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Small-Molecule Targeting of RNA Structures: From Cryptic RNA Pockets to Sequence-Selective Therapeutics

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Abstract: RNA is emerging as a chemically tractable therapeutic target rather than merely an intermediary between DNA and proteins. Its diverse secondary, tertiary, and quaternary structures create ligandable environments for small molecules, although dynamic conformations, electrostatic surfaces, and recurrent motifs complicate selective targeting. Cryptic RNA pockets are particularly promising sites that are weakly populated or inaccessible in the unbound state but become exposed or reorganized upon ligand binding. Riboswitches provide natural models for this mechanism, while recent studies have extended RNA targeting to hairpins, internal loops, bulges, G-quadruplexes, viral regulatory elements, precursor microRNAs, long non-coding RNAs, and RNA–protein interfaces. Advances in dynamic-ensemble screening, fragment-based discovery, RNA-focused libraries, sequence-based design, chemical probing, and computational pocket prediction are expanding the druggable RNA landscape. The Cbl riboswitch illustrates how ligand-induced nucleotide displacement can reveal a cryptic pocket for synthetic ligand recognition. RIBOTACs and targeted-cleavage conjugates further connect RNA binding with cellular function, while risdiplam demonstrates clinical feasibility of small-molecule RNA modulation. This mini-review discusses structural principles, discovery strategies, representative applications, challenges, and emerging technologies for developing sequence-selective RNA therapeutics through integrated structure-guided medicinal chemistry.

Keywords: RNA targeting; small molecules; cryptic pockets; riboswitch; RIBOTAC; Inforna.

1. Introduction

RNA occupies a central position in essentially every layer of gene regulation, translation, RNA processing, signaling, and cellular adaptation. Beyond messenger RNA, the transcriptome contains riboswitches, precursor mRNAs, microRNAs, long non-coding RNAs, ribosomal RNAs, viral RNAs, repeat expansions, and numerous regulatory RNA elements whose biological activities depend strongly on their three-dimensional structures [1–6]. These structural features create opportunities for chemical recognition that are fundamentally different from conventional protein-oriented drug discovery.

Historically, the perception that RNA was difficult to target pharmacologically was driven by its highly negatively charged backbone, structural heterogeneity, conformational mobility, and the relatively shallow or solvent-exposed nature of many RNA surfaces. Nevertheless, naturally occurring metabolites and antibiotics demonstrate that RNA can present high-affinity binding pockets. The discovery of ligand-responsive riboswitches established that RNA folds can generate highly organized molecular-recognition sites that

discriminate closely related metabolites [7,17–19]. Contemporary RNA-targeting research builds on this principle by identifying additional pockets and designing ligands that convert RNA recognition into functional cellular outcomes [3,4].

The uploaded source highlights a major conceptual transition: RNA ligand discovery is moving from the identification of obvious preformed cavities toward systematic exploration of dynamic and cryptic binding sites that may be revealed by ligand-induced conformational rearrangement. Recent studies additionally show that RNA-targeted small molecules can be complemented by host–guest systems, motif-based design, and targeted degradation, thereby expanding mechanisms beyond simple occupancy [20–24]. Contemporary reviews likewise emphasize structure determination, computational prediction, focused libraries, and functionalization as major components of the emerging RNA drug-discovery framework.

The central premise of this review is that sequence selectivity and functional selectivity should be treated as related but distinct design objectives. A ligand can bind an RNA motif with high affinity yet remain biologically silent if the interaction occurs at a nonfunctional site. Conversely, a ligand with moderate affinity may produce a strong biological effect when it binds a structurally critical or regulatory element and is coupled to an appropriate effector function [1–3,22]. Thus, successful RNA therapeutics require the integration of structural recognition, cellular localization, target engagement, functional consequence, and medicinal-chemistry optimization.

