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Viral RNA Targets and Their Small Molecule Ligands

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Abstract RNA genomes and transcripts of viruses contain conserved structured motifs which are attractive targets for small molecule inhibitors of viral replication. Ligand binding affects conformational states, stability, and interactions of these viral RNA targets which play key roles in the infection process. Inhibition of viral RNA function by small molecule ligands has been extensively studied for human immunodeficiency virus (HIV) and hepatitis C virus (HCV) which provide valuable insight for the future exploration of RNA targets in other viral pathogens including severe respiratory syndrome coronavirus (SARS CoV), influenza A, and insect-borne flaviviruses (Dengue, Zika, and West Nile) as well as filoviruses (Ebola and Marburg). Here, I will review recent progress on the discovery and design of small molecule ligands targeting structured viral RNA motifs.

Keywords Antiviral drugs, Drug targets, Hepatitis C virus, Human immunodeficiency virus, Influenza A virus, Noncoding RNA, Viral inhibitors

Contents

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Abbreviations

dsRNA Double-stranded RNA
ssRNA Single-stranded RNA

For abbreviations of virus names, see Table 1.

1 Introduction

The compact genomes of viruses offer a limited number of protein targets for the development of anti-infective therapy. Structured RNA elements in viral genomes and transcripts have the potential to expand the target space for antiviral drug discovery. Precedent for clinically approved RNA-binding drugs is found in natural product-derived antibiotics including macrolides, tetracyclins, oxazolidinones, and aminoglycosides which interact with ribosomal RNA (rRNA) of bacteria and block protein synthesis in the pathogens [1, 2]. The well-defined structure of rRNA provides selective binding sites for these antibiotics which serve as a paradigm for RNA recognition by small molecule ligands. RNA elements in viruses have been extensively explored as potential drug targets in the human immunodeficiency virus (HIV) and hepatitis C virus (HCV) [3, 4] whose genomes include conserved noncoding regions (ncRNA) that may present structured binding sites for small molecules [5, 6]. Challenges and successes in the discovery and design of compounds targeting RNA have been discussed in the previous comprehensive review articles which also provide a historic perspective on past efforts to explore viral RNA targets for small molecule inhibitors [7–12]. In the current chapter, I describe progress on discovery and investigation of small molecule ligands for viral RNA targets over the last 2–3 years and include perspectives on potential new viral RNA targets which have not yet been widely explored but may attract interest in pathogens of unmet or emerging medical needs (Table 1).

Table 1 Viral RNA targets

Family	Virus	Genome	RNA target	Small molecule ligands
Retrovirus	Human immunodeficiency virus (HIV)	(+) ssRNA	• Transactivation response (TAR) element • Rev response element (RRE) • Dimer initiation sequence (DIS) • Packaging signal (Ψ) stem-loop 3 (SL-3) • Frameshifting signal (FSS)	Reported for all HIV targets; previously reviewed [3, 13–15], and in this chapter
Flavivirus (genus hepacivirus)	Hepatitis C virus (HCV)	(+) ssRNA	• Internal ribosome entry site (IRES) • G-quadruplex in the C (nucleocapsid) gene (p22)	Previously reviewed [4], and here reviewed here
Insect-borne flavivirus (arbovirus; genus flavivirus)	Dengue (DENV) West Nile (WNV) Yellow fever (YFV) Zika (ZIKV) Tick-borne encephalitis (TBEV)	(+) ssRNA	• 5′ UTR (including RNA promoter in stem-loop A, SLA; RNA long-range interacting stem-loop B, SLB) • Structured elements in the coding region (including capsid coding region hairpin, cHP; pseudoknot C1) • 3′ UTR (including RNA long-range interacting structures) • 3′ UTR-derived ncRNA (including subgenomic flavivirus RNA, sfRNA, compromising host defense)	None published yet
Coronavirus	Severe acute respiratory syndrome coronavirus (SARS CoV)	(+) ssRNA	• Frameshifting pseudoknot (PK)	Reviewed here
Orthomyxovirus	Influenza A virus	(−) ssRNA	• RNA promoter for the viral RNA-dependent RNA polymerase (RdRp)	Previously reviewed [16], and reviewed here
Filovirus	Ebola (EBOV) Marburg (MARV)	(−) ssRNA	• RNA promoter for the viral RNA-dependent RNA	None published yet

(continued)

Table 1 (continued)

Family	Virus	Genome	RNA target	Small molecule ligands
			polymerase (RdRp) • Structured intergenic regions (IGR) of the viral genome • 5′ and 3′ UTR in viral transcripts	
Herpesvirus	Kaposi's sarcoma associated herpesvirus (KSHV)	dsDNA	• IRES in the transcript for the viral homolog of the FLICE inhibitory protein (vFLIP) • Polyadenylated nuclear (PAN) noncoding RNA	None published yet
Hepadnavirus	Hepatitis B (HBV)	ds/ssDNA	• Encapsidation signal epsilon of viral pregenomic RNA (pgRNA)	None published yet

2 Viral RNA Targets

While many RNA virus genomes and viral transcripts contain structured and conserved noncoding elements, not all RNA motifs may be accessible to selective targeting with drug-like small molecule ligands. In the following, I will discuss previously validated and new prospective RNA targets of viruses along with their structural properties.

2.1 Human Immunodeficiency Virus

The (+) ssRNA genome of HIV contains multiple regulatory elements that play key roles in transcriptional regulation, reverse transcription, viral protein translation, nucleocytoplasmic transport, genome dimerization, and virion packaging [3]. The HIV transactivation response (TAR) and Rev response (RRE) regulatory elements were among the first non-ribosomal RNA targets investigated for the discovery of small molecule inhibitors [17–23]. Other potential HIV RNA targets for small molecule ligands include the dimer initiation sequence (DIS), the packaging signal (Ψ), and the Gag/Pol frameshift site (FSS). Three-dimensional structures have been determined for all HIV RNA regulatory elements by NMR and crystallography studies (Fig. 1). Previous efforts targeting HIV RNA have been reviewed comprehensively

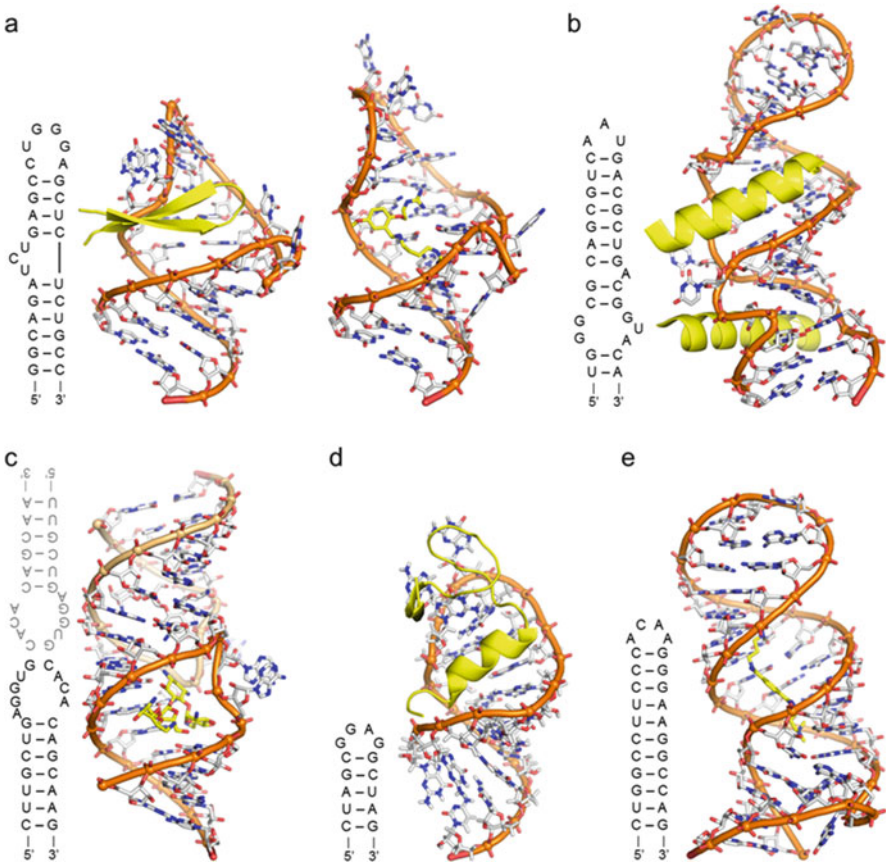

Fig. 1 Secondary and three-dimensional structures of HIV RNA elements which were previously explored as targets for small molecule ligands. Codes for atom coordinate files in the Protein Data Bank (PDB) are indicated. (**a**) The transactivation response (TAR) element in complex with a peptide mimetic of the Tat protein (PDB: 2KX5) [24] (*left*) and with a synthetic small molecule ligand (PDB: 1UUD) [25] (*right*). (**b**) Complex of the Rev response (RRE) RNA with Rev protein (PDB: 4PMI) [26]. (**c**) Kissing loop dimer of the dimer initiation sequence (DIS) in complex with the natural aminoglycoside neomycin (PDB: 2FCY) [27]. (**d**) Complex of the packaging signal (Ψ) stem-loop 3 (SL-3) with nucleocapsid protein (PDB: 1A1T) [28]. (**e**) The Gag/Pol frameshift site (FSS) in complex with a synthetic small molecule ligand (PDB: 2L94) [29]

(Table 1) [3, 10, 11, 13–15]. In Sect. 3, I will discuss more recent studies on discovery of inhibitors targeting the TAR and RRE RNA by screening and scaffold-based design.

