REVIEW

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Synthetic small-molecule RNA ligands: future prospects as therapeutic agents

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RNA is one of the most intriguing and promising biological targets for the discovery of innovative drugs in many pathologies and various biologically relevant RNAs that could serve as drug targets have already been identified. Among the most important ones, one can mention prokaryotic ribosomal RNA which is the target of several marketed antibiotics, viral RNAs or oncogenic microRNAs that are tightly involved in the development and progression of various cancers. Oligonucleotides are efficient and specific RNA targeting agents but suffer from poor pharmacodynamic and pharmacokinetic properties. For this reason, a number of synthetic small-molecule ligands have been identified and studied upon screening of chemical libraries or focused design of RNA binders. In this review, we report the most relevant examples of synthetic compounds bearing sufficient selectivity to envisage clinical studies and future therapeutic applications with a particular attention for the main strategies that can be undertaken toward the improvement of selectivity and biological activity.

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1. Introduction

Therapeutic targeting of biologically relevant RNAs represents an original and promising approach toward the discovery of new tools against a number of pathological processes.¹ Beyond the role of coding RNAs in transcription and translation, non-coding RNAs have pivotal functions in the control of gene expression as well as in a wide range of regulation processes² thus being particularly relevant therapeutic targets.³ The association of single-stranded regions with double-stranded ones makes these non-coding RNAs adopt upon folding a well-defined three-dimensional structure that can be targeted not only by oligonucleotides but also using selective small-molecule modulators.^{4–9} Indeed, while oligonucleotides can be very specific and efficient inhibitors of a particular RNA target upon complementary recognition of the RNA sequence, small molecules can recognize a specific three-dimensional structure and overcome known oligonucleotides drawbacks such as their poor pharmacological properties. Various classes of marketed antimicrobials stand indeed as the most successful examples of RNA-targeting small molecules.¹⁰ Compounds such as aminoglycosides, tetracyclines, macrolides or oxazolidinones, act upon binding to a particular site of the prokaryotic ribosomal RNA with various degrees of selectivity thus impairing protein synthesis in bacteria. These compounds demonstrate the feasibility of the small-molecule approach for RNA targeting even if they suffer from

a lack of specificity. Nevertheless, they remain an important source of inspiration for the design of new compounds together with the development of new approaches toward the identification of potential drugs with the ability to target specific RNAs.

Various non-coding RNAs have been considered for small-molecule targeting, in particular viral RNAs (HCV IRES, HIV-1 TAR),¹¹ nucleotide repeat expansions as in fragile X syndrome, myotonic dystrophy or spinocerebellar ataxia¹² and more recently oncogenic ncRNAs such as microRNAs.¹³ As mentioned above, all these RNAs bear a particular three-dimensional structure and small synthetic compounds are rather structure-specific than sequence-specific. It is thus important to understand the targeted structure in order to design efficient and selective ligands. However, it is somehow difficult to know the exact structure in solution as most biologically relevant RNAs are present as multiple isoforms thus rendering structural studies by NMR and/or molecular modeling difficult. For this reason, the major part of identified RNA ligands have been discovered after the screening of large libraries and the mode of binding has been studied afterwards. Recently, some new efficient strategies for the design of rational ligands bearing selectivity for a particular RNA target have also been developed thus leading to major improvements in the field.^{14,15} Clinical applications of synthetic RNA binders have never been so close illustrating that a whole new set of drug targets different from proteins could soon open whole new avenues in drug discovery.

Major reviews on small-molecule RNA ligands have been published recently.^{5–7} Most reviews report more particularly on the targeting of selected RNAs, such as microRNAs and

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viral RNAs.^{11,16,17} In this review, we have chosen to focus our attention on the small-molecule RNA ligands that have led to the most advanced results in cells as well as in animal models. This led us to perform an overview of the most promising compounds that could lead in the near future to therapeutic applications. In this context, we will describe three main classes of RNA targets, *i.e.* viral RNAs, trinucleotide repeat expansions and oncogenic microRNAs, whose study as targets for small-molecule binders has led to important milestones in the field and to promising results in intracellular assays and in animal models. Beside these three classes of RNA targets, some bacterial RNAs have been particularly studied over the years, *i.e.* prokaryotic ribosomal RNA and riboswitches.^{10,18} As mentioned above, prokaryotic ribosomal RNA is the target of various classes of marketed antimicrobial compounds and various analogs have been described in the literature to increase their activity and lower their side effects.^{5,19,20} On the other hand, riboswitches raised as another potential target for the development of new antibiotics. These are *cis* regulatory structural elements located in the 5' untranslated region (UTR) of mRNAs that specifically bind to natural compounds, such as amino acids, vitamins, glucosamine-6-phosphate, S-adenosylmethionine, thiamine pyrophosphate, ions and flavin mononucleotide, leading to the regulation of gene expression.²¹ Riboswitches are composed of two functionally distinct domains: an aptamer ligand binding domain, and an expression platform. The conformation change induced upon binding to each specific metabolite leads to the alteration of gene expression. Modified analogs of riboswitches natural ligands have thus been developed in order to selectively downregulate riboswitches in bacteria and could thus serve as novel therapeutic agents that have been studied in detail as antibiotic compounds.^{22,23} The search for new antimicrobial compounds needs to take into account specific issues such as penetration of the bacterial membranes as well as the emergence of resistance thus leading to a medicinal chemistry approach that is different from the one used for compounds targeting viral or eukaryotic targets. We thus decided to focus this review on the three RNA targets mentioned above and on the most relevant chemical candidates for future biological applications and on the medicinal chemistry approaches that led to their discovery.

2. Targeting of viral RNAs

RNA molecules play significant roles in infectious diseases, especially in the case of RNA viruses that rely on a single-stranded RNA genome. Indeed, non-coding RNAs (ncRNAs) can be found in viral genomes and transcripts and, due to their three dimensional folding structures, they represent potential targets for the development of antiviral drugs.^{11,16} The most advanced studies in the targeting of viral RNA structures by small-molecule ligands are described in the context of the human immunodeficiency virus (HIV)¹⁶ and the hepatitis C virus (HCV),¹⁷ but a few examples of RNA li-

gands targeting other RNA viruses have also been reported.^{24–28}

Human immunodeficiency virus (HIV) bases its replication on specific RNA structures and RNA/protein interactions that, being crucial for viral proliferation, represent potential therapeutic targets.^{11,16} One of the most studied HIV RNAs is the HIV-1 transactivation response (TAR) element, which binds to the Tat protein and is necessary for efficient HIV replication (Fig. 1A).²⁹ TAR RNA is a stem-loop structure that is spontaneously generated within the first 59 nucleotides of each viral transcript. Upon interaction with the viral protein Tat along with positive transcription elongation factor b (P-TEFb) composed of cyclin T1 and cyclin-dependent kinase 9 (CDK9), TAR RNA controls transcription. Tat interacts with TAR through an arginine-rich region that recognizes the bulged region of TAR. Inhibition of Tat/TAR interaction induces the inhibition of viral replication. A wide number of RNA ligands able to block this interaction have been reported¹⁶ and TAR RNA represents an invaluable model system to study ligand binding to RNA as well as a promising target for the discovery of compounds with anti-HIV activity. Even though TAR RNA target has been largely studied in the literature only few compounds actually reached intracellular assays probably because of a general lack of selectivity. In fact, most of the RNA binding agents described so far presents a highly positively charged chemical structure inherently limiting their selectivity. In the following paragraphs we will only describe the compounds that showed a selective effect in infected cells thus confirming the potential of the small-molecule approach.