2. RNA as a Structured and Dynamic Small-Molecule Target

2.1 Structural diversity of RNA

RNA can form hairpins, internal loops, bulges, pseudoknots, triplexes, G-quadruplexes, tertiary contacts, and RNA–protein assemblies. These structural elements generate pockets through base stacking, hydrogen bonding, electrostatic interactions, cation coordination, and hydrophobic surfaces [5–8]. Unlike proteins, RNA often uses nucleobases as both recognition elements and architectural components. Consequently, ligand binding can reorganize the same structural elements that create the binding site.

The physicochemical properties of known bioactive RNA ligands suggest that successful compounds occupy a specialized chemical space that balances electrostatic contacts with hydrophobicity, shape complementarity, and molecular rigidity [10–12]. Excessively cationic compounds may bind RNA broadly, whereas highly lipophilic ligands may suffer from poor aqueous solubility or nonspecific membrane association. These observations have motivated the development of RNA-focused libraries and cheminformatic resources such as R-BIND, which systematically organize experimentally validated RNA-targeted chemical matter [10–12].

2.2 RNA pockets are not always static

A major difference between RNA and conventional rigid protein targets is the importance of conformational ensembles. RNA frequently undergoes structural interconversion among low- and high-energy conformations, and ligand binding can shift the population toward a previously underrepresented state [14]. Therefore, an RNA pocket should not necessarily be conceptualized as an invariant geometric cavity. Instead, it may exist transiently within a conformational ensemble.

Dynamic-ensemble screening provided an early experimental demonstration that low-population conformations can be exploited to discover selective RNA ligands [14]. Fragment-

based screening against riboswitches similarly showed that weak interactions can identify recognition points that are subsequently optimized into higher-affinity ligands [15]. These observations underpin the modern concept of cryptic RNA pockets.

3. Cryptic RNA Pockets: A New Dimension of RNA Druggability

3.1 Definition and structural basis

A cryptic RNA pocket is a ligandable site that is absent, poorly exposed, or sterically restricted in a major unliganded RNA conformation but becomes accessible following local rearrangement. Such sites can emerge through nucleotide displacement, base flipping, loop reorientation, tertiary-contact disruption, or cooperative restructuring of neighboring residues. This concept is particularly valuable because RNA structures often contain tightly packed nucleobases that can function as movable structural gates. Rather than requiring a large pre-existing cavity, a ligand may initiate pocket formation through an induced-fit or population-shift mechanism. Consequently, the energetic cost of pocket opening becomes an integral part of ligand recognition [14,20,21].

3.2 The cobalamin riboswitch as a model system

Riboswitches represent some of the best-characterized small-molecule-binding RNAs because their receptor domains have evolved to recognize endogenous metabolites. FMN, thiamine pyrophosphate, guanine, and cobalamin riboswitches have therefore served as model systems for RNA-focused drug discovery [15–19]. A particularly instructive recent example involves the cobalamin (Cbl) riboswitch. In this system, chemically modified cobalamin derivatives can displace adenine A20 and expose a previously hidden binding region. Subsequent medicinal-chemistry optimization identified synthetic ligands capable of exploiting the newly revealed pocket and strengthening π-stacking interactions [20]. The study illustrates a general principle: a ligand does not simply "fit" an existing RNA pocket; it can create its own binding site by remodeling the RNA architecture. The study further showed that substituent geometry could strongly influence affinity, consistent with a spatially constrained cryptic site.

More recent work has strengthened this concept by examining cryptic RNA sites as energetically accessible states rather than exceptional structural anomalies [21]. This view suggests that cryptic pockets may represent a broader and underexplored component of the RNA ligandome (Fig. 1).

