Non-nucleoside inhibitors of the HCV polymerase

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Chronic hepatitis C virus (HCV) infection is the leading cause of chronic liver disease. Current therapy using pegylated interferon-α with ribavirin is poorly tolerated and confers an overall sustained virological response around 56%. Compounds exhibiting an improved safety profile with similar or enhanced antiviral properties may represent future treatment options. Several drug discovery programmes are ongoing to directly target the viral enzymes involved in HCV replication. Recent clinical success using a peptidomimetic inhibitor of the viral serine protease has demonstrated proof-of-concept for the use of direct antiviral agents in reducing viral load. The RNA-dependent RNA polymerase (RdRp) of HCV is also required for viral RNA replication and thus represents an attractive drug discovery target. Preclinical characterization of several non-nucleoside inhibitors (NNIs) of the HCV RdRp have been described, including a promising series of benzothiadiazine derivatives which have been shown to efficiently block viral RNA synthesis in HCV replicon cell systems. Herein, the antiviral activity, mode of action, resistance profiling and therapeutic potential of the benzothiadiazine class of compounds for clinical development are explored.

Keywords: benzothiadiazines, NS5B, IFN-α, RdRp inhibitors

HCV antivirals

Discovery of potential therapeutics for hepatitis C virus (HCV) has been hampered by the inability to grow the virus in culture and by the lack of robust small animal models. To that end, identification and evaluation of inhibitors has been progressed by screening candidate compounds in both biochemical enzymic assays and cell-based HCV replicon systems. Several structurally distinct non-nucleoside inhibitors (NNIs) of the HCV RNA-dependent RNA polymerase (RdRp) have recently been reported, including a benzothiazadiazine, a disubstituted phenylalanine, and two benzimidazole derivatives.1–4 The benzothiazadiazine and benzimidazole classes have been well characterized and shown to inhibit HCV replication effectively in cell-based replicon systems with no apparent cytotoxicity. Extensive biochemical characterization of these derivatives suggests an allosteric mode of inhibition. Specifically, both classes of compounds directly bind the viral polymerase (NS5B), inhibit RNA synthesis non-competitively with respect to NTPs and have been confirmed to block RdRp activity before the formation of an elongative complex based on single-turnover studies.5–8 Interestingly, recent data indicate that the putative binding site on the viral enzyme for these two classes of NNIs may indeed be distinct, thereby implicating multiple regions of the NS5B thumb domain surface as potential antiviral targets for allosteric inhibition.7,9 Taken together, consideration of combination therapy regimens based on the use of multiple NNIs may be a future option for antiviral intervention which is in contrast to HIV reverse transcriptase for which all known NNIs bind to the same site.10

Although a shallow substrate-binding channel of the HCV protease challenged drug discovery efforts, proof-of-concept for a small molecule anti-HCV agent has been attained in short-term trials with an orally administered inhibitor of the viral protease, BILN2061, showing a rapid 100–1000-fold reduction in viral load.11 Longer-term safety and efficacy trials represent the next challenge for this antiviral. While peptidomimetics represent potent, specific inhibitors of the viral serine protease, advances in HCV polymerase inhibitors are now beginning to mature. For example, an orally bioavailable benzimidazole analogue (JTK-003) is currently under investigation in a Phase Ib multicentre, placebo-controlled clinical study on HCV patients refractory to interferon-based therapy.12 Although the benzothiadiazine class of compounds has not yet achieved progression into the clinic, they certainly represent an exciting chemical scaffold for further exploration. Herein, we summarize the antiviral activity, mode of action, resistance profile and therapeutic potential of this compound class.

Polymerase inhibitors

High-throughput screening of the GlaxoSmithKline proprietary compound collection using an NS5B-directed RNA synthesis
HCV RdRp inhibitors

Mode of action
Mechanistic studies confirmed that the reduction in viral RNA synthesis was due to direct catalytic inhibition rather than titration of nucleic acid or competition with nucleotides. Specifically, CD spectroscopy, analytical ultracentrifugation, isothermal titration and enzymology studies showed a direct interaction of the benzothiadiazines with NS5B and a kinetic behaviour consistent with a reversible, non-competitive mode of inhibition with respect to GTP. The midpoint melting transition temperature \( T_m \) of NS5B (46.4°C) increased 3.4°C when monitoring thermal denaturation in the presence of compound 4. This increased shift in \( T_m \) and change in the unfolding enthalpy was consistent with direct binding of the compounds to NS5B.6

Testing of the benzothiadiazine derivatives in a gel-based assay using short RNA templates showed a block in the formation of the initial phosphodiester bond, thereby confirming the mode of action as inhibition of RNA synthesis initiation.8 In contrast, these agents did not interfere with elongative synthesis. Surface plasmon resonance was utilized to explore higher-order complex formation and provided evidence that a ternary complex is indeed capable of forming between RNA template, \( \Delta 21 \) HCV RdRp and compound 4.9 Together with other studies, these data indicate that the heterocyclic benzothiadiazine agents interact with both the apoenzyme form and the RNA-bound form of \( \Delta 21 \) HCV RdRp and do not interfere directly with RdRp–RNA interaction.

Combination testing of compound 4 with interferon-\( \alpha \) (IFN-\( \alpha \)) was carried out in the replicon system to assess the potential for synergy, additivity or antagonism. The deviation from dose-dependent additivity and fractional inhibitory concentration was presented as a combination isobologram, showing that combination testing resulted in inhibition of viral replication greater than expected from additive inhibition. A synergy coefficient of \(-0.28 (P=0.009)\) indicated that this combination was highly synergic, consistent with compound 4 and IFN-\( \alpha \) having distinct mechanisms of action.10

A time course analysis after a single pulse treatment of replicon cells with 5 \( \mu \)M compound 4 showed a loss of viral RNA consistent with the replicon RNA half-life, suggesting greater than 90% inhibition of ongoing or newly initiated replicative intermediates. This finding is consistent with the mechanism of action, an RNA synthesis initiation inhibitor. Further, unlike IFN-\( \alpha \), an immediate reduction in HCV replicon RNA synthesis was apparent upon addition of compound 4. Treatment of replicon cells with IFN-\( \alpha \) showed a delay of approximately 4h before inhibition of viral RNA replication, consistent with IFN-\( \alpha \)’s signalling kinetics.11

Resistance profile
A compound 4-resistant replicon was selected by repeat passage at a drug concentration 20 times the replicon IC\(_{50}\) and was shown to have significantly reduced susceptibility to compound 4. Cross-resistance to compound 1, a closely-related analogue of compound 4, was confirmed in these resistant replicon cells \((\text{IC}_{50}>50\ \mu\text{M})\), and as expected, inhibition by IFN-\( \alpha \) remained unchanged. The mutation conferring resistance to compound 4 was mapped to residue 414 within NS5B, a methionine to threonine substitution, and computer modelling revealed that the mutation occurred at a site near motif E. A recombinant replicon was engineered to contain the Met414Thr mutation, and this stable replicon cell line was indeed impaired for susceptibility to compound 4.9 Although selection of resistance in culture identified one residue involved in benzothiadiazine susceptibility, the effects of natural variations on their activity have yet to be explored.

Therapeutic potential
Combination therapy of pegylated IFN-\( \alpha \) with ribavirin has proven efficacious in treating chronic HCV infection, although a significant proportion of patients, especially those with genotype 1, remain unable to achieve a sustained virological response. Further, discontinuation of treatment due to side effects is a growing concern. Newer agents targeted against the viral replicative enzymes may provide future promise, especially in combination therapy, a strategy successfully used for HIV. Recent advances in the structural profile of some HCV enzymes as well as the widely available HCV replicon system have proven invaluable for identifying and selecting drug candidates to progress into clinical development. Anti-virals targeting conserved regions on HCV enzymes aimed at minimizing the development of drug resistance or those having synergy with IFN-\( \alpha \) and other small molecule HCV enzyme inhibitors to offer a range of combination treatment options may be most likely to play a role in future treatment strategies. Given that the benzothiadiazine compounds target a site within the more highly conserved active site of NS5B polymerase, interest in further developing this compound class for eventual exploration in clinical trials is quite high. Chemistry focused on understanding the structure–activity relationship should enhance the physicochemical properties and potency profile of this compound class. Selectivity screening of the benzothiadiazine derivatives against known human receptors and ion channels as well as preclinical toxicology studies will help to evaluate the future potential of this chemical series and assist in the design of improved compounds with the required pharmacokinetic and safety parameters to justify clinical investigation of their efficacy.
References