Aminoglycoside antibiotics represent the main source of inspiration for the design of new RNA ligands.⁶ The scaffold of the aminoglycoside neomycin has been widely employed as a starting point for the design and synthesis of new RNA ligands since it binds to various structured RNAs thus explaining its lack of specificity for prokaryotic ribosomal RNA.⁶ This specificity issue was improved upon chemical modifications and various analogs were designed and synthesized to specifically target TAR RNA.^{30,31} Neomycin binds to three different sites on TAR RNA allosteric to the Tat binding site as suggested by ESI-MS data and ribonuclease footprint experiments.³¹ Based on these results, Arya and co-workers envisaged a series of dimeric neomycin derivatives able to bind TAR RNA on two sites different from the Tat binding site.³² Upon binding, these dimers (for example compound 1, Fig. 1B) stabilize TAR in one particular conformation (increase in the melting temperature of TAR RNA up to 10 °C) thus preventing Tat interaction. Compound 1 is an effective binder (K_D in the nM range) and an inhibitor of HIV proliferation in MT-2 infected cells with a 70% inhibition at a low micromolar concentration (9 μ M) although toxicity is observed at twice the active concentration. This work demonstrates therefore that rational design of multivalent ligands can lead to promising RNA-targeting agents.

Molecular docking has also been used to discover TAR small-molecule binders upon quantitative prediction of

Similarly, other large-scale screenings have also been used to select RNA ligands able to inhibit another crucial interaction of the HIV replication cycle: Rev/RRE. The complex between Rev protein and RRE RNA is involved in the formation of a host export complex containing mRNAs to be transcribed in viral proteins, such as Gag, Tat, Nef and Rev itself (Fig. 2A),³⁷ thus representing a promising target for RNA ligands. An example of efficient RRE binder was reported by Garvey and co-workers who performed a high-throughput scintillation proximity assay with biotinylated Rev protein and tritiated RRE RNA for the screening of 500 000 compounds.³⁸ Various hits bearing EC₅₀ in the low micromolar range were identified and several molecules were confirmed as inhibitors of the Rev/RRE interaction *in vitro*. Unfortunately, intracellular assays revealed that most compounds were cytotoxic for the host cells. The screening of 1120 small-molecule FDA-approved drugs using an assay based on the displacement of Rev from RRE also led to the identification of various compounds able to inhibit Rev–RRE complex formation.³⁹ The two most active compounds were clomiphen and cyproheptadine (5 and 6, Fig. 2B) that show K_D in the low μ M range, good selectivity in the presence of tRNA and DNA and IC₅₀ of 3.7 and 4.2 μ M, respectively, for the *in vitro* inhibition of Rev/RRE interaction. Interestingly, they hold the

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Fig. 2 A) Primary sequence and secondary structure of HIV-1 RRE RNA in complex with Rev peptide; B) chemical structure of ligands 5–7 inhibiting the proliferation of HIV-1 infected cells upon binding to RRE.

inhibitory potential in cellular assays since they can block HIV-1 gene expression and viral transcription in intracellular assays with EC_{50} of 4.3 and 4.2 μ M, respectively. A larger screening of 10 000 diverse drug-like compounds performed by the same authors allowed for the identification of benfluron (compound 7 in Fig. 2B) as an efficient inhibitor of Rev/RRE complex both

in vitro and in cells.⁴⁰ This compound blocks HIV-1 replication with a submicromolar EC_{50} of 830 nM that is 34 times below the toxic concentration.

Gag/SL3 interaction has also been proposed as a new RNA/protein target for future anti-HIV-1 therapies.⁴¹ HIV-1 Gag proteins are central players in virus particle assembly,

Fig. 3 A) Primary sequence and secondary structure of HIV-1 5'-UTR which consists of a series of stem-loops (SL1–SL4). SL3 is the binding site of Gag proteins; B) chemical structure of ligand 8 that inhibits SL4/Gag interactions; C) primary sequence and secondary structure of HIV-1 DIS loop–loop interaction; D) chemical structure of ligands 9–11 stabilizing the DIS kissing loop.

release and maturation and also function in the establishment of a productive infection.⁴² For what concerns their interaction with RNA, it occurs specifically between the nucleocapsid (NC) site of Gag and a stable helix known as SL3 of the highly conserved 5'-UTR during encapsidation of the two copies of viral RNA genome in the assembly process (Fig. 3A). Compound NSC260594 (8 in Fig. 3B) was identified from a high-throughput screening thanks to its ability to interfere with SL3/Gag interaction.⁴³ This compound is able to bind to SL3 terminal loop where Gag is known to interact and disrupts the interaction of SL3 with Gag, stabilizes SL3 and leads to reduced conformational flexibility of the wider backbone structure of the RNA. This eventually led to the specific inhibition of RNA incorporation into virions when 293T cells were treated with 50 μM of compound 8 (IC_{50} = 4.5 μM) without affecting any other step of viral replication and without toxicity. The above example clearly show that it is possible to identify diverse chemical structures able to efficiently inhibit various RNA/protein interactions intracellularly.

Dimerization of the viral genomic RNA is a key step in HIV replication. The dimerization initiation site (DIS) is a strongly conserved RNA stem-loop structure located in the 5'-non-coding region of the genome (Fig. 3C). The nine-nucleotide apical DIS loop contains a six-nucleotide self-complementary sequence that promotes genome dimerization by forming a loop-loop RNA kissing complex. Because of its functional importance for viral replication, DIS represents another attractive therapeutic target and many efforts have been devoted to structural studies. Only few small molecules were reported as efficient DIS binders with the ability to stabilize the kissing complex, except natural or modified aminoglycosides. For example, neomycin, paromomycin and lividomycin (compounds 9, 10 and 11, respectively, Fig. 3D) stabilize the kissing-loop complex by bridging the two HIV-1 RNA molecules both *in vitro* and in infected cells.⁴⁴ The X-ray structures of these aminoglycosides in complex with DIS kissing-loop have given access to useful information for the design of optimized binders. However, even if these compounds prevent dissociation of the kissing-loop complex in infected cells, they do not bear any antiviral activity probably because of poor penetration in eukaryotic cells or the fact that stabilizing DIS is not sufficient to induce the inhibition of HIV-1 replication. It therefore seems easier to identify RNA ligands that are effective in inhibiting HIV replication in cells by targeting RNA/protein interactions rather than RNA/RNA interactions. Some recent works reported new chemical structures with the ability to bind TAR RNA that could find future applications in biological studies, but further studies are necessary in order to validate the efficacy that so far has been demonstrated only during *in vitro* studies.⁴⁵

Another RNA virus that has been largely studied as a potential application for RNA binders is hepatitis C virus (HCV). HCV is a pathogenic flavivirus with a positive-sense single-stranded RNA genome whose translation is regulated by an internal ribosome entry site (IRES) in the 5' untranslated region (UTR) upstream of the open reading frame. The HCV