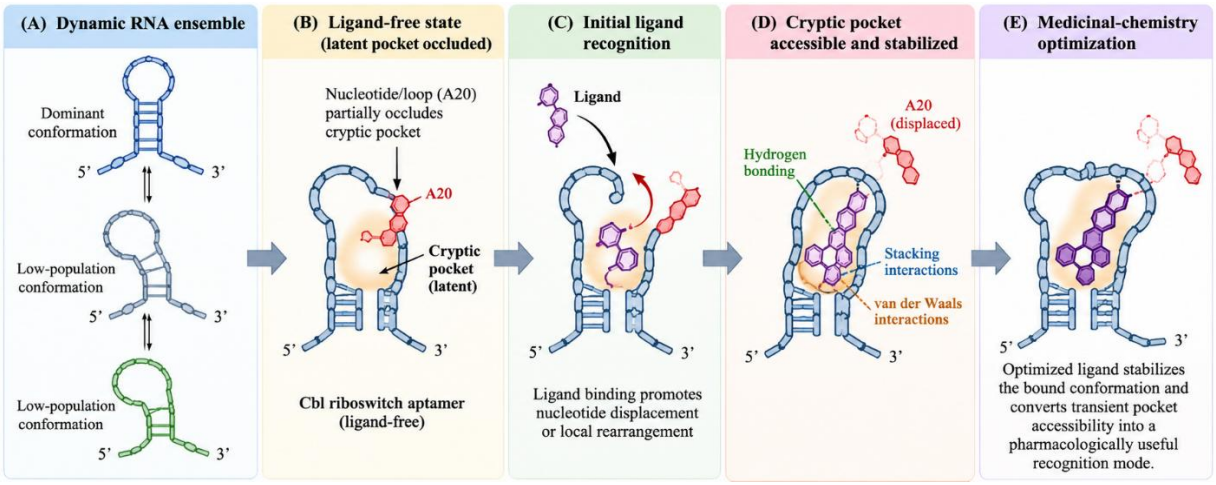

Figure 1. Conceptual model of cryptic RNA-pocket formation and ligand recognition.

(A) RNA exists as a dynamic ensemble containing dominant and low-population conformations. **(B)** In the ligand-free state, a nucleotide or loop partially occludes a latent pocket. **(C)** Initial ligand recognition promotes nucleotide displacement or local backbone rearrangement. **(D)** The cryptic pocket becomes accessible and provides additional stacking, hydrogen-bonding, and van der Waals interactions. **(E)** Medicinal-chemistry optimization stabilizes the ligand-bound conformation and converts transient pocket accessibility into a pharmacologically useful recognition mode. The Cbl riboswitch/A20-displacement paradigm should be used as the representative structural example [14,20,21].

4. Strategies for Discovering RNA Binding Pockets

4.1 X-ray crystallography, NMR, and cryo-EM

High-resolution structural methods remain fundamental to RNA-targeted drug discovery. X-ray crystallography and NMR have historically provided atomic-level descriptions of RNA–ligand complexes, while cryo-electron microscopy is increasingly useful for large RNA, viral RNA, and ribonucleoprotein assemblies [5,6,29,33]. Structural snapshots are particularly informative when they reveal ligand-induced conformational changes. However, a single structure can underestimate RNA conformational heterogeneity. The increasing integration of multiple structural states, molecular dynamics, chemical probing, and fragment screening therefore provides a more realistic representation of RNA ligandability [14,29,33,34].

4.2 Chemical probing and in-cell structure mapping

Selective 2′-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP), dimethyl sulfate mutational profiling (DMS-MaPseq), and related approaches allow structural information to be obtained for long RNAs and, importantly, inside cells [29–32]. SHAPE-MaP reports nucleotide reactivity patterns that can reveal flexible or conformationally responsive positions, whereas DMS-based methods preferentially interrogate accessible adenosine and cytidine residues. These techniques can be used in a ligand-dependent manner. Differences in reactivity between untreated and compound-treated RNA may reveal conformational remodeling, ligand protection, or indirect allosteric effects. In-cell approaches are particularly important because RNA structures measured under purified conditions may differ from those in the crowded cellular environment [29–32]. Targeted structure mapping has also been applied to long non-coding RNAs such as Xist, demonstrating the feasibility of high-resolution RNA structural analysis in cells [31].

4.3 High-throughput and library-versus-library screening

Classical high-throughput screening evaluates compound libraries against individual RNA targets, whereas RNA-focused and library-versus-library strategies systematically interrogate **multiple RNA motifs against multