One of the major challenges of current medicinal chemistry is targeting noncoding RNAs using small-molecule drugs. Unlike classical drug discovery methodologies, it requires a change of perspective to be successful.

As described by Disney and co-workers in this issue of ACS Central Science, the discovery of specific ligands for targeting SARS-CoV-2 RNA, the development of ribonuclease targeting chimeras (RIBOTAC), and the detailed study of the intracellular mechanisms of action using cutting-edge chemical biology tools highlight the impact that new chemical modalities could have in future therapies.

This work's field of application involves targeting SARS-CoV-2, the virus that caused the current global health crisis. Many efforts have been made toward drug repositioning, large screenings, and vaccine development; however, research toward new targets and new bioactive molecules for innovative therapeutic approaches is clearly needed to increase the antiviral drug arsenal.

One of the major challenges of current medicinal chemistry is targeting noncoding RNAs using small-molecule drugs. Unlike classical drug discovery methodologies, it requires a change of perspective to be successful.

RNAs, once considered mere intermediates in the process of gene expression, are now valid targets for drug discovery. RNA is a macromolecule involved in essential biological processes such as transcription, translation, and regulation of gene expression. In fact, many deregulations in noncoding RNAs expression and/or functions have been directly linked to a number of pathologies such as neurological disorders, cardiological diseases, or cancers. Most notably, more than 70% of the human genome is transcribed in noncoding RNAs, while only 1.5% codes for proteins. Since only a small portion of these proteins represents the actual target of marketed drugs, it is clear that adding noncoding RNAs to potential therapeutic targets would greatly increase the landscape of drug development. The coronavirus pandemic caused by the new SARS-CoV-2, an RNA virus, highlights the potential of RNA-targeted therapies for the treatment of infections caused by RNA viruses. These therapies were previously explored in the literature for HIV, HCV, or influenza viruses.

There has been significant progress in the discovery of new RNA-targeting drugs since the first RNA ligands entered the market as antibiotics in the 1940s, such as aminoglycosides or tetracyclines, and more recently oxazolidinones. Progress made during recent years in RNA binders include different kinds of original, and sometimes unusual, drug discovery strategies. Lead identification strategies for the identification of very specific RNA ligands also have been developed such as Inforna. The latter is a technique based on the combination of two-dimensional combinatorial screening (2-DCS) and...
structure—activity relationships through sequencing (StARTS). The application of this statistical method to screening results allows anticipating the affinity and selectivity of members of a RNA library and scoring binding interactions. This method has proven to be very effective for successfully predicting compounds that are specific for a particular RNA secondary structure.

Various screening technologies, such as microarrays or fluorescence-based assays, have also been used for the discovery of RNA ligands together with the structure-based design of selective ligands. The results gathered from these experimental screenings and biological studies allow the construction of databases such as R-BIND that will likely accelerate the drug discovery process in the field of RNA targeting. Finally, various tools of structural biology and chemical probing, such as NMR and SHAPE technologies, have also been developed to understand small molecules/RNA interactions. Altogether, the efforts made toward the discovery of RNA-targeting small molecules recently showed remarkable success with the marketing of Risdiplam as a mRNA splicing modulator against spinal muscular atrophy (SMA).

The tools used for drug discovery can be adapted specifically to address the challenging targets represented by RNAs. This strategy can be combined with the development of original drug modalities, such as precise genome editing, modified peptides, oligonucleotides, macrocycles, and various conjugates, to open new ways to modulate targets. The study by Disney and co-workers applies such technologies for the identification of precise tools for specific RNA targeting and RNA degradation as well as for the study of the intracellular molecular mechanism of action (Figure 1).

In their work, Disney and co-workers first screened an RNA-focused small molecule collection housed in the Informa database against the SARS-CoV-2 frameshifting element RNA that bears a 1×1 nucleotide UU internal loop in its stem important for frameshifting element function. Various hits were identified and then studied in detail for their affinity and selectivity in vitro, but also for their ability to bind and inhibit the target in cells using a transfected luciferase reporter system. This led to the discovery of an RNA binder called Covidicil-19 (Figure 1, left) that bears a nanomolar affinity for this RNA target and has the ability to stabilize the hairpin’s folded state and impair frameshifting in cells.

Chemical cross-linking and isolation by pull-down (Chem-CLIP) was then used to study the exact intracellular target (Figure 1, center). Chem-CLIP is a proximity-based reaction that covalently links an RNA-binding small molecule to its target(s). With this aim, a chlorambucyl moiety able to
form a covalent link with the RNA target and a biotin residue for the immunoprecipitation of the formed covalent adducts were conjugated in positions of the compound that could be modified without losing the affinity for the target. The use of such a conjugate (Covidcil-CLIP) for cell treatment and the following immunoprecipitation showed a 3-fold increase in the targeted RNA in the pull-down fraction, which confirms the affinity and the selectivity for FSE RNA. Finally, Covidcil-19 was modified to prepare a RIBOTAC (Figure 1, right). RIBOTACs, analogous to PROTACs that have been developed in the context of protein targeting and degradation, are new chemical modalities where a specific RNA binder is covalently attached to a chemical compound that is able to recruit a cellular ribonuclease. The latter induces the cleavage and degradation of the targeted RNA. Covidcil-19 was, thus, conjugated to a heterocyclic recruiter of RNase L that led to the Covidcil RIBOTAC compound. This compound showed the ability to induce targeted cleavage and degradation of the entire SARS-CoV construct. The RIBOTAC lead optimization strategy improved the bioactivity of the compound at least 10-fold as demonstrated by intracellular luciferase reporter assays.

While multiple strategies and therapeutic approaches are needed and currently under investigation to tackle SARS-CoV-2, targeting its RNA genome with small-molecule drugs could lead to promising developments with wider applications, and this study demonstrates how the SARS-CoV-2 RNA genome can be considered druggable.

The ever-increasing understanding of RNA biology and targets structure/function provides exciting opportunities for the development of new chemical modalities, such as RIBOTACs, that explore innovative mechanisms of action.

Targeting RNA with drug-like compounds holds great promise for developments in drug discovery. In fact, recent successes with new RNA binders reaching the clinic further underline the potential for new applications in the very near future. Besides this potential, the ever-increasing understanding of RNA biology and targets structure/function provides exciting opportunities for the development of new chemical modalities, such as RIBOTACs, that explore innovative mechanisms of action. However, these hybrid compounds contain different moieties conjugated to take advantage of various intracellular mechanisms. The ones described in this work are binding, covalent linkage, isolation of ligand/target complex, and finally RNase recruitment and induced degradation. This increases the chemical complexity adding new challenges for scale-up, formulation, stability, and/or toxicity. While the clinical application of such complex chemical tools and challenging targets might seem hardly attainable, recent progress in clinical trials of the PROTAC approach suggests that this kind of drug modalities could represent the future of medicinal chemistry.

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Notes

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