IRES is a highly structured ncRNA element which recruits host cell ribosomes directly at the viral start codon without the requirement for most translation initiation factors.⁴⁶ The IRES element first recruits host cell small (40S) ribosomal subunits as well as eukaryotic initiation factor 3 (eIF3), which is a large multi-protein complex required to prevent premature association of 40S and 60S ribosomal subunits. The IRES RNA is a potential target for HCV translation inhibitors due to its unique function and high conservation in clinical virus isolates. IRES contains three independently folding domains (II–IV) connected by flexible linkers (Fig. 4A). The assays for the search of IRES inhibitors are essentially based on replicons thus leading to information about both the ability of compounds to inhibit IRES-mediated translation in cells and their toxicity. Among the small molecules identified so far, phenazine-like compounds such as 12 (Fig. 4B) showed a specific efficient inhibition of IRES activity of 79 nM in HCF cells (pooled Huh-7 cells stably transfected with a molecular construct CAT-IRES-Fluciferin under a CMV promoter).⁴⁷ Another screening of about 180 000 compounds biased toward structured RNA targets led to the identification of biaryl guanidines as selective inhibitors.⁴⁸ A SAR study led to optimized compound 13 (Fig. 4B) as a 2 μM inhibitor of HCV-IRES translation. Beside screenings based on the replicon assays, studies reported on inhibitors selected for their ability to strongly bind *in vitro* a particular IRES subdomain and their inhibition activity was then confirmed in cells. Two domains have been considered for targeting with small molecules: (i) domain II that mediates the release of a key initiation factor, eIF2-GDP, from the 40S subunit to promote 80S ribosomal assembly and (ii) domain III that is essential for the interaction with the 40S ribosomal subunit.¹⁷ Griffey and co-workers employed MS-based high-throughput screening against subdomain IIA 29-mer to identify imidazole derivatives as promising IRES binders and HCV replication inhibitors. Structure-activity relationship studies led to new analogs such as 14 (Fig. 4B) bearing a K_D of 0.86 μM and an intracellular IC_{50} of 1.7 μM with minimal toxicity in a HCV replicon assay.⁴⁹ Among these series of imidazole derivatives, Hermann and co-workers chose compound 15 (Fig. 4B) for further studies about molecular mechanism of action.⁵⁰ The combination of fluorescence-based experiments on wild-type and mutated IIA RNAs revealed that 15 acts as an HCV-specific translation inhibitor by conformational induction of a widened RNA interhelical angle at the IRES subdomain IIA. The μM affinities observed *in vitro* correlated well with μM intracellular activities. The 3D structure of IIA in complex with 15 was also determined at 2.2 Å resolution revealing that RNA undergoes a dramatic ligand-induced conformational adaptation to form a deep pocket that resembles the substrate binding sites in riboswitches.⁵¹ The same authors also studied a series of 3,5-diaminopiperidine heterocycles as IIA ligands and discovered μM binders such as 16 (Fig. 4B) bearing selective activity in cells based HCV replicon assay.⁵² Compared to benzimidazole inhibitors, that induced a widened interhelical angle in the subdomain IIA

Fig. 4 A) Primary sequence and secondary structure of HCV IRES RNA; B) chemical structure of ligands **12–16** targeting various sites of IRES and inhibiting viral transcription.

leading to inhibition of IRES-driven translation, these modular ligands affect HCV translation by arresting subdomain IIA in a 90° bent state thereby interfering with translation initiation.

HIV and HCV studies therefore validate the RNA-targeting approach and assess its feasibility and limitations in the anti-viral therapy context. Consequently, in recent years, a few other RNA viruses have been investigated.^{24–28} As an example, in influenza A viruses, the 5′-end and the 3′-end of each RNA segment are highly conserved and fold to form a partial duplex which is recognized by the RNA-dependent RNA polymerase (RdRp) and referred to as the promoter.²⁷ This latter represents an attractive target for anti-influenza drug development. A NMR-based fragment screening revealed that 6,7-dimethoxy-2-(1-piperazinyl)-4-quinazolinamine (DPQ) is able to bind to this RNA structure ($K_D = 50.5 \mu\text{M}$) and to inhibit viral replication in MDCK infected cells with EC_{50} between 71 and 275 μM depending on the viral strain.²⁵ Additional screenings and SAR studies led to improved cellular activity in the micromolar range.²⁴

Altogether, these works show that discovery of new anti-viral strategies based on the use of RNA ligands is challenging but still presents a great interest since several RNA targets would be suitable for efficient therapies. The new insights in the field of RNA targeting by small molecules that have been gained over the years and that will be described in the following sections, will undoubtedly enrich the arsenal of available binders and contribute to new discoveries in the search for innovative antiviral compounds for emerging and/or incurable viral infections.

3. Targeting of RNA nucleotide repeats

Expanded RNA repeats have been identified as the cause of various diseases and have then been exploited as potential targets of small-molecule RNA ligands for potential therapeutic applications.⁵³ To date, over 25 human genes with tandem repeat expansions have been identified. These disease-causing repeats can be located in 5′ and 3′ untranslated regions (UTRs) such as in fragile X-associated tremor ataxia syndrome (FXTAS) and myotonic dystrophy type 1 (DM1), in introns such as in spinocerebellar ataxia type 10 (SCA10) and myotonic dystrophy type 2 (DM2) or in coding regions such as in Huntington's disease (HD). Even if the molecular causes of these disorders are still not entirely elucidated, it is now clear that expanded repeats in RNA transcripts can cause cellular toxicity and neurodegeneration by altering the splicing machinery and leading to the production of aberrant proteins. Indeed, the RNA nucleotide repeats sequester splicing proteins and the subsequent splicing defects cause the perturbation of numerous cellular functions. The discovery of small molecules that target these RNAs thus blocking their effects on splicing factors would inhibit the related dysfunctions and could represent a promising therapeutic approach. The majority of reported examples of small molecules targeting this kind of RNA structures have been designed against DM1 and DM2. In DM1, the expansion is an rCUG triplet repeat ($\text{r(CUG)}^{\text{exp}}$) located in the 3′ untranslated region of the dystrophin myotonia protein kinase (*DMPK*) gene, whereas in DM2 expansion is an rCCUG ($\text{r(CCUG)}^{\text{exp}}$)

quadruplet repeat in intron 1 of the zinc finger 9 protein (*ZNF9*) gene (Fig. 5A). Both RNAs fold into hairpin structures with periodically repeating internal loops separated by two 5' GC/3'CG base pairs. The sizes of the loops, however, are different: the DM1 repeat forms 1×1 nucleotide UU loops while the DM2 repeat forms 2×2 nucleotide 5'CU/3'UC loops. Even though these mutations are present in functionally unrelated genes, DM1 and DM2 have similar disease manifestations and share a common biochemical mechanism: sequestration of the essential splicing regulator Muscleblind-like 1 protein (MBNL1) by expanded RNA repeats. This understanding of the biochemical causes of DMs led to the development of approaches aimed at the targeting of RNA repeats in order to inhibit the formation of MBNL1 complex with these RNAs. First reports about small molecules as specific nucleotide repeats binders described modularly assembled ligands as par-

ticularly suited for this aim. As an example, the aminoglycoside derivative 6'-N-5-hexynoate kanamycin A (Fig. 5B), that was identified as a tight binder of pyrimidine-rich internal loops like those formed in DM1 and DM2 associated repeats,⁵⁴ was employed for the assembling of multiple residues onto a peptoid backbone such as in 17 (Fig. 5B). This kind of compound gave very promising results in terms of activity with IC₅₀ in the low nanomolar range *in vitro* for the inhibition of MBNL1/rCUG repeat interaction. The spacing of ligand modules has been precisely tuned in order to increase the selectivity for the targeting of rCUG repeats over other kind of repeats.⁵⁵ The main issue of these modular ligands is their poor cell permeability that strongly limits their intracellular and *in vivo* applications. Addition of a D-Arg₉ (DR9) transporter conjugated to these compounds as in 18 led to a second generation of modularly assembled compounds

Fig. 5 A) r(CUG)^{exp} and r(CUUG)^{exp} found in DM1 and DM2, respectively; B) chemical structure of 6'-N-5-hexynoate kanamycin A and of ligands 17–22 selective for RNA nucleotide repeats.

effective in cell culture and animal models of DM1 thanks to a strong improvement of cellular penetration.⁵⁶ Compound **18** shows *in vitro* IC₅₀ for the disruption of r(CUG)10–MBNL1 complex of 240 nM, and was able to improve the MD1-associated defects in cells at micromolar concentrations. A partial rescue of splicing was also observed in a mouse model of DM1.⁵⁶ These kanamycin A derivatives were also modulated upon modification of the number of modules for a selectivity toward DM2 *versus* DM1 repeats.^{57,58} Taking advantage of these discoveries, and to overcome cell permeability limitations of these multivalent compounds, an *in situ* click chemistry approach based on the Huisgen 1,3-dipolar cycloaddition reaction was developed in order to take advantage of the better permeabilities of small modules and of the ability of the target to catalyze the synthesis of the multivalent inhibitor inside cells.⁵⁹ Cells treatment with equimolar azido- and alkyne-containing kanamycin moieties led to the formation of dimeric and trimeric reaction products specifically in DM2-affected cells and with desired intracellular effects at nM concentrations.

Structures less polycharged than aminoglycoside derivatives were also identified as inhibitors of rCUG–MBNL1 interaction, such as peptides^{60,61} as well as aromatic and hetero-aromatic moieties (Hoechst derivatives, acridine–triazole conjugates or pentamidine)^{62–64} Hoechst 33258 and pentamidine are interesting lead ligands suitable for a chemical similarity searching approach. This kind of methodology was thus applied to identify small molecules that are similar in shape and/or the positioning of functional groups and study these analogs for their ability to bind rCUG repeats and inhibit MD1-associated defects.⁶⁵ This study led for example to the identification of Hoechst derivative **19** (Fig. 5B) as a potential hit. This compound was indeed the strongest binder of rCUG repeats with *K_D* of 70 nM and the ability to inhibit MBNL1/rCUG complex formation in the low micromolar range. However, intracellular activity revealed to be in the mM range probably because of membrane permeability or selectivity issues. Other Hoechst derivatives were then employed for the preparation of modularly assembled compounds as previously described for kanamycin A thus leading to compounds such as **20** that were able to improve all effects induced by trinucleotide repeats such as alternative splicing defects, translational defects and disruption formation of nuclear foci in cells.⁶⁶ Further studies on this series of compounds revealed that the nature of the scaffold significantly affects potency and bioactivity. In particular, polyamines, α -peptides, β -peptides and peptide-tertiary amides (PTAs) were employed for the preparation of modular ligands thus revealing that among the used scaffolds, PTA is optimal for intracellular activity, cellular permeability, stability against proteases and toxicity.⁶⁷ The addition of an electrophilic nucleic acid-reactive module, such as chlorambucil, strongly improved the potency of **20** through the formation of a covalent interaction with the target.⁶⁸ This adduct formation enabled the identification of cellular RNA targets and the mutant DMPK allele containing r(CUG)^{exp} was selectively

targeted. Attachment of a cross-linker thus rendered the RNA binder more potent upon forming a covalent linkage. Further addition of a cleavage domain, such as bleomycin, which is known to induce RNA cleavage *in vitro*, led to reduced DMPK mRNA levels and enabled target profiling in cells. An on-site probe synthesis approach was also developed in order to react small molecules bound to adjacent sites in r(CUG)^{exp}. This afforded picomolar inhibitors *via* a proximity-based click reaction only in DM1-affected cells.⁶⁹

Zimmerman and co-workers also identified compound **21** as a binder of CUG repeats and a selective inhibitor of CUG–MBNL1 interaction *in vitro*.⁷⁰ This compound was rationally designed based on the X-ray structure of a short CUG sequence and is composed of a well-known acridine DNA intercalator and a triaminotriazine unit to recognize U–U through Janus-wedge hydrogen bonding.⁷¹ Thus, the two edges of the triazine heterocycle have the potential to form simultaneously a full set of hydrogen bonds with the poorly paired uracil bases, while stacking of the triaminotriazine and acridine units decreases non-specific, intercalative binding to duplex RNA. Unfortunately, no activity was observed in cells both because of its low water solubility and its inability to penetrate the cell membrane.⁶² The conjugation of **21** to a cationic polyamine demonstrated to be favorable for cellular permeation and intracellular activity leading to *in vitro* *K_D* of 5.2 nM and IC₅₀ of 15 μ M as well as to intracellular activities in the high micromolar range.⁷⁰ A structure-based approach was also used to identify efficient binders of r(CUG)^{exp} leading to the discovery of a novel class of groove-binding ligands carrying two triaminotriazine units such as ligand **22**.⁷² Similarly to **21**, this compound showed *in vitro* IC₅₀ in the low micromolar range and *K_D* in the low nanomolar range, but the concentrations needed to observe an intracellular effect were much higher (over 100 μ M). Nakatani and co-workers also reported 2,9-diaminoalkyl-1,10-phenanthroline (DAP, compound **23** in Fig. 5B) as an efficient CUG-binding compound. They thus developed its dimeric form (DDAP, compound **24** in Fig. 5B) and demonstrated that it was able to bind r(CUG)^{exp} with nanomolar affinities and intracellular efficacy in rescuing splicing defects induced by these expanded repeats with micromolar activities. *In vivo* activity in a DM1-mouse model was also observed even if it is clear that activity and selectivity should be improved in order to reach a satisfying therapeutic effect.⁷³

Beside DM1 and DM2 trinucleotide repeats, the rCGG repeats involved in fragile X-associated tremor ataxia syndrome (FXTAS) have been explored as targets for small molecules (Fig. 6A). FXTAS is caused by an RNA gain of function since the sequestration of splicing proteins by expanded repeats induce the aberrant splicing of a variety of pre-mRNAs leading to the expression of defective proteins. A high-throughput screening assay was developed to identify inhibitors of the interaction between r(CGG)₁₂ and DCGR8D protein involved in the regulation of pre-mRNA splicing.⁷⁴ This assay led to identification of various compounds with low micromolar activities in particular compound **25** (Fig. 6B) that bears an

Disney and co-workers recently focused on the search for small molecules able to bind selectively to AU base pairs in order to target the expanded r(AUUCU) repeat that causes SCA10, an incurable neuromuscular disorder. These repeats contain stretches of AU base pairs, in particular repeating 5' AU/3'UA pair steps (Fig. 6C). After screening a set of 104 compounds selected using chemical similarity searching for small molecules that should bind RNA internal loops, the authors identified benzamidine compound 27 (Fig. 6D) as a selective binder of AU pairs and of r(AUUCU)^{exp} repeats with a 5–15 fold selectivity over other repeats.⁷⁷ This compound bears a K_D of 300 nM and a binding stoichiometry of 11 : 1 suggesting that a modular strategy similar to the one previously described for r(CUG)^{exp} targeting could apply also in this case. A modular derivative of 27 was thus prepared using a peptoid scaffold leading to 28 (Fig. 6D) as the most efficient compound. Indeed, 28 significantly improves SCA10-associated

In conclusion, high-throughput screenings (HTS), chemical similarity searches as well as rational design led to promising compounds able to target RNA repeat expansions efficiently and selectively. Major intracellular effects are difficult to reach mainly because the compounds bearing the best biological activity *in vitro* reveal to have issues of solubility or cellular penetration, but also because binding to these repeats could not be sufficient for a relevant biological effect. A lack of specificity could also be responsible for the high concentrations that are sometimes needed to have a satisfying biological effect. However, the small-molecule approach for the targeting of RNA repeat expansions remains an extremely promising strategy for the development of new drugs against these incurable pathologies.

The third major target in the field of biologically relevant RNAs is represented by the class of small non-coding RNAs called microRNAs that are involved in the regulation of gene expression.⁷⁸ These short RNAs are constituted of about 22–24 nucleotides and are ubiquitously present in eukaryotic cells. Their biosynthesis starts from the transcription of miRNAs genes into primary miRNAs (pri-miRNAs) constituted of various kilobases.⁷⁹ After ribonuclease processing by Drosha enzyme, pri-miRNAs are converted into shorter pre-miRNAs of about 70 nucleotides. These precursors are then moved to the cytoplasm where a second ribonuclease called

Dicer leads to the formation of a miRNA duplex. After unwinding, this latter forms a multiprotein complex called miRISC able to recognize various mRNAs and leading to the inhibition of mRNA translation into proteins.⁸⁰ This physiological mechanism is essential for the regulation of protein synthesis and cellular homeostasis, but it can be affected by modification in the expression of miRNAs that can be overexpressed or underexpressed compared to healthy cells. These deregulations processes have been linked to a wide number of pathologies such as cardiovascular diseases, neurological pathologies or cancers.⁸¹ It is thus clear that restoring physiological expression of deregulated miRNAs by increasing the underexpressed ones or inhibiting the overexpressed ones would lead to promising therapeutic strategies.¹ For this reason, various approaches applying small molecules have been developed and are aimed at targeting more specifically the biogenesis of deregulated miRNAs, *i.e.* inhibiting their transcription from the miRNA gene or the processing of pri- and pre-miRNAs by Drosha and Dicer ribonucleases, instead of the miRNA itself.⁷ More recently, compounds interacting with the RNA/protein complexes along miRNAs pathways (such as Lin28 interactions) have been described and are promising tools for future therapeutic applications. Most of these works have been devoted to the inhibition of oncogenic miRNAs or to the induction of production of tumor suppressors miRNAs. Various reviews have recently covered these ever-increasing reports about

miRNAs targeting using small molecules.^{4,7,82} Here, we selected the most recent and advanced examples as performed in previous sections concerning targeting of viral RNAs and of trinucleotide repeats.

The seminal works about the identification of small molecules interfering with the production of disease-related miRNAs were based on high-throughput screening of large compounds libraries. In this context, Deiters and co-workers reported a screening against the widely distributed oncogenic miR-21 and against miR-122 which is tumor suppressor in hepatocellular carcinoma.⁸³ This led to the identification of compounds 29–31 (Fig. 7) able to inhibit miR-21 and miR-122 production in cells with EC₅₀ in the low μ M range. Among the most advanced examples of oncogenic miRNAs inhibitors, one should mention AC1MMYR2 (compound 32 in Fig. 7) that is able to specifically inhibit miR-21 expression in cancer cells leading to antitumor effect *in vivo* in both glioblastoma and breast cancer orthotopic models.^{84–86}

We recently designed new RNA ligands to inhibit Dicer-mediated processing of oncogenic miRNAs and these compounds showed promising biological activity in cells.^{87–90} The targeted miRNAs were miR-372 and miR-373 involved in the development of various cancers such as gastric cancers. The designed compounds were based on the conjugation of artificial nucleobases able to selectively interact with RNA base pairs such as nucleobase S in compound 33 (Fig. 7) with aminoglycoside neomycin that is known to interact with

Fig. 7 Chemical structure of ligands 27–34 that represents promising inhibitors of oncogenic miRNAs biogenesis upon binding to miRNAs precursors (pri-miRNAs and pre-miRNAs).

various RNAs with strong affinity. The combination of these RNA binding motifs allowed the inhibition of miR-372 processing *in vitro* and in cells with IC₅₀s in the low micromolar range. The specific inhibition of proliferation of gastric cancer cells overexpressing targeted miRNAs was also observed. The study of the site of interaction of the most active compounds on the targeted miRNA allowed the design of optimized compounds *via* the addition of a third RNA binding domain represented by basic amino acids. Another screening for efficient inhibitors of the same oncogenic miRNA led to the identification of functionalized polyamines, such as compound 34 (Fig. 7) as promising structures for intracellular inhibition of oncogenic miRNAs.⁹¹

Very efficient compounds inhibiting oncogenic miRNA biogenesis in a specific manner were also reported by Disney and co-workers who developed a lead identification approach called Inforna as a strategy to identify such compounds.^{92,93} First, they developed a library *vs.* library approach where a large set of small-molecule RNA binders immobilized on microarrays are screened against a large number of RNA motifs, such as bulges or internal loops.⁹⁴ This 2-dimensional combinatorial screening (2-DCS) was then combined with structure–activity relationships through sequencing (StARTS) approach that is a statistical method allowing for anticipating the affinity and selectivity of members of a RNA library thus scoring binding interactions.⁹⁵ This led to the identification of the RNA motifs that are most suitable targets and of the small molecules that are the most probable specific binders. This lead identification strategy was called Inforna and allowed for the identification of various compounds that bear extremely specific activities in cells and in animal models against a particular miRNA.^{96–98} One of the most successful examples is targaprimir-96 (compound 35, Fig. 7) that binds with low nanomolar K_D to the target miR-96 hairpin precursor and shows IC₅₀ of about 50 nM in intracellular studies. This compound can also inhibit tumor growth in animals treated with 10 mg kg⁻¹ by IP injection and this inhibition was correlated to the silencing of miR-96 and the restoration of its protein target expression (FOXO1).⁹⁷ Similarly, targapremir-210 (compound 36, Fig. 7) was also discovered as a compound able to inhibit the production of miR-210, a central regulator of the hypoxic response that affects expression of hypoxia inducible factors (HIFs) in solid tumor masses. MiR-210 represses levels of the glycerol phosphate dehydrogenase 1-like (GPD1L) enzyme. Targapremir-210 binds pre-miR-210 Dicer processing site and modulates the miR-210 hypoxic circuit in triple negative breast cancer cells and in a mouse xenograft model in a very specific manner.⁹⁹

Altogether, these intracellular and *in vivo* successes demonstrate that the biogenesis of microRNAs is a viable target for small molecules and these studies hold the promise for a therapeutic perspective in the near future. In the same way as for other medicinal chemistry programs aimed at protein targeting, innovative approaches, such as small molecule induced cleavage of the target can be envisaged and are already becoming a reality.

5. The future of RNA ligands: development of precise tools for the manipulation of protein/RNA interactions

Even if the development of small molecule therapeutics that act by targeting defined RNA sequences associated with human diseases remains a challenge for medicinal chemists and chemical biologists, some new drug candidates, beside the marketed antibiotics targeting prokaryotic ribosome, are already raising. The pyrido-pyrimidinone RG-7916 (compound 37, Fig. 8) is a very advanced clinical candidate already in phase II clinical trials for various types of spinal muscular atrophy (SMA).¹⁰⁰ SMA is the leading genetic cause of infant mortality and is caused by deletion or mutation of the survival of motor neuron 1 (SMN1) gene which encodes the SMN protein. This causes a progressive degeneration of spinal motoneurons leading to muscle weakness and atrophy. Humans carry two nearly identical copies of the SMN genes that are SMN1 and SMN2. It was demonstrated that regulation of SMN2 exon 7 splicing is one of the best alternative splicing processes leading to the increase in the expression of the SMN protein. In 2014, the screening for small molecules able to increase SMN levels upon modulation of SMN2 pre-mRNA splicing to include exon 7 led to the identification of SMN-C class of compounds, such as SMN-C2 (compound 38, Fig. 8).¹⁰⁰ These compounds were orally available and increased the production of SMN protein in different cell types and across SMA disease severities with nM activities as well as in mouse models increasing life span and preventing motor dysfunction and neuromuscular deficits. This class of compounds acts *via* interaction with a tertiary RNA structure in the mRNA–protein complex in a selective manner in particular by binding a region of exon 7 and a RNA helix formed by the 5'-ss in intron 7 and the 5' terminus of U1 snRNA, thus increasing the interaction with U1 snRNP.¹⁰¹ Detailed mechanistic studies on this class of RNA binders revealed that they directly bind to the AGGAAG motif on exon 7 of the SMN2 pre-mRNA and promote a conformational change both in cells and *in vivo*. These successful results in terms of innovative therapeutic applications for RNA binders further confirm that nucleic acid targeted molecules may have a promising future in the modulation of disease processes involving RNA deregulations, such as in this particular case pre-mRNA splicing.¹⁰²

Fig. 8 Chemical structure of compound RG-7916 (35) and SMN-C2 (36).