Transcription of full-length HIV transcripts is stimulated by a complex mechanism that involves host cell factors and a complex of the viral Tat protein bound to the TAR element in the 5′ leader region of the virus genome [13]. Tat recognizes a conserved RNA stem-loop in TAR with a flexible pyrimidine-rich bulge which adopts a stable conformation in complex with the viral protein, peptide fragments, and small molecule ligands. Structures of TAR complexes determined by NMR revealed a relatively

shallow and solvent-exposed ligand binding site in the widened RNA major groove, which in case of Tat-derived peptides extends to the terminal hairpin loop (Fig. 1a). Disruption of the Tat/TAR complex by competing RNA-binding ligands, including peptides, natural products such as aminoglycosides, and synthetic small molecules, blocks HIV replication [13, 18, 23].

Similarly, the viral Rev protein–RRE RNA complex has been extensively studied as a target for HIV inhibitors [3]. The RRE sequence of ~250 bases in the second intron of the viral RNA genome adopts a complex secondary structure which contains a stem-loop (SL-IIB) that serves as the binding site for Rev. Nucleocytoplasmic export of full-length and singly spliced viral transcripts depends on Rev binding to RRE. In contrast to Tat, which recognizes TAR RNA through a beta-sheet domain (Fig. 1a), the RNA binding of Rev is mediated by an alpha helix that inserts in a widened major groove at the purine-rich internal loop of RRE SL-IIB (Fig. 1b).

The packaging signal resides in the 5′ leader of the HIV genome, downstream of the TAR element, and directs selective packaging of unspliced viral RNA as a dimer into assembling virus particles. Genome dimerization initiates through kissing loop interaction between DIS hairpins and requires in addition the packaging signal (Ψ) stem-loop 3 (SL-3) which binds the viral nucleocapsid protein (NCp7). Both, the DIS and Ψ SL-3 have been explored as targets for ligands that affect viral genome packaging. Three-dimensional structures have been determined for the DIS kissing loop dimer in complexes with aminoglycoside ligands (Fig. 1c) and the Ψ SL-3 bound to NCp7 (Fig. 1d). The aminoglycoside binding site is located in the interface region between the kissing loops and resembles the structure of the internal loop of the bacterial ribosomal decoding site (A-site). The SL-3 interacts with NCp7 in the RNA major groove and residues of the loop. Recently, NMR analysis has revealed the three-dimensional structure of a 155-nucleotide region of the viral genome 5′ leader that contains the core encapsidation signal, including the Ψ SL-3 and DIS elements [30].

The Gag/Pol FSS regulates the transition of highly expressed HIV structural proteins to enzymes expressed at low levels by a programmed −1 frameshift during translation. Ribosomal frameshifting allows to maximize the coding content of viral genomes by giving access to overlapping reading frames [31]. Frameshifting depends on two distinct RNA motifs, including a slippery sequence for the reading frame change and a downstream motif whose relatively stable secondary structure stalls the ribosome. In HIV, an RNA hairpin with a long GC-rich stem serves as the frameshift motif. Ligands binding at the HIV FSS target may disrupt or stabilize the RNA hairpin and thereby affect the equilibrium between translation of structural and enzymatically functional viral proteins. The three-dimensional structure of the FFS RNA in complex with a synthetic compound has been determined by NMR, revealing ligand binding along the major groove of the hairpin stem (Fig. 1e).

2.2 *Hepatitis C Virus*

The HCV is a member of the genus hepacivirus in the flavivirus family. HCV proteins are translated by a cap-independent mechanism under the control of an internal ribosome entry site (IRES) in the 5′ untranslated region (UTR) of the viral (+) ssRNA genome. The HCV IRES adopts a structured fold comprised of four discrete domains which play key roles in the recruitment and assembly of host cell ribosomes. An RNA internal loop motif in subdomain IIa serves as a conformational switch during translation initiation and provides the binding site for selective inhibitors of viral translation. The small molecule ligands capture an extended conformation of the RNA switch and inhibit IRES-driven translation [32]. Discovery of the IRES binding HCV translation inhibitors and studies of their mechanism-of-action have been described in a comprehensive previous review [4]. Here, I will discuss the HCV IRES target (Fig. 2a) as well as a recently described G-quadruplex target in the C (nucleocapsid) gene. Progress in the discovery and characterization of HCV translation inhibitors will be outlined in Sect. 3.

The HCV IRES element recruits ribosomes to the translation start site of the viral genome, without the involvement of most canoncial eukaryotic initiation factors. Because of this crucial role for viral propagation and the high conservation of the IRES RNA sequence in clinical isolates, this ncRNA element has been recognized early as a potential drug target [35, 36]. Among the first inhibitors of IRES-driven translation described were phenazines [37] and biaryl guanidines [38] identified by high throughput screening against reporter translation in cells. IRES binding was not revealed in these studies but structural features of the two chemical series suggest that the compounds may interact with RNA. Screening for direct binding to the viral RNA was the basis of a high-throughput mass-spectrometry approach that identified 2-aminobenzimidazoles as ligands

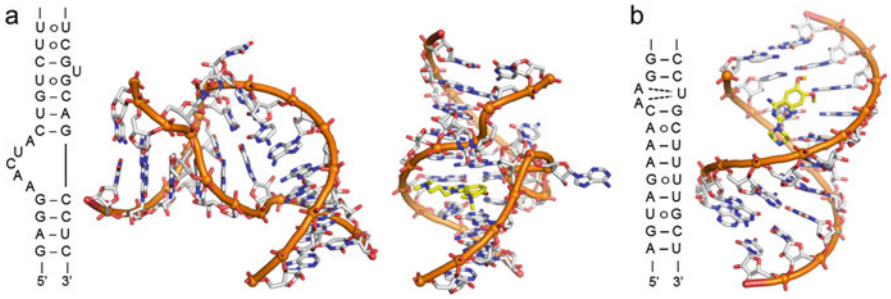

Fig. 2 Secondary and three-dimensional structures of RNA targets for small molecule inhibitors of HCV translation and influenza A virus replication. Codes for atom coordinate files in the PDB are indicated. (**a**) The HCV IRES subdomain IIa internal loop. Crystal structures have been determined for both, the free RNA and the target in complex with a benzimidazole translation inhibitor (*yellow sticks*) [33] (PDB: 1UUD) [25] (**b**) The influenza A virus RNA promoter. A three-dimensional model of a ligand–target complex was determined by NMR spectroscopy [34]. The ligand is shown in *yellow stick* representation. The added tetraloop is indicated in *grey* (PDB: 2LWK) [34]

of the subdomain IIa internal loop in the HCV IRES (Fig. 2a) [39]. Mechanism of action studies demonstrated that these compounds act as allosteric inhibitors of an RNA conformational switch [32]. Further investigations revealed that the HCV IRES subdomain IIa motif is the prototype of a new class of RNA conformational switches occurring in the IRES elements of flavi- and picornaviruses. Unlike traditional metabolite-sensing riboswitches, the viral RNA switches are structurally well-defined in both ligand-free and bound states and function as ligand-responsive, purely mechanical switches [40].

The structural signature of the IIa-like viral switches is an RNA internal loop flanked by two extended helices which adopt an overall bent conformation in the absence of bound ligand (Fig. 2a). The L-shaped fold provides a scaffold that directs the IRES subdomain IIa hairpin towards the ribosomal E site, at the interface of the small and large subunits. Crystal structure determination has provided insight into the conformational states of the HCV subdomain IIa switch in the absence [41] and presence [33] of ligands. It has been suggested that the 2-amino-benzimidazoles are fortuitous ligands of a guanosine binding site [42] which lock the subdomain IIa target in an extended conformation and thereby inhibit IRES function. In the RNA complex, the 2-aminobenzimidazole inhibitor binds in a deep solvent-excluded RNA pocket that resembles ligand interaction sites in aptamers and riboswitches (Fig. 2a) [33].

Recently, an RNA G-quadruplex (RG4) motif has been discovered in the HCV genome which may serve as a potential target for viral inhibitors [43]. A conserved guanine-rich sequence of the HCV core (C) nucleocapsid gene may transiently fold into an RG4 motif under physiological conditions. Porphyrin derivatives such as tetra-(N-methyl-4-pyridyl)porphyrin (TMPyP4) bind to the RG4 fold and stabilize the RNA motif sufficiently to inhibit viral replication and translation in HCV-infected cell culture [43]. While these findings support a potential RG4 motif in HCV as a new target for antivirals, a recent genome-wide study in yeast and human cells suggests that RG4 motifs are globally unfolded in eukaryotes, likely due to association with abundant single-stranded RNA-binding proteins [44]. However, it is conceivable that RG4-binding ligands may trap guanine-rich sequences in the quadruplex conformation and thereby affect biological processes.

