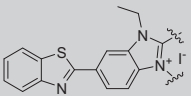
**Figure 22** Hit-to-lead optimization of WNV PIs. [Colour figure can be viewed at wileyonlinelibrary.com]



**Table 1** Benzothiazoles as antiviral agents

Chemical classes	Pharmacophore	Mechanisms of action	Viruses	Clinical status
8-Hydroxyquinoline analogue of benzothiazole		NS2B/NS3 protease inhibitor	DENV2 & WNV	NA
Benzothiazolyl carboxamide		RNA helicase inhibitor Bcl-2i inhibitor	DENV Influenza A	NA
Pyridobenzothiazole		RNA polymerase (RdRp) Inhibitor NS5B polymerase inhibitor	DENV2 CV	NA
Benzothiazolyl oligomers (Dyes)		NS3 helicase inhibitors	HCV	NA
Benzothiazolyl carbanucleoside		NS5B polymerase inhibitors	HCV	NA
Disulphonamide analogue of benzothiazole		HCV replicon inhibitors	HCV	NA
Benzothiazolyl urea	 Frentizole	–	HSV & Influenza	Immunosuppressive drug
Benzoisothiazolyl-dihydropyridine-3-carboxamide		Reverse transcriptase Inhibitors	HIV	NA
bis-Tetrahydrofuranyl-urethane analogue of benzothiazole	 64 TMC-310911 ASC-09	Protease Inhibitor	HIV & COVID-19	Phase I & IIa-completed (HIV) <sup>[104]</sup> Phase 3-ongoing (COVID-19) <sup>[105]</sup> (Janssen R&D Ireland)

**Table 1** (Continued)

Chemical classes	Pharmacophore	Mechanisms of action	Viruses	Clinical status
Isoindoline-1,3-dione analogue of 2-thioxobenzothiazole		HIV-1 protease	MDR-HIV	NA
Coumarin analogue of benzothiazole		Integrase inhibitors	HIV	NA
Benzobisthiazole		IRF3 agonists	Influenza virus A	NA
Cationic benzimidazole analogue of benzothiazole		Protein-kinase-B inhibitor	Parainfluenza virus 5 (PIV5)	NA

NA, Not available.



**Figure 23** Suggested pharmacophore model of ABT analogues for eliciting antiviral activities. [Colour figure can be viewed at wileyonlinelibrary.com]

potency of compounds could be attributed due to the favourable hydrophobic interactions of lipophilic benzothiazole and 8-HQ moieties at S2 and S1 hydrophobic pockets, respectively. Another ABT derivative containing pyrimidine nucleus (**4**) strongly inhibited DENV NS3 helicase. The amino group of compound **4** formed a hydrogen bond with Arg599 at the active site. Docking analysis of 6-methyl-*N*-(4-nitrophenyl)benzothiazol-2-amine (**24**) at the active site of HCV RdRp protein structure showed that it occupied the thumb domain of the enzyme and made close contacts with the surrounding residues, Leu419, Leu497, Arg501 and Trp528. It is thus observed that, in general, ABT analogues could interact strongly with varied molecular targets of different viruses and these ligands have broad-spectrum structural features that make them proficient for inhibiting numerous significant target proteins. Figure 23 reveals a common structural features ABTs where the lipophilic benzothiazole moiety is linked to the terminal aryl/heteroaryl nucleus through amino/amide linkage as hydrogen bonding domain.

In addition, *bis*-THF analogue containing ABTs (**34**) have proved to be effective HIV-1 PIs. *Bis*-THF analogues

with HIV-1 protease shows extensive interactions at the active site. The pharmacophore model suggests the hydrophobic, electrostatic and hydrogen bonding with the backbone atoms located in the S2 to S2'-subsites whereas the hydroxyl group binds to a catalytic aspartate while a P1 and P1' ligand occupy the S1 and S1' subsites (Fig 12). Using the X-ray crystallography, the *bis*-THF template was further optimised and developed into the first clinical candidate ABT derivative TMC310911 (ASC09, **64**) with improved resistance profile and higher genetic barrier to resistance. TMC310911 has completed phase I and phase IIa clinical trials and the drug was reported to be safe and well tolerated. TMC310911 formed hydrogen bond networks with the ASP25, ASP25<sup>o</sup>, ASP29, ASP30, ASP30<sup>o</sup> and GLY27 residues.<sup>[103]</sup>

## Conclusion, authors comments and future perspective

Despite some progress in recent years, the fight against antiviral diseases remains a great challenge. Antiviral drug discovery has seen huge leaps in scientific research and

technological innovations. During the past few years, significant advancements have been made in understanding the molecular targets of viral proteins and based on these many biochemical assays have been developed. *In silico* screening, robust *in vitro* HTS and *in vivo* models have become increasingly important tools for the discovery of antiviral leads. Several antiviral compounds containing a benzothiazole scaffold have emerged as excellent leads in HTS viral enzyme assay and subgenomic replicon cells assay. After an overview of the antiviral benzothiazole analogues, it was noticed that there is a general trend of designing molecules with simple substitutions at the C-2 position with amide or aryl ring. Some of these compounds were unusually effective against a broad spectrum of viruses, as their target genome shares a common homology. This clearly indicates that there is still room for improvement in terms of efficacy, toxicity and physicochemical properties.

Moreover, the ongoing pandemic of COVID-19 is defining global health crisis of our time and underscores the urgency to develop effective vaccine or medicine against this virus. In this regards, hundreds of reports have been published uncovering the whole genome sequence of SARS-CoV-2 variants. Experts around the world have been gearing up to repurpose existing drugs especially antiviral drugs against the coronavirus. One PI, benzothiazolyl analogue ASC09 (64), is currently under evaluation in clinical

trials as combinational therapy to assess the efficacy of ASC09F and ritonavir for 2019-nCoV pneumonia.

No doubt, significant advancement in the discovery of novel benzothiazolyl antiviral agents has been made, and there is a plethora of preclinical candidates. There are still lots of benzothiazoles having drug like properties with varied biological activities have not been investigated for their antiviral potential. Putting drug repurposing of these large-scale data will play a principal role in clinical development of novel antiviral drugs in the future. Various computational approaches along with HTS can be used to design novel antiviral agents not only effective against COVID-19 but against any deadly viruses.

## Declarations

### Conflict of interest

Authors have no conflicts of interest.

### Acknowledgements

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