Future of RNA ligands could thus rely on a better understanding of small molecules/RNA/protein interactions toward the precise modulation of RNA functions. A recent work reported small molecules that recruit ribonuclease (RNases) that naturally regulate RNA lifetime to specific transcripts thus leading to the first examples of ribonuclease targeting chimeras or RIBOTACs.¹⁰³ For the preparation of such tool, compound 33 that selectively binds the oncogenic miR-96 precursor was appended with a short 2'-5' poly(A) oligonucleotide recruiting endogenously the latent ribonuclease (RNase L) which selectively cleaved the miR-96 precursor in cancer cells in a catalytic and substoichiometric way. Similarly to proteolysis targeting chimeras (PROTACs), this approach will largely broaden the view of RNA as a viable target in drug discovery.

Conflicts of interest

The authors declare no conflict of interests.

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References

- 1 M. Matsui and D. R. Corey, *Nat. Rev. Drug Discovery*, 2017, **16**, 167–179.
- 2 K. V. Morris and J. S. Mattick, *Nat. Rev. Genet.*, 2014, **15**, 423–437.
- 3 M. Esteller, *Nat. Rev. Genet.*, 2011, **12**, 861–874.
- 4 A. Donlic and A. E. Hargrove, *Wiley Interdiscip. Rev.: RNA*, 2018, **9**, e1477.
- 5 L. Guan and M. D. Disney, *ACS Chem. Biol.*, 2012, **7**, 73–86.
- 6 J. R. Thomas and P. J. Hergenrother, *Chem. Rev.*, 2008, **108**, 1171–1224.
- 7 K. D. Warner, C. E. Hajdin and K. M. Weeks, *Nat. Rev. Drug Discovery*, 2018, **17**, 547–558.
- 8 C. M. Connelly, M. H. Moon and J. S. Schneekloth, Jr., *Cell Chem. Biol.*, 2016, **23**, 1077–1090.
- 9 M. D. Shortridge and G. Varani, *Curr. Opin. Struct. Biol.*, 2015, **30**, 79–88.
- 10 D. N. Wilson, *Nat. Rev. Microbiol.*, 2014, **12**, 35–48.
- 11 T. Hermann, *Wiley Interdiscip. Rev.: RNA*, 2016, **7**, 726–743.
- 12 A. Lopez Castel, J. D. Cleary and C. E. Pearson, *Nat. Rev. Mol. Cell Biol.*, 2010, **11**, 165–170.
- 13 H. Ling, M. Fabbri and G. A. Calin, *Nat. Rev. Drug Discovery*, 2013, **12**, 847–865.
- 14 S. P. Velagapudi, Y. Luo, T. Tran, H. S. Haniff, Y. Nakai, M. Fallahi, G. J. Martinez, J. L. Childs-Disney and M. D. Disney, *ACS Cent. Sci.*, 2017, **3**, 205–216.
- 15 S. P. Velagapudi, B. R. Vummidi and M. D. Disney, *Curr. Opin. Chem. Biol.*, 2015, **24**, 97–103.
- 16 A. Blond, E. Ennifar, C. Tisne and L. Micouin, *ChemMedChem*, 2014, **9**, 1982–1996.
- 17 S. M. Dibrov, J. Parsons, M. Carnevali, S. Zhou, K. D. Rynearson, K. Ding, E. Garcia Segal, N. D. Brunn, M. A. Boerneke, M. P. Castaldi and T. Hermann, *J. Med. Chem.*, 2014, **57**, 1694–1707.
- 18 P. J. McCown, K. A. Corbino, S. Stav, M. E. Sherlock and R. R. Breaker, *RNA*, 2017, **23**, 995–1011.
- 19 K. Bodoor, V. Boyapati, V. Gopu, M. Boisdore, K. Allam, J. Miller, W. D. Treleaven, T. Weldeghiorghis and F. Aboul-ela, *J. Med. Chem.*, 2009, **52**, 3753–3761.
- 20 I. A. Katsoulis, G. Kythreoti, A. Papakyriakou, K. Koltsida, P. Anastasopoulou, C. I. Stathakis, I. Mavridis, T. Cottin, E. Saridakis and D. Vourloumis, *ChemBioChem*, 2011, **12**, 1188–1192.
- 21 K. E. Deigan and A. R. Ferre-D'Amare, *Acc. Chem. Res.*, 2011, **44**, 1329–1338.
- 22 J. A. Howe, H. Wang, T. O. Fischmann, C. J. Balibar, L. Xiao, A. M. Galgoci, J. C. Malinverni, T. Mayhood, A. Villafania, A. Nahvi, N. Murgolo, C. M. Barbieri, P. A. Mann, D. Carr, E. Xia, P. Zuck, D. Riley, R. E. Painter, S. S. Walker, B. Sherborne, R. de Jesus, W. Pan, M. A. Plotkin, J. Wu, D. Rindgen, J. Cummings, C. G. Garlisi, R. Zhang, P. R. Sheth, C. J. Gill, H. Tang and T. Roemer, *Nature*, 2015, **526**, 672–677.
- 23 N. F. Rizvi, J. A. Howe, A. Nahvi, D. J. Klein, T. O. Fischmann, H. Y. Kim, M. A. McCoy, S. S. Walker, A. Hruza, M. P. Richards, C. Chamberlin, P. Saradjian, M. T. Butko, G. Mercado, J. Burchard, C. Strickland, P. J. Dandliker, G. F. Smith and E. B. Nickbarg, *ACS Chem. Biol.*, 2018, **13**, 820–831.
- 24 A. Bottini, S. K. De, B. Wu, C. Tang, G. Varani and M. Pellecchia, *Chem. Biol. Drug Des.*, 2015, **86**, 663–673.
- 25 M. K. Lee, A. Bottini, M. Kim, M. F. Bardaro, Jr., Z. Zhang, M. Pellecchia, B. S. Choi and G. Varani, *Chem. Commun.*, 2014, **50**, 368–370.
- 26 S. J. Park, Y. G. Kim and H. J. Park, *J. Am. Chem. Soc.*, 2011, **133**, 10094–10100.
- 27 A. Pflug, D. Guilligay, S. Reich and S. Cusack, *Nature*, 2014, **516**, 355–360.
- 28 D. B. Ritchie, J. Soong, W. K. Sikkema and M. T. Woodside, *J. Am. Chem. Soc.*, 2014, **136**, 2196–2199.
- 29 M. Stevens, E. De Clercq and J. Balzarini, *Med. Res. Rev.*, 2006, **26**, 595–625.
- 30 K. F. Blount and Y. Tor, *ChemBioChem*, 2006, **7**, 1612–1621.
- 31 S. Wang, P. W. Huber, M. Cui, A. W. Czarnik and H. Y. Mei, *Biochemistry*, 1998, **37**, 5549–5557.
- 32 S. Kumar, P. Kellish, W. E. Robinson, Jr., D. Wang, D. H. Appella and D. P. Arya, *Biochemistry*, 2012, **51**, 2331–2347.
- 33 A. C. Stelzer, A. T. Frank, J. D. Kratz, M. D. Swanson, M. J. Gonzalez-Hernandez, J. Lee, I. Andricioaei, D. M. Markovitz and H. M. Al-Hashimi, *Nat. Chem. Biol.*, 2011, **7**, 553–559.
- 34 D. Guianvarc'h, R. Benhida, J. L. Fourrey, R. Maurisse and J. S. Sun, *Chem. Commun.*, 2001, 1814–1815.
- 35 J. P. Joly, G. Mata, P. Eldin, L. Briant, F. Fontaine-Vive, M. Duca and R. Benhida, *Chemistry*, 2014, **20**, 2071–2079.
- 36 J. Sztuba-Solinska, S. R. Shenoy, P. Gareiss, L. R. Krumpe, S. F. Le Grice, B. R. O'Keefe and J. S. Schneekloth, Jr., *J. Am. Chem. Soc.*, 2014, **136**, 8402–8410.