2.3 Influenza A Virus

The (−) ssRNA genome of the influenza A virus contains eight protein-coding segments (vRNA) which are transcribed to mRNA and replicated to complementary sequences (cRNA). The viral replicase is an RNA-dependent RNA polymerase (RdRp) that recognizes a partial duplex motif [45] formed through hybridization of complementary sequences at the 5′ and 3′ end of each vRNA segment [46, 47]. Duplex formation between ends of segments leads to circularization of the vRNA and produces a promoter for transcription and replication [48]. NMR studies revealed the RNA promoter as an A-form duplex containing a noncanonical A•C base pair next to a uracil base that forms a bifurcated hydrogen bond interaction with two consecutive adenine residues in the opposite strand (Fig. 2b) [49]. These structural features induce widening

of the RNA major groove in the promoter helix near the polymerase initiation site and may provide a selective recognition motif for small molecule inhibitors of influenza virus RNA replication. Ligands that interfere with replication by binding to the promoter structure would provide a novel route for the development of anti-influenza drugs. In Sect. 3, I will discuss recent studies of such RNA promoter-binding ligands [34].

2.4 *Severe Respiratory Syndrome Coronavirus*

In SARS CoV, the expression of viral replicase proteins such as the RdRp involves a −1 programmed frameshift during translation of the (+) ssRNA genome. Ribosomal frameshifting maximizes the coding content of the viral genome by regulating translation of overlapping reading frames [31]. In some RNA viruses such as SARS CoV and HIV, a −1 frameshift during translation enables a transition in the production of highly expressed structural proteins to viral enzymes expressed at low levels. Ribosomal frameshifting occurs at a slippery sequence and is triggered by a downstream structured RNA motif that stalls the ribosome. The frameshift in HIV translation is triggered by a stable RNA hairpin that has been explored as a target for ligands aimed at stabilizing or disrupting the RNA fold. These earlier efforts on targeting the HIV frameshift signal have been summarized in recent reviews [3, 15]. The SARS CoV frameshift motif is an RNA pseudoknot [50] which has recently been studied as a target for small molecule ligands that inhibit ribosomal frameshifting [51, 52]. Ligand discovery efforts will be discussed in Sect. 3.

2.5 *Insect-Borne Flaviviruses*

Insect-borne flaviviruses including West Nile, Dengue, and Zika viruses contain a (+) ssRNA genome. Unlike members of the hepacivirus family (e.g., HCV), these pathogens do not rely on an IRES element for translation but employ other structured RNA motifs for translational control, replication, and host defense suppression (Table 1) [53]. Conservation and structural features of flaviviral RNA elements suggest that they might be the viable targets for selective small molecule ligands interfering with the biological function of these RNAs. The best-studied motif among such elements is the replication promoter in the 5′ UTR of the Dengue virus (DENV) genome which recruits the viral RdRp [54]. During replication initiation, the viral RdRp binds at an RNA three-way junction, designated as stem-loop A (SLA), which comprises the first 70 nucleotides of the 5′ UTR [54, 55]. Replication of the DENV genome is preceded by circularization through complementary sequences in the 5′ and 3′ regions of the UTR and ORF, similar as in the influenza A virus. However, unlike in influenza A, circularization of the DENV genome does not involve the RNA promoter motif SLA [55]. The DENV SLA was discovered by secondary structure prediction and confirmed by enzymatic as well as chemical probing [56–58]. Key

structural elements of the DENV SLA three-way junction are highly conserved in different serotypes and clinical isolates. Mutation studies demonstrated that structural and conformational integrity of the SLA is essential for the function of the RNA promoter [54, 56], which suggests that ligand binding at the RNA may achieve a similar inhibitory effect.

2.6 *Filoviruses*

The etiologic agents of Ebola and Marburg hemorrhagic fever are filoviruses which carry a (−) ssRNA genome. Similar as in the insect-borne flaviviruses and the influenza A virus, filovirus replication requires a structured RNA promoter but unlike in the former viruses the 5′ and 3′ termini of the filovirus genome do not interact during replication. Chemical probing has established an extended hairpin within the first 55 nucleotides of the filovirus genome which serves as the replication promoter in both EBOV and MARV [59, 60] and represents a potential target for small molecule inhibitors of viral replication. Other potential RNA targets in the filovirus genome occur in the long intergenic regions (IGR) which separate the reading frames for seven structural virus proteins. Specifically, the IGR between VP24 and VP30 has been proposed to adopt a two-armed stem-loop fold including an RNA four-way junction [61]. RNA hairpin motifs as potential targets in filoviruses are found at transcription start sites and the 5′ UTR of viral transcripts. A hairpin loop at the transcription start site binds the VP30 nucleocapsid protein which serves as an anti-termination factor [62, 63]. The 5′ UTR elements of viral transcripts derive from IGR and contain hairpin structures which have been proposed to regulate transcription and translation [64]. A recent bioinformatics analysis of the EBOV genome suggests that a conserved guanine-rich sequence within the L gene coding for the viral RdRp may fold into a G-quadruplex (RG4) that is stabilized by a porphyrin ligand (TMPyP4), similar to the RG4 motif discovered in the HCV core gene [65].

2.7 *Kaposi-Sarcoma Associated Herpesvirus*

Among DNA viruses that contain potential RNA targets for the development of antiviral drugs is the oncogenic Kaposi's sarcoma associated herpesvirus (KSHV) which causes malignancies in AIDS patients. KSHV-induced tumorigenesis involves the viral homolog of the FLICE inhibitory protein (vFLIP) [66]. Translation of this protein is driven by a structured RNA element that contains an IRES including a conserved segment of 252 nucleotides [67, 68]. The vFLIP IRES consists of an independently folding RNA core domain whose secondary structure has been determined by chemical probing and mutational analysis [69]. The IRES RNA, together with a series of hairpin motifs following immediately downstream, provides structurally well-defined sites for ligands that may suppress vFLIP expression. A second RNA target in KSHV for

potential antiviral intervention with small molecule ligands is a conserved hairpin motif which acts as an enhancer of nuclear retention element (ENE) [70]. The ENE is a 79-nucleotide sequence in the 3′ terminus of the 1,077-nucleotide polyadenylated nuclear (PAN) ncRNA, which is the most abundant viral transcript during lytic KSHV replication [71]. PAN is an essential component required for viral propagation whose accumulation relies on posttranscriptional stabilization dependent on the cis-acting ENE RNA motif [70]. The ENE sequesters in cis the PAN poly(A) tail in an RNA triple helix that protects the ncRNA from decay and leads to PAN accumulation [72]. The ENE hairpin, which contains a U-rich internal loop, and the ENE–poly(A) triple helix complex are potential targets for small molecule ligands that may interfere with KSHV replication.

2.8 Hepatitis B Virus

The Hepatitis B virus (HBV) contains a DNA genome that is replicated through reverse transcription of an intermediate pregenomic RNA template (pgRNA) [73]. The HBV pgRNA is sequestered together with polymerase into subviral particles prior to reverse transcription. Initiation of reverse transcription requires a conserved sequence in the 5′ terminal region of the pgRNA which is also involved in virus encapsidation. The initiation and encapsidation motif adopts a stem loop structure with a uridine-rich internal loop referred to as the epsilon encapsidation signal [74–77]. Small molecule ligands of this RNA motif have not been reported yet, but RNA decoys of the epsilon sequence have been used to sequester reverse transcriptase, thereby providing proof-of-principle that disruption of the pgRNA–polymerase interaction suppresses HBV replication [78].

3 Ligands Targeting Viral RNAs

In the following, I will discuss recent progress on the discovery and design of small molecule ligands for RNA targets from viruses including HIV, HCV, influenza A, and SARS CoV, which were outlined in Sect. 2.

3.1 Human Immunodeficiency Virus

Among the RNA targets in HIV, the TAR element has been an early and primary focus for efforts to develop ligands that disrupt binding of the viral Tat protein [13, 18, 23]. Previously reported TAR-binding inhibitor ligands include synthetic molecules, natural products, and peptides whose discovery and design have been summarized in several previous reviews [3, 10, 11, 13, 14]. In a more recent study,

small molecule microarray (SMM) screening of a TAR hairpin RNA conjugated with a fluorescent dye has been used to identify selective ligands from a library of ~20,000 drug-like immobilized synthetic molecules [79]. The thienopyridine derivative **1** (Fig. 3) was identified as a hit compound with a target affinity of 2.4 μM and anti-HIV activity in T-lymphoblasts (EC_{50} value of 12 μM). The ligand **1** represents a new and more drug-like chemotype compared to previously reported TAR binders, and lead candidates for the development of antiviral drugs may emerge from future improvement of similar thienopyridine derivatives.

In another recent effort to discover TAR-binding ligands, a fragment screen of 29 small molecules selected to represent molecular motifs beneficial for RNA recognition has been performed by applying a fluorimetric competition assay that measured ligand-induced displacement of a dye-labeled Tat peptide from a TAR complex [80]. The fragments were chosen to include hydrogen bond donors such as amines, guanidines, and amidines as well as aromatic rings to engage in stacking interactions. The most potent competitor ligands of the Tat–TAR interaction identified in the fragment screen were quinazoline derivatives (**2**; Fig. 3) which inhibited complex formation with IC_{50} values between 40 and 60 μM. Proton NMR spectroscopy confirmed the interaction of the quinazolines with the TAR RNA target as indicated by changes in imino-proton signals upon compound titration. While the ligands emerging from the fragment screening study were not tested for cellular activity, the quinazoline **2a**

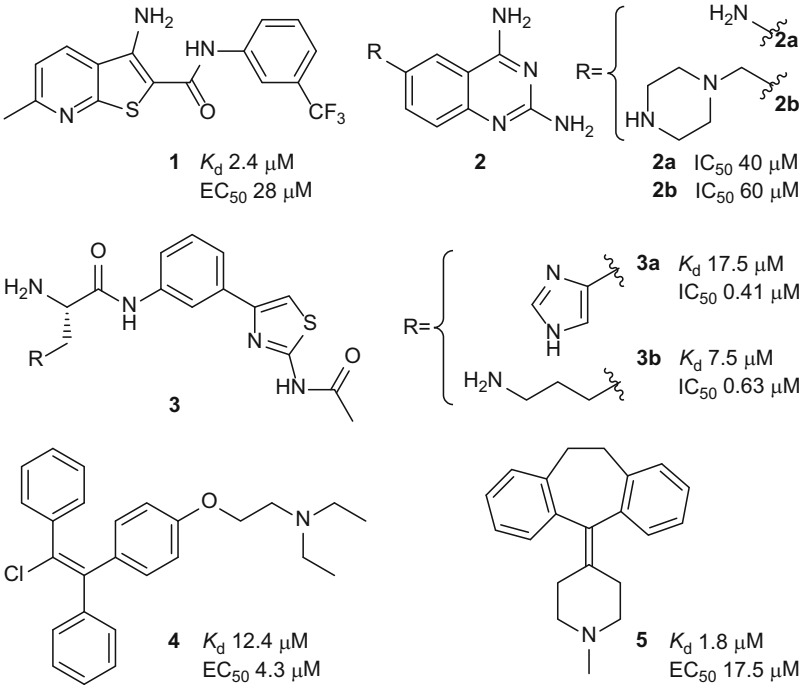

Fig. 3 Ligands for HIV TAR (**1**–**3**) and RRE (**4**, **5**) RNA targets

had previously been identified as an inhibitor of the Tat/TAR complex with biological activity to downregulate Tat transactivation in HIV-infected cells [23].

A previously reported approach of ligand discovery for the HIV TAR target focused on derivatives of amino-phenylthiazole (termed "S nucleobase") which had previously been developed as a scaffold designed to interact with A–U pairs through hydrogen bonding at the Hoogsteen edge of adenine [81, 82]. A set of 15 amino acid and dipeptide conjugates of the amino-phenylthiazole scaffold (**3**; Fig. 3) was tested for TAR target binding and antiviral activity in cell culture. While several derivatives showed binding to TAR in an assay that measured fluorescence changes upon compound titration to a terminally dye-conjugated RNA, only a histidine conjugate (**3a**; Fig. 3) was a selective ligand whose target interaction was not affected in the presence of competitor nucleic acids. A tighter binding lysine derivative (**3b**; Fig. 3) was compromised by promiscuous binding to other nucleic acids. Antiviral activity testing of the S nucleobase conjugates **3** in HIV-infected human cells resulted in IC_{50} values over tenfold lower than TAR binding affinity which suggests that these compounds may act also on targets other than TAR.

Structurally more complex ligands of TAR which have been reported recently include aminoglycoside-benzimidazole conjugates [83, 84] and nucleobase-linked aminoglycosides [85, 86] for which nanomolar affinity for the TAR RNA has been reported while antiviral activity in cells has not been tested yet.

For the HIV RRE–Rev complex target, inhibitors have mostly been explored by ligand-based design in the past, as summarized in previews reviews [10, 14], and two studies report small molecule high-throughput screens [87, 88]. However, these efforts have not resulted in confirmed inhibitors of the Rev–RRE complex that also showed antiviral activity in cells. Recent research suggests that post-transcriptional modification of HIV-1 RRE by N6-methylation of adenine bases in SL-IIB may play a key role in the activity of the RRE/Rev complex [89], indicating that authentic model systems are requisite for the study of RNA targets.

A binding competition screen for inhibitors of the RRE–Rev interaction has been used to identify inhibitors of HIV RNA biogenesis. Around 1,120 FDA-approved drugs were tested for the ability to block complex formation between the RRE SL-IIB RNA and a fluorescent dye-conjugated Rev peptide [90]. Two drugs, clomiphene and cyproheptadine (**4** and **5**; Fig. 3), were identified as inhibitors of HIV transcription that affected levels of spliced versus unspliced viral transcripts. It was shown that clomiphene (**4**), which is approved as a selective estrogen receptor modulator, bound to the RRE SL-IIB RNA with a K_d of 12.4 μM and had antiviral activity with an EC_{50} value of 4.3 μM in cells. Cyproheptadine (**5**), which is used as an antihistamine H1 receptor antagonist, bound the RRE RNA with a K_d of 1.8 μM and inhibited viral replication with an EC_{50} value of 17.5 μM. While the interaction of clomiphene (**4**) with the RRE RNA target was specific, target binding of cyproheptadine (**5**) was compromised in the presence of competitor nucleic acids. Interaction sites of the drugs **4** and **5** with the RRE target were investigated by NMR, revealing the G-rich internal loop in the lower stem of the SL-IIB RNA as the binding site. This region overlaps with the binding site of Rev, consistent with the proposed mechanism of inhibition by competition between the small molecule ligands and the viral protein. Interestingly, compounds **4** and **5** are

quite hydrophobic and lack hydrogen bond donors which suggest that a large number of heteroatom hydrogen bond donors and acceptors are not required to confer RNA targeting properties to small molecule ligands.

3.2 Hepatitis C Virus

The HCV IRES subdomain IIa RNA was identified as a target for selective inhibitors of viral translation, as outlined above in Sect. 2.2, including 2-aminobenzimidazoles (**6**–**8**; Fig. 4) and diaminopiperidines (**10**; Fig. 4). The 2-aminobenzimidazole ligands, which were initially discovered in a high-throughput mass-spectrometry approach, were optimized for target binding by using structure–activity relationship data, resulting in inhibitors such as **6** (Fig. 4) which had an affinity of 0.9 μM (K_d) for the IRES target and showed anti-HCV activity in cell culture with an EC_{50} value of 3.9 μM [39]. Mechanism of action studies demonstrated that the 2-aminobenzimidazole compounds act as allosteric inhibitors of an RNA conformational switch in the subdomain IIa [32]. A FRET-based assay was developed to test compounds for the ability to bind and lock the conformation of subdomain IIa, leading to viral translation inhibition, and thereby identifying inhibitors that capture the IRES RNA switch in an extended state [32, 91]. Crystal structure analysis of the subdomain IIa target in complex with inhibitor **6a** revealed the ligand binding in a deep solvent-excluded pocket of the RNA [33]. Structural characteristics, depth, and complexity of the ligand binding pocket suggest that drug-like inhibitors may be developed that target this RNA as selective inhibitors of HCV translation.

A different fluorescence assay, which did not rely on FRET, was used to identify diaminopiperidines (**10**; Fig. 4) as ligands of the HCV IRES which lock the RNA conformational switch in a bent state and thereby inhibit viral translation initiation [92]. An abundance of polar groups renders the diaminopiperidines hydrophilic compounds whose binding affinity for the subdomain IIa RNA decreases in the presence of salt, including physiological concentrations of sodium or magnesium [92]. The discovery, optimization, structure, and mechanism of action studies of 2-aminobenzimidazole and diaminopiperidine HCV translation inhibitors have been comprehensively reviewed recently [4].

In attempts to optimize the synthesis of 2-aminobenzimidazoles such as inhibitor **6**, which required a lengthy route to construct the pyran ring, we designed second-generation ligands for the IRES subdomain IIa target. We synthesized N1-coupled aryl derivatives (**7**) in which sterical hindrance of the aryl substituent induces a non-planar conformation of the resulting compounds [93]. To address the basicity of the 2-aminobenzimidazole ligands, which increases the overall charge of the inhibitors under physiological conditions, we replaced the imidazole ring with the less basic oxazole ring to obtain compounds such as **8a** and **8b** [94]. Neither the N1-coupled aryl benzimidazoles (**7**) [93] nor the oxazoles (**8**) [94] had an affinity for the IIa RNA target better than the original 2-aminobenzimidazoles (Fig. 4).

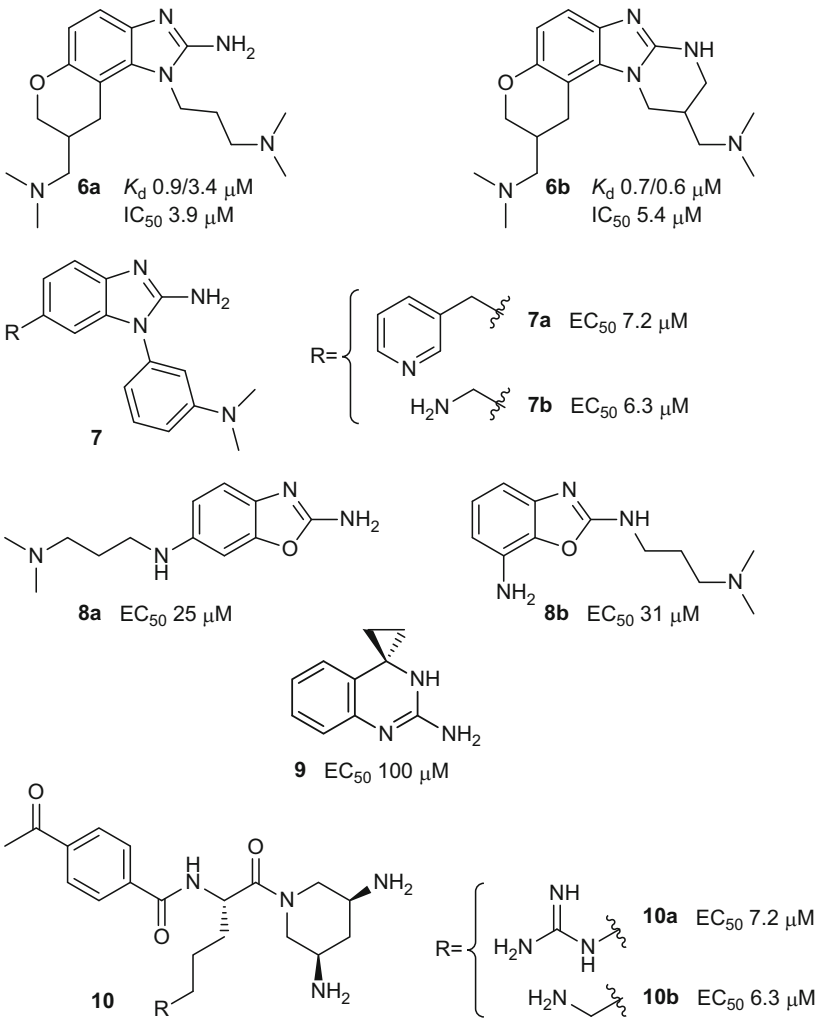

Fig. 4 Ligands for the HCV IRES subdomain IIa RNA target

Based on the finding that 2-aminobenzimidazoles are fortuitous ligands of a guanosine binding site in the subdomain IIa RNA switch, we explored amino-quinazoline derivatives as more drug-like scaffolds to develop ligands for the HCV IRES target. Closer analysis of the ligand binding site in the subdomain IIa led us to the design of the amino-quinazoline fragment **9** (Fig. 4) whose spiro-cyclopropyl modification targets a small pocket at the backside of the inhibitor interaction site [95]. While the fragment **9** showed only moderate binding affinity to the HCV IRES target, the positive impact of the hydrophobic spiro-cyclopropyl substituent on ligand binding suggests that inclusion of carefully placed nonpolar groups that improve shape complementarity is a promising strategy for optimization of compounds binding to RNA. Compared to

the 2-aminobenzimidazole and oxazole compounds **6–8**, the fragment **9** stands out for the simplicity of synthesis which is achieved in only two steps from commercial starting material, thereby allowing straightforward preparation of more potent derivatives in the future.

Ligands for the recently described putative G-quadruplex (R4G) motif in a guanine-rich sequence of the HCV core gene include the porphyrin derivative **11** (TMPyP4) [96] and the pyridostatin derivative **12** (PDP) [97] (Fig. 5) which stabilize the R4G RNA fold sufficiently to inhibit viral replication and translation [43]. Porphyrins such as **11** have been used before to target DNA G-quadruplexes, for example, in telomeric regions and the c-MYC promoter [98–100] and were recently found to stabilize an RG4 motif in the EBOV genome [65]. Efficacy studies of TMPyP4 (**11**) in rodent xenograft tumor models revealed that despite the cationic nature of the porphyrin derivative, intraperitoneal administration of the compound resulted in systemic distribution and decreased tumor growth, presumably by action on the c-MYC G-quadruplex DNA target [96, 100].

In addition to small molecule ligands of the HCV IRES, copper-binding metallopeptides have been reported recently which bind at IRES domains and are proposed to inhibit viral translation by damaging the RNA through metal-catalyzed cleavage [101–103]. The IRES-targeting metallopeptides were 7–27 amino acids in length, including a Cu-binding Gly-Gly-His motif followed by an RNA-binding sequence, and inhibited HCV in cell culture with sub-micromolar activity [101–103].

3.3 Influenza A Virus

The RNA promoter motif, which provides the initiation site for the influenza A virus replicase, has been proposed as a target for ligands that inhibit viral replication. While aminoglycoside antibiotics were shown to bind the promoter RNA with micromolar affinity, the impact of these promiscuously RNA-binding natural products on replication

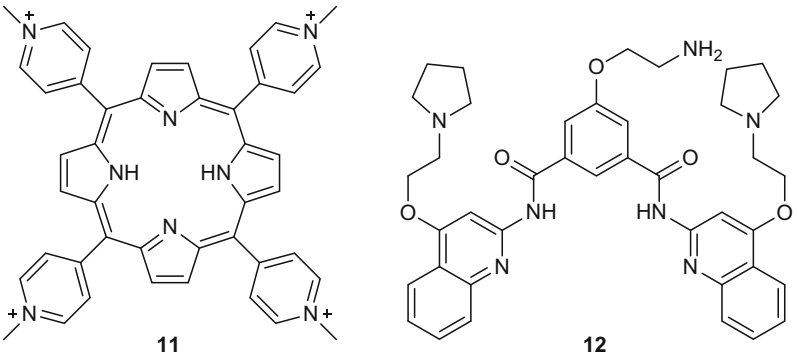

Fig. 5 Ligands for RNA G-quadruplex (RG4) targets, the porphyrin derivative TMPyP4 (**11**) and PDP (**12**)

was not reported [104]. In a recent study, NMR fragment screening of an oligonucleotide representing the RNA promoter against over 4,000 compounds identified the amino-quinazoline derivative **13a** (Fig. 6) as a selective ligand with a target affinity of 50 μM [34, 105]. Modeling of the RNA–ligand complex based on NMR NOE distance constraints suggested binding of the quinazoline ligand **13a** in the major groove at a motif including a bifurcated U < A/A motif (Fig. 2b). While the NMR model of the promoter complex shows the quinazoline **13a** interacting with the RNA target by close contacts of ligand methoxy substituents, it is not clear how much contribution to binding may be attributed to hydrogen bonds involving C–H donor groups which are weak and quite rare but not without precedent [106].

Antiviral activity of compound **13a** against different strains of influenza A was demonstrated by measuring inhibition of virus cytopathic effect, with the highest activity achieved on H1N1 with an EC_{50} value of 72 μM [34]. However, a cell-based viral replication assay returned the antiviral potency of **13a** corresponding to an EC_{50} value in the range of 430–550 μM [34, 105]. Synthesis of analogs derived from **13a** furnished compounds **13b** and **13c** which had slightly better binding affinity for the RNA promoter and improved activity as inhibitors of viral replication (Fig. 6) [105]. However, the investigators noted that direct inhibition of the viral RdRp may contribute to the antiviral activity of the quinazoline derivatives **13** [105].

3.4 *Severe Respiratory Syndrome Coronavirus*

An RNA pseudoknot in the genome of SARS CoV which triggers a −1 frameshift during translation and thereby enables the transition from production of structural proteins to viral enzymes has been proposed as a target for small molecule ligands that inhibit ribosomal frameshifting. A three-dimensional structure model of the RNA

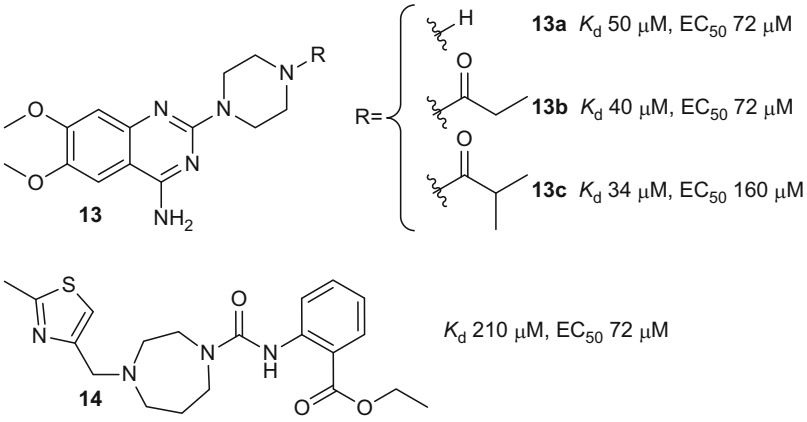

Fig. 6 Ligands of the SARS CoV frameshifting pseudoknot RNA (**13**) and the influenza A virus RNA promoter (**14**)

pseudoknot was used for in silico docking which identified the 1,4-diazepane **14** (Fig. 6) as a potential ligand [50]. Subsequent testing revealed **14** as an inhibitor of SARS CoV translational frameshifting in vitro and in virus-infected cells [50]. More recently, binding of **14** at the viral pseudoknot was confirmed by surface plasmon resonance (SPR), however with a relatively weak K_d of 210 μM [107]. Comparison of single-molecule unfolding of the SARS CoV pseudoknot RNA in the absence and presence of **14** suggested that ligand binding reduces the conformational plasticity of the RNA fold which, in turn, affects ribosomal frameshifting [108]. The ability of the RNA fold to adopt alternate conformations and structures are determinants of frameshifting efficiency rather than thermodynamic stability of the RNA fold or its impact on ribosomal pausing. Therefore, ligand-induced frameshifting modulation may only partially rely on stabilization of an RNA fold. Previously described inhibitors of HIV translational frameshifting may affect ribosome function through promiscuous RNA binding rather than by binding to the viral genomic frameshifting signal [15]. Similarly, the SARS CoV pseudoknot-binding ligand **14** may interact with other RNA targets as well, which may explain the over 450-fold higher potency of this compound as a frameshifting inhibitor in a cell-based assay [50] compared to its binding affinity for the pseudoknot RNA [107].

4 Summary

While viruses show high genetic variability, regulatory motifs in viral transcripts and RNA genomes are often conserved in clinical isolates and, therefore, may provide potential drug targets with a high barrier to resistance development. Development of small molecule inhibitors is challenging for structured RNA, however, as target drugability and ligand selectivity have to be carefully evaluated. RNA folds rarely contain deep and structurally rigid binding pockets which are the most promising targets for drug-like ligands. Among viral RNAs, such characteristics are most prominently found in the HCV IRES subdomain IIa which offers additional advantages in targeting as a switch motif whose conformational states may be affected by ligand binding in a deep RNA pocket. Similar well-defined ligand binding sites are present in bacterial riboswitches which have been explored as antibiotic targets [109, 110]. Just recently, a novel class of synthetic antibacterial compounds has been discovered, which exert their activity through an unprecedented mechanism of action that involves targeting a bacterial riboswitch involved in cofactor metabolism [111]. This success story of antibiotic discovery for a bacterial RNA target sets a promising precedent for ligand discovery directed at viral RNAs which provide future therapeutic opportunities defined by the targets' structural complexity, participation in key processes of infection as well as high conservation in the pathogens.

References

1. Hermann T (2005) Drugs targeting the ribosome. Curr Opin Struct Biol 15:355–366
2. McCoy LS, Xie Y, Tor Y (2011) Antibiotics that target protein synthesis. Wiley Interdiscip Rev RNA 2:209–232
3. Le Grice SF (2015) Targeting the HIV RNA genome: high-hanging fruit only needs a longer ladder. Curr Top Microbiol Immunol 389:147–169
4. Dibrov SM, Parsons J, Carnevali M, Zhou S, Rynearson KD, Ding K, Garcia Sega E, Brunn ND, Boerneke MA, Castaldi MP et al (2014) Hepatitis C virus translation inhibitors targeting the internal ribosomal entry site. J Med Chem 57:1694–1707
5. Gallego J, Varani G (2002) The hepatitis C virus internal ribosome-entry site: a new target for antiviral research. Biochem Soc Trans 30:140–145
6. Jubin R (2003) Targeting hepatitis C virus translation: stopping HCV where it starts. Curr Opin Investig Drugs 4:162–167
7. Hermann T, Westhof E (1998) RNA as a drug target: chemical, modelling, and evolutionary tools. Curr Opin Biotechnol 9:66–73
8. Hermann T (2000) Strategies for the design of drugs targeting RNA and RNA-protein complexes. Angew Chem Int Ed Engl 39:1890–1904
9. Gallego J, Varani G (2001) Targeting rna with small-molecule drugs: therapeutic promise and chemical challenges. Acc Chem Res 34:836–843
10. Thomas JR, Hergenrother PJ (2008) Targeting RNA with small molecules. Chem Rev 108:1171–1224
11. Guan L, Disney MD (2012) Recent advances in developing small molecules targeting RNA. ACS Chem Biol 7:73–86
12. Disney MD, Yildirim I, Childs-Disney JL (2014) Methods to enable the design of bioactive small molecules targeting RNA. Org Biomol Chem 12:1029–1039
13. Mousseau G, Mediouni S, Valente ST (2015) Targeting HIV transcription: the quest for a functional cure. Curr Top Microbiol Immunol 389:121–145
14. Blond A, Ennifar E, Tisne C, Micouin L (2014) The design of RNA binders: targeting the HIV replication cycle as a case study. ChemMedChem 9:1982–1996
15. Brakier-Gingras L, Charbonneau J, Butcher SE (2012) Targeting frameshifting in the human immunodeficiency virus. Expert Opin Ther Targets 16:249–258
16. Shortridge MD, Varani G (2015) Structure based approaches for targeting non-coding RNAs with small molecules. Curr Opin Struct Biol 30:79–88
17. Zapp ML, Stern S, Green MR (1993) Small molecules that selectively block RNA binding of HIV-1 Rev protein inhibit Rev function and viral production. Cell 74:969–978
18. Mei H-Y, Galan AA, Halim NS, Mack DP, Moreland DW, Sanders KB, Truong HN, Czarnik AW (1995) Inhibition of an HIV-1 Tat-derived peptide binding to TAR RNA by aminoglycoside antibiotics. Bioorg Med Chem Lett 5:2755–2760
19. Ratmeyer L, Zapp ML, Green MR, Vinayak R, Kumar A, Boykin DW, Wilson WD (1996) Inhibition of HIV-1 Rev-RRE interaction by diphenylfuran derivatives. Biochemistry 35:13689–13696
20. Park WKC, Auer M, Jaksche H, Wong C-H (1996) Rapid combinatorial synthesis of aminoglycoside antibiotic mimetics: use of a polyethylene glycol-linked amine and a neamine-derived aldehyde in multiple component condensation as a strategy for the discovery of new inhibitors of the HIV RNA Rev responsive element. J Am Chem Soc 118:10150–10155
21. Wang S, Huber PW, Cui M, Czarnik AW, Mei HY (1998) Binding of neomycin to the TAR element of HIV-1 RNA induces dissociation of Tat protein by an allosteric mechanism. Biochemistry 37:5549–5557
22. Mei HY, Cui M, Heldsinger A, Lemrow SM, Loo JA, Sannes-Lowery KA, Sharmeen L, Czarnik AW (1998) Inhibitors of protein-RNA complexation that target the RNA: specific recognition of human immunodeficiency virus type 1 TAR RNA by small organic molecules. Biochemistry 37:14204–14212