- 37 Y. Cao, X. Liu and E. De Clercq, *Curr. HIV Res.*, 2009, **7**, 101–108.
- 38 R. L. Chapman, T. B. Stanley, R. Hazen and E. P. Garvey, *Antiviral Res.*, 2002, **54**, 149–162.
- 39 S. Prado, M. Beltran, M. Coiras, L. M. Bedoya, J. Alcamí and J. Gallego, *Biochem. Pharmacol.*, 2016, **107**, 14–28.
- 40 S. Prado, M. Beltran, A. Moreno, L. M. Bedoya, J. Alcamí and J. Gallego, *Biochem. Pharmacol.*, 2018, **156**, 68–77.
- 41 C. K. Ingemarsdotter, J. Zeng, Z. Long, A. M. L. Lever and J. C. Kenyon, *Retrovirology*, 2018, **15**, 25.
- 42 A. A. Waheed and E. O. Freed, *AIDS Res. Hum. Retroviruses*, 2012, **28**, 54–75.
- 43 N. M. Bell, A. L'Hernault, P. Murat, J. E. Richards, A. M. Lever and S. Balasubramanian, *Biochemistry*, 2013, **52**, 9269–9274.
- 44 E. Ennifar, J. C. Paillart, A. Bodlender, P. Walter, J. M. Weibel, A. M. Aubertin, P. Pale, P. Dumas and R. Marquet, *Nucleic Acids Res.*, 2006, **34**, 2328–2339.
- 45 N. N. Patwardhan, L. R. Ganser, G. J. Kapral, C. S. Eubanks, J. Lee, B. Sathyamoorthy, H. M. Al-Hashimi and A. E. Hargrove, *MedChemComm*, 2017, **8**, 1022–1036.
- 46 H. Ji, C. S. Fraser, Y. Yu, J. Leary and J. A. Doudna, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 16990–16995.
- 47 W. Wang, P. Preville, N. Morin, S. Mounir, W. Cai and M. A. Siddiqui, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1151–1154.
- 48 E. A. Jefferson, P. P. Seth, D. E. Robinson, D. K. Winter, A. Miyaji, S. A. Osgood, E. E. Swayze and L. M. Risen, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 5139–5143.
- 49 P. P. Seth, A. Miyaji, E. A. Jefferson, K. A. Sannes-Lowery, S. A. Osgood, S. S. Propp, R. Ranken, C. Massire, R. Sampath, D. J. Ecker, E. E. Swayze and R. H. Griffey, *J. Med. Chem.*, 2005, **48**, 7099–7102.
- 50 J. Parsons, M. P. Castaldi, S. Dutta, S. M. Dibrov, D. L. Wyles and T. Hermann, *Nat. Chem. Biol.*, 2009, **5**, 823–825.
- 51 S. M. Dibrov, K. Ding, N. D. Brunn, M. A. Parker, B. M. Bergdahl, D. L. Wyles and T. Hermann, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 5223–5228.
- 52 M. Carnevali, J. Parsons, D. L. Wyles and T. Hermann, *ChemBioChem*, 2010, **11**, 1364–1367.
- 53 C. Misra, F. Lin and A. Kalsotra, *Adv. Neurobiol.*, 2018, **20**, 213–238.
- 54 J. L. Childs-Disney, M. Wu, A. Pushechnikov, O. Aminova and M. D. Disney, *ACS Chem. Biol.*, 2007, **2**, 745–754.
- 55 M. M. Lee, J. L. Childs-Disney, A. Pushechnikov, J. M. French, K. Sobczak, C. A. Thornton and M. D. Disney, *J. Am. Chem. Soc.*, 2009, **131**, 17464–17472.
- 56 J. L. Childs-Disney, R. Parkesh, M. Nakamori, C. A. Thornton and M. D. Disney, *ACS Chem. Biol.*, 2012, **7**, 1984–1993.
- 57 J. L. Childs-Disney, I. Yildirim, H. Park, J. R. Lohman, L. Guan, T. Tran, P. Sarkar, G. C. Schatz and M. D. Disney, *ACS Chem. Biol.*, 2014, **9**, 538–550.
- 58 M. M. Lee, A. Pushechnikov and M. D. Disney, *ACS Chem. Biol.*, 2009, **4**, 345–355.
- 59 S. G. Rzuczek, H. Park and M. D. Disney, *Angew. Chem., Int. Ed.*, 2014, **53**, 10956–10959.
- 60 A. Garcia-Lopez, B. Llamusi, M. Orzaez, E. Perez-Paya and R. D. Artero, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 11866–11871.
- 61 P. C. Gareiss, K. Sobczak, B. R. McNaughton, P. B. Palde, C. A. Thornton and B. L. Miller, *J. Am. Chem. Soc.*, 2008, **130**, 16254–16261.
- 62 J. F. Arambula, S. R. Ramisetty, A. M. Baranger and S. C. Zimmerman, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 16068–16073.
- 63 A. Pushechnikov, M. M. Lee, J. L. Childs-Disney, K. Sobczak, J. M. French, C. A. Thornton and M. D. Disney, *J. Am. Chem. Soc.*, 2009, **131**, 9767–9779.
- 64 M. B. Warf, M. Nakamori, C. M. Matthys, C. A. Thornton and J. A. Berglund, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 18551–18556.
- 65 R. Parkesh, J. L. Childs-Disney, M. Nakamori, A. Kumar, E. Wang, T. Wang, J. Hoskins, T. Tran, D. Housman, C. A. Thornton and M. D. Disney, *J. Am. Chem. Soc.*, 2012, **134**, 4731–4742.
- 66 J. L. Childs-Disney, J. Hoskins, S. G. Rzuczek, C. A. Thornton and M. D. Disney, *ACS Chem. Biol.*, 2012, **7**, 856–862.
- 67 S. G. Rzuczek, Y. Gao, Z. Z. Tang, C. A. Thornton, T. Kodadek and M. D. Disney, *ACS Chem. Biol.*, 2013, **8**, 2312–2321.
- 68 L. Guan and M. D. Disney, *Angew. Chem., Int. Ed.*, 2013, **52**, 10010–10013.
- 69 S. G. Rzuczek, L. A. Colgan, Y. Nakai, M. D. Cameron, D. Furling, R. Yasuda and M. D. Disney, *Nat. Chem. Biol.*, 2017, **13**, 188–193.
- 70 A. H. Jahromi, L. Nguyen, Y. Fu, K. A. Miller, A. M. Baranger and S. C. Zimmerman, *ACS Chem. Biol.*, 2013, **8**, 1037–1043.
- 71 H. Chen, Meena and L. W. McLaughlin, *J. Am. Chem. Soc.*, 2008, **130**, 13190–13191.
- 72 C. H. Wong, L. Nguyen, J. Peh, L. M. Luu, J. S. Sanchez, S. L. Richardson, T. Tuccinardi, H. Tsoi, W. Y. Chan, H. Y. Chan, A. M. Baranger, P. J. Hergenrother and S. C. Zimmerman, *J. Am. Chem. Soc.*, 2014, **136**, 6355–6361.
- 73 J. Li, M. Nakamori, J. Matsumoto, A. Murata, C. Dohno, A. Kiliszek, K. Taylor, K. Sobczak and K. Nakatani, *Chemistry*, 2018, **24**, 18115–18122.
- 74 M. D. Disney, B. Liu, W. Y. Yang, C. Sellier, T. Tran, N. Charlet-Berguerand and J. L. Childs-Disney, *ACS Chem. Biol.*, 2012, **7**, 1711–1718.
- 75 W. Y. Yang, F. He, R. L. Strack, S. Y. Oh, M. Frazer, S. R. Jaffrey, P. K. Todd and M. D. Disney, *ACS Chem. Biol.*, 2016, **11**, 2456–2465.
- 76 T. Tran, J. L. Childs-Disney, B. Liu, L. Guan, S. Rzuczek and M. D. Disney, *ACS Chem. Biol.*, 2014, **9**, 904–912.
- 77 W. Y. Yang, R. Gao, M. Southern, P. S. Sarkar and M. D. Disney, *Nat. Commun.*, 2016, **7**, 11647.
- 78 V. Ambros, *Nat. Med.*, 2008, **14**, 1036–1040.