23. Mei HY, Mack DP, Galan AA, Halim NS, Heldsinger A, Loo JA, Moreland DW, Sannes-Lowery KA, Sharmeen L, Truong HN et al (1997) Discovery of selective, small-molecule inhibitors of RNA complexes – I. The Tat protein/TAR RNA complexes required for HIV-1 transcription. Bioorg Med Chem 5:1173–1184
24. Davidson A, Patora-Komisarska K, Robinson JA, Varani G (2011) Essential structural requirements for specific recognition of HIV TAR RNA by peptide mimetics of Tat protein. Nucleic Acids Res 39:248–256
25. Davis B, Afshar M, Varani G, Murchie AI, Karn J, Lentzen G, Drysdale M, Bower J, Potter AJ, Starkey ID et al (2004) Rational design of inhibitors of HIV-1 TAR RNA through the stabilisation of electrostatic "hot spots". J Mol Biol 336:343–356
26. Jayaraman B, Crosby DC, Homer C, Ribeiro I, Mavor D, Frankel AD (2014) RNA-directed remodeling of the HIV-1 protein Rev orchestrates assembly of the Rev-Rev response element complex. Elife 3:e04120
27. Ennifar E, Paillart JC, Bodlenner A, Walter P, Weibel JM, Aubertin AM, Pale P, Dumas P, Marquet R (2006) Targeting the dimerization initiation site of HIV-1 RNA with aminoglycosides: from crystal to cell. Nucleic Acids Res 34:2328–2339
28. De Guzman RN, Wu ZR, Stalling CC, Pappalardo L, Borer PN, Summers MF (1998) Structure of the HIV-1 nucleocapsid protein bound to the SL3 psi-RNA recognition element. Science 279:384–388
29. Marcheschi RJ, Tonelli M, Kumar A, Butcher SE (2011) Structure of the HIV-1 frameshift site RNA bound to a small molecule inhibitor of viral replication. ACS Chem Biol 6:857–864
30. Keane SC, Heng X, Lu K, Kharytonchyk S, Ramakrishnan V, Carter G, Barton S, Hosic A, Florwick A, Santos J et al (2015) RNA structure. Structure of the HIV-1 RNA packaging signal. Science 348:917–921
31. Dinman JD (2012) Mechanisms and implications of programmed translational frameshifting. Wiley Interdiscip Rev RNA 3:661–673
32. Parsons J, Castaldi MP, Dutta S, Dibrov SM, Wyles DL, Hermann T (2009) Conformational inhibition of the hepatitis C virus internal ribosome entry site RNA. Nat Chem Biol 5:823–825
33. Dibrov SM, Ding K, Brunn ND, Parker MA, Bergdahl BM, Wyles DL, Hermann T (2012) Structure of a hepatitis C virus RNA domain in complex with a translation inhibitor reveals a binding mode reminiscent of riboswitches. Proc Natl Acad Sci U S A 109:5223–5228
34. Lee MK, Bottini A, Kim M, Bardaro MF Jr, Zhang Z, Pellecchia M, Choi BS, Varani G (2014) A novel small-molecule binds to the influenza A virus RNA promoter and inhibits viral replication. Chem Commun (Camb) 50:368–370
35. Jubin R (2001) Hepatitis C IRES: translating translation into a therapeutic target. Curr Opin Mol Ther 3:278–287
36. Tan SL, Pause A, Shi Y, Sonenberg N (2002) Hepatitis C therapeutics: current status and emerging strategies. Nat Rev Drug Discov 1:867–881
37. Wang W, Preville P, Morin N, Mounir S, Cai W, Siddiqui MA (2000) Hepatitis C viral IRES inhibition by phenazine and phenazine-like molecules. Bioorg Med Chem Lett 10:1151–1154
38. Jefferson EA, Seth PP, Robinson DE, Winter DK, Miyaji A, Osgood SA, Swayze EE, Risen LM (2004) Biaryl guanidine inhibitors of in vitro HCV-IRES activity. Bioorg Med Chem Lett 14:5139–5143
39. Seth PP, Miyaji A, Jefferson EA, Sannes-Lowery KA, Osgood SA, Propp SS, Ranken R, Massire C, Sampath R, Ecker DJ et al (2005) SAR by MS: discovery of a new class of RNA-binding small molecules for the hepatitis C virus: internal ribosome entry site IIA subdomain. J Med Chem 48:7099–7102
40. Boerneke MA, Hermann T (2015) Ligand-responsive RNA mechanical switches. RNA Biol 12:780–786
41. Dibrov SM, Johnston-Cox H, Weng YH, Hermann T (2007) Functional architecture of HCV IRES domain II stabilized by divalent metal ions in the crystal and in solution. Angew Chem Int Ed Engl 46:226–229

42. Boerneke MA, Dibrov SM, Gu J, Wyles DL, Hermann T (2014) Functional conservation despite structural divergence in ligand-responsive RNA switches. Proc Natl Acad Sci U S A 111:15952–15957
43. Wang SR, Min YQ, Wang JQ, Liu CX, Fu BS, Wu F, Wu LY, Qiao ZX, Song YY, Xu GH et al (2016) A highly conserved G-rich consensus sequence in hepatitis C virus core gene represents a new anti-hepatitis C target. Sci Adv 2:e1501535
44. Guo JU, Bartel DP (2016) RNA G-quadruplexes are globally unfolded in eukaryotic cells and depleted in bacteria. Science 353
45. Pflug A, Guilligay D, Reich S, Cusack S (2014) Structure of influenza A polymerase bound to the viral RNA promoter. Nature 516:355–360
46. Fodor E, Pritlove DC, Brownlee GG (1994) The influenza virus panhandle is involved in the initiation of transcription. J Virol 68:4092–4096
47. Flick R, Neumann G, Hoffmann E, Neumeier E, Hobom G (1996) Promoter elements in the influenza vRNA terminal structure. RNA 2:1046–1057
48. Noble E, Mathews DH, Chen JL, Turner DH, Takimoto T, Kim B (2011) Biophysical analysis of influenza A virus RNA promoter at physiological temperatures. J Biol Chem 286:22965–22970
49. Bae SH, Cheong HK, Lee JH, Cheong C, Kainosho M, Choi BS (2001) Structural features of an influenza virus promoter and their implications for viral RNA synthesis. Proc Natl Acad Sci U S A 98:10602–10607
50. Park SJ, Kim YG, Park HJ (2011) Identification of RNA pseudoknot-binding ligand that inhibits the -1 ribosomal frameshifting of SARS-coronavirus by structure-based virtual screening. J Am Chem Soc 133:10094–10100
51. Plant EP, Perez-Alvarado GC, Jacobs JL, Mukhopadhyay B, Hennig M, Dinman JD (2005) A three-stemmed mRNA pseudoknot in the SARS coronavirus frameshift signal. PLoS Biol 3: e172
52. Su MC, Chang CT, Chu CH, Tsai CH, Chang KY (2005) An atypical RNA pseudoknot stimulator and an upstream attenuation signal for -1 ribosomal frameshifting of SARS coronavirus. Nucleic Acids Res 33:4265–4275
53. Villordo SM, Carballeda JM, Filomatori CV, Gamarnik AV (2016) RNA structure duplications and flavivirus host adaptation. Trends Microbiol 24(4):270–283
54. Filomatori CV, Iglesias NG, Villordo SM, Alvarez DE, Gamarnik AV (2011) RNA sequences and structures required for the recruitment and activity of the dengue virus polymerase. J Biol Chem 286:6929–6939
55. Gebhard LG, Filomatori CV, Gamarnik AV (2011) Functional RNA elements in the dengue virus genome. Viruses 3:1739–1756
56. Lodeiro MF, Filomatori CV, Gamarnik AV (2009) Structural and functional studies of the promoter element for dengue virus RNA replication. J Virol 83:993–1008
57. Sztuba-Solinska J, Le Grice SF (2014) Insights into secondary and tertiary interactions of dengue virus RNA by SHAPE. Methods Mol Biol 1138:225–239
58. Sztuba-Solinska J, Teramoto T, Rausch JW, Shapiro BA, Padmanabhan R, Le Grice SF (2013) Structural complexity of dengue virus untranslated regions: cis-acting RNA motifs and pseudoknot interactions modulating functionality of the viral genome. Nucleic Acids Res 41:5075–5089
59. Crary SM, Towner JS, Honig JE, Shoemaker TR, Nichol ST (2003) Analysis of the role of predicted RNA secondary structures in Ebola virus replication. Virology 306:210–218
60. Weik M, Enterlein S, Schlenz K, Muhlberger E (2005) The Ebola virus genomic replication promoter is bipartite and follows the rule of six. J Virol 79:10660–10671
61. Neumann G, Watanabe S, Kawaoka Y (2009) Characterization of Ebolavirus regulatory genomic regions. Virus Res 144:1–7
62. Weik M, Modrof J, Klenk HD, Becker S, Muhlberger E (2002) Ebola virus VP30-mediated transcription is regulated by RNA secondary structure formation. J Virol 76:8532–8539

63. Enterlein S, Schmidt KM, Schumann M, Conrad D, Krahling V, Olejnik J, Muhlberger E (2009) The Marburg virus 3′ noncoding region structurally and functionally differs from that of ebola virus. J Virol 83:4508–4519
64. Brauburger K, Boehmann Y, Krahling V, Muhlberger E (2015) Transcriptional regulation in Ebola virus: effects of gene border structure and regulatory elements on gene expression and polymerase scanning behavior. J Virol 90:1898–1909
65. Wang SR, Zhang QY, Wang JQ, Ge XY, Song YY, Wang YF, Li XD, Fu BS, Xu GH, Shu B et al (2016) Chemical targeting of a G-quadruplex RNA in the Ebola virus L gene. Cell Chem Biol 23:1113–1122
66. Guasparri I, Keller SA, Cesarman E (2004) KSHV vFLIP is essential for the survival of infected lymphoma cells. J Exp Med 199:993–1003
67. Bieleski L, Talbot SJ (2001) Kaposi's sarcoma-associated herpesvirus vCyclin open reading frame contains an internal ribosome entry site. J Virol 75:1864–1869
68. Bieleski L, Hindley C, Talbot SJ (2004) A polypyrimidine tract facilitates the expression of Kaposi's sarcoma-associated herpesvirus vFLIP through an internal ribosome entry site. J Gen Virol 85:615–620
69. Othman Z, Sulaiman MK, Willcocks MM, Ulryck N, Blackbourn DJ, Sargueil B, Roberts LO, Locker N (2014) Functional analysis of Kaposi's sarcoma-associated herpesvirus vFLIP expression reveals a new mode of IRES-mediated translation. RNA 20:1803–1814
70. Tycowski KT, Shu MD, Borah S, Shi M, Steitz JA (2012) Conservation of a triple-helix-forming RNA stability element in noncoding and genomic RNAs of diverse viruses. Cell Rep 2:26–32
71. Conrad NK (2016) New insights into the expression and functions of the Kaposi's sarcoma-associated herpesvirus long noncoding PAN RNA. Virus Res 212:53–63
72. Mitton-Fry RM, DeGregorio SJ, Wang J, Steitz TA, Steitz JA (2010) Poly(A) tail recognition by a viral RNA element through assembly of a triple helix. Science 330:1244–1247
73. Beck J, Nassal M (2007) Hepatitis B virus replication. World J Gastroenterol 13:48–64
74. Kramvis A, Kew MC (1998) Structure and function of the encapsidation signal of hepadnaviridae. J Viral Hepat 5:357–367
75. Jones SA, Boregowda R, Spratt TE, Hu J (2012) In vitro epsilon RNA-dependent protein priming activity of human hepatitis B virus polymerase. J Virol 86:5134–5150
76. Feng H, Chen P, Zhao F, Nassal M, Hu K (2013) Evidence for multiple distinct interactions between hepatitis B virus P protein and its cognate RNA encapsidation signal during initiation of reverse transcription. PLoS One 8:e72798
77. Cao F, Jones S, Li W, Cheng X, Hu Y, Hu J, Tavis JE (2014) Sequences in the terminal protein and reverse transcriptase domains of the hepatitis B virus polymerase contribute to RNA binding and encapsidation. J Viral Hepat 21:882–893
78. Feng H, Beck J, Nassal M, Hu KH (2011) A SELEX-screened aptamer of human hepatitis B virus RNA encapsidation signal suppresses viral replication. PLoS One 6:e27862
79. Sztuba-Solinska J, Shenoy SR, Gareiss P, Krumpe LR, Le Grice SF, O'Keefe BR, Schneekloth JS Jr (2014) Identification of biologically active, HIV TAR RNA-binding small molecules using small molecule microarrays. J Am Chem Soc 136:8402–8410
80. Zeiger M, Stark S, Kalden E, Ackermann B, Ferner J, Scheffer U, Shoja-Bazargani F, Erdel V, Schwalbe H, Gobel MW (2014) Fragment based search for small molecule inhibitors of HIV-1 Tat-TAR. Bioorg Med Chem Lett 24:5576–5580
81. Joly JP, Mata G, Eldin P, Briant L, Fontaine-Vive F, Duca M, Benhida R (2014) Artificial nucleobase-amino acid conjugates: a new class of TAR RNA binding agents. Chemistry 20:2071–2079
82. Duca M, Malnuit V, Barbault F, Benhida R (2010) Design of novel RNA ligands that bind stem-bulge HIV-1 TAR RNA. Chem Commun (Camb) 46:6162–6164
83. Ranjan N, Kumar S, Watkins D, Wang D, Appella DH, Arya DP (2013) Recognition of HIV-TAR RNA using neomycin-benzimidazole conjugates. Bioorg Med Chem Lett 23:5689–5693

84. Kumar S, Ranjan N, Kellish P, Gong C, Watkins D, Arya DP (2016) Multivalency in the recognition and antagonism of a HIV TAR RNA-TAT assembly using an aminoglycoside benzimidazole scaffold. Org Biomol Chem 14:2052–2056
85. Watanabe K, Katou T, Ikezawa Y, Yajima S, Shionoya H, Akagi T, Hamasaki K (2007) Nucleobase modified neamines, their synthesis and binding specificity for HIV TAR RNA. Nucleic Acids Symp Ser (Oxf):209–210
86. Inoue R, Watanabe K, Katou T, Ikezawa Y, Hamasaki K (2015) Nucleobase modified neamines with a lysine as a linker, their inhibition specificity for TAR-Tat derived from HIV-1. Bioorg Med Chem 23:2139–2147
87. Chapman RL, Stanley TB, Hazen R, Garvey EP (2002) Small molecule modulators of HIV Rev/Rev response element interaction identified by random screening. Antiviral Res 54:149–162
88. Shuck-Lee D, Chen FF, Willard R, Raman S, Ptak R, Hammarskjold ML, Rekosh D (2008) Heterocyclic compounds that inhibit Rev-RRE function and human immunodeficiency virus type 1 replication. Antimicrob Agents Chemother 52:3169–3179
89. Lichinchi G, Gao S, Saletore Y, Gonzalez GM, Bansal V, Wang Y, Mason CE, Rana TM (2016) Dynamics of the human and viral m6A RNA methylomes during HIV-1 infection of T cells. Nat Microbiol 1:16011
90. Prado S, Beltran M, Coiras M, Bedoya LM, Alcami J, Gallego J (2016) Bioavailable inhibitors of HIV-1 RNA biogenesis identified through a Rev-based screen. Biochem Pharmacol 107:14–28
91. Zhou S, Rynearson KD, Ding K, Brunn ND, Hermann T (2013) Screening for inhibitors of the hepatitis C virus internal ribosome entry site RNA. Bioorg Med Chem 21:6139–6144
92. Carnevali M, Parsons J, Wyles DL, Hermann T (2010) A modular approach to synthetic RNA binders of the hepatitis C virus internal ribosome entry site. Chembiochem 11:1364–1367
93. Ding K, Wang A, Boerneke MA, Dibrov SM, Hermann T (2014) Aryl-substituted aminobenzimidazoles targeting the hepatitis C virus internal ribosome entry site. Bioorg Med Chem Lett 24(14):3113–3117
94. Rynearson KD, Charrette B, Gabriel C, Moreno J, Boerneke MA, Dibrov SM, Hermann T (2014) 2-Aminobenzoxazole ligands of the hepatitis C virus internal ribosome entry site. Bioorg Med Chem Lett 24:3521–3525
95. Charrette BP, Boerneke MA, Hermann T (2016) Ligand optimization by improving shape complementarity at a hepatitis C virus RNA target. ACS Chem Biol 11(12):3263–3267
96. Grand CL, Han H, Munoz RM, Weitman S, Von Hoff DD, Hurley LH, Bearss DJ (2002) The cationic porphyrin TMPyP4 down-regulates c-MYC and human telomerase reverse transcriptase expression and inhibits tumor growth in vivo. Mol Cancer Ther 1:565–573
97. Muller S, Kumari S, Rodriguez R, Balasubramanian S (2010) Small-molecule-mediated G-quadruplex isolation from human cells. Nat Chem 2:1095–1098
98. Huppert JL (2008) Four-stranded nucleic acids: structure, function and targeting of G-quadruplexes. Chem Soc Rev 37:1375–1384
99. Parkinson GN, Ghosh R, Neidle S (2007) Structural basis for binding of porphyrin to human telomeres. Biochemistry 46:2390–2397
100. Hurley LH, Von Hoff DD, Siddiqui-Jain A, Yang D (2006) Drug targeting of the c-MYC promoter to repress gene expression via a G-quadruplex silencer element. Semin Oncol 33:498–512
101. Bradford S, Cowan JA (2012) Catalytic metallodrugs targeting HCV IRES RNA. Chem Commun (Camb) 48:3118–3120
102. Bradford SS, Ross MJ, Fidai I, Cowan JA (2014) Insight into the recognition, binding, and reactivity of catalytic metallodrugs targeting stem loop IIb of hepatitis C IRES RNA. ChemMedChem 9:1275–1285
103. Ross MJ, Bradford SS, Cowan JA (2015) Catalytic metallodrugs based on the LaR2C peptide target HCV SLIV IRES RNA. Dalton Trans 44:20972–20982
104. Kim H, Lee MK, Ko J, Park CJ, Kim M, Jeong Y, Hong S, Varani G, Choi BS (2012) Aminoglycoside antibiotics bind to the influenza A virus RNA promoter. Mol Biosyst 8:2857–2859

105. Bottini A, De SK, Wu B, Tang C, Varani G, Pellecchia M (2015) Targeting influenza A virus RNA promoter. Chem Biol Drug Des 86:663–673
106. Hermann T (2016) Small molecules targeting viral RNA. Wiley Interdiscip Rev RNA 7:726–743
107. Ritchie DB, Soong J, Sikkema WK, Woodside MT (2014) Anti-frameshifting ligand reduces the conformational plasticity of the SARS virus pseudoknot. J Am Chem Soc 136:2196–2199
108. Ritchie DB, Foster DA, Woodside MT (2012) Programmed -1 frameshifting efficiency correlates with RNA pseudoknot conformational plasticity, not resistance to mechanical unfolding. Proc Natl Acad Sci U S A 109:16167–16172
109. Blount KF, Breaker RR (2006) Riboswitches as antibacterial drug targets. Nat Biotechnol 24:1558–1564
110. Matzner D, Mayer G (2015) (Dis)similar analogues of riboswitch metabolites as antibacterial lead compounds. J Med Chem 58:3275–3286
111. Howe JA, Wang H, Fischmann TO, Balibar CJ, Xiao L, Galgoci AM, Malinverni JC, Mayhood T, Villafania A, Nahvi A et al (2015) Selective small-molecule inhibition of an RNA structural element. Nature 526:672–677