- 79 V. N. Kim, J. Han and M. C. Siomi, *Nat. Rev. Mol. Cell Biol.*, 2009, **10**, 126–139.
- 80 M. Ha and V. N. Kim, *Nat. Rev. Mol. Cell Biol.*, 2014, **15**, 509–524.
- 81 A. Drusco and C. M. Croce, *Adv. Cancer Res.*, 2017, **135**, 1–24.
- 82 A. Di Giorgio, T. P. Tran and M. Duca, *Future Med. Chem.*, 2016, **8**, 803–816.
- 83 K. Gumireddy, D. D. Young, X. Xiong, J. B. Hogenesch, Q. Huang and A. Deiters, *Angew. Chem., Int. Ed.*, 2008, **47**, 7482–7484.
- 84 Y. Ren, X. Zhou, X. Liu, H. H. Jia, X. H. Zhao, Q. X. Wang, L. Han, X. Song, Z. Y. Zhu, T. Sun, H. X. Jiao, W. P. Tian, Y. Q. Yang, X. L. Zhao, L. Zhang, M. Mei and C. S. Kang, *Cancer Lett.*, 2016, **374**, 96–106.
- 85 Y. Ren, X. Zhou, J. J. Yang, X. Liu, X. H. Zhao, Q. X. Wang, L. Han, X. Song, Z. Y. Zhu, W. P. Tian, L. Zhang, M. Mei and C. S. Kang, *Cancer Lett.*, 2015, **362**, 174–182.
- 86 Z. Shi, J. Zhang, X. Qian, L. Han, K. Zhang, L. Chen, J. Liu, Y. Ren, M. Yang, A. Zhang, P. Pu and C. Kang, *Cancer Res.*, 2013, **73**, 5519–5531.
- 87 D. D. Vo, C. Becquart, T. P. A. Tran, A. Di Giorgio, F. Darfeuille, C. Staedel and M. Duca, *Org. Biomol. Chem.*, 2018, **16**, 6262–6274.
- 88 D. D. Vo and M. Duca, *Methods Mol. Biol.*, 2017, **1517**, 137–154.
- 89 D. D. Vo, C. Staedel, L. Zehnacker, R. Benhida, F. Darfeuille and M. Duca, *ACS Chem. Biol.*, 2014, **9**, 711–721.
- 90 D. D. Vo, T. P. Tran, C. Staedel, R. Benhida, F. Darfeuille, A. Di Giorgio and M. Duca, *Chemistry*, 2016, **22**, 5350–5362.
- 91 C. Staedel, T. P. A. Tran, J. Giraud, F. Darfeuille, A. Di Giorgio, N. J. Tourasse, F. Salin, P. Uriac and M. Duca, *Sci. Rep.*, 2018, **8**, 1667.
- 92 A. J. Angelbello, J. L. Chen, J. L. Childs-Disney, P. Zhang, Z. F. Wang and M. D. Disney, *Chem. Rev.*, 2018, **118**, 1599–1663.
- 93 M. D. Disney and A. J. Angelbello, *Acc. Chem. Res.*, 2016, **49**, 2698–2704.
- 94 O. Aminova, D. J. Paul, J. L. Childs-Disney and M. D. Disney, *Biochemistry*, 2008, **47**, 12670–12679.
- 95 S. P. Velagapudi, S. J. Seedhouse and M. D. Disney, *Angew. Chem., Int. Ed.*, 2010, **49**, 3816–3818.
- 96 C. L. Haga, S. P. Velagapudi, J. R. Strivelli, W. Y. Yang, M. D. Disney and D. G. Phinney, *ACS Chem. Biol.*, 2015, **10**, 2267–2276.
- 97 S. P. Velagapudi, M. D. Cameron, C. L. Haga, L. H. Rosenberg, M. Lafitte, D. R. Duckett, D. G. Phinney and M. D. Disney, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, 5898–5903.
- 98 S. P. Velagapudi, S. M. Gallo and M. D. Disney, *Nat. Chem. Biol.*, 2014, **10**, 291–297.
- 99 M. G. Costales, C. L. Haga, S. P. Velagapudi, J. L. Childs-Disney, D. G. Phinney and M. D. Disney, *J. Am. Chem. Soc.*, 2017, **139**, 3446–3455.
- 100 N. A. Naryshkin, M. Weetall, A. Dakka, J. Narasimhan, X. Zhao, Z. Feng, K. K. Ling, G. M. Karp, H. Qi, M. G. Woll, G. Chen, N. Zhang, V. Gabbeta, P. Vazirani, A. Bhattacharyya, B. Furia, N. Risher, J. Sheedy, R. Kong, J. Ma, A. Turpoff, C. S. Lee, X. Zhang, Y. C. Moon, P. Trifillis, E. M. Welch, J. M. Colacino, J. Babiak, N. G. Almstead, S. W. Peltz, L. A. Eng, K. S. Chen, J. L. Mull, M. S. Lynes, L. L. Rubin, P. Fontoura, L. Santarelli, D. Haehnke, K. D. McCarthy, R. Schmucki, M. Ebeling, M. Sivaramakrishnan, C. P. Ko, S. V. Paushkin, H. Ratni, I. Gerlach, A. Ghosh and F. Metzger, *Science*, 2014, **345**, 688–693.
- 101 M. Sivaramakrishnan, K. D. McCarthy, S. Campagne, S. Huber, S. Meier, A. Augustin, T. Heckel, H. Meistermann, M. N. Hug, P. Birrer, A. Moursy, S. Khawaja, R. Schmucki, N. Berntenis, N. Giroud, S. Golling, M. Tzouros, B. Banfai, G. Duran-Pacheco, J. Lamerz, Y. Hsiu Liu, T. Luebbers, H. Ratni, M. Ebeling, A. Clery, S. Paushkin, A. R. Krainer, F. H. Allain and F. Metzger, *Nat. Commun.*, 2017, **8**, 1476.
- 102 J. Wang, P. G. Schultz and K. A. Johnson, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, E4604–E4612.
- 103 M. G. Costales, Y. Matsumoto, S. P. Velagapudi and M. D. Disney, *J. Am. Chem. Soc.*, 2018, **140**, 6741–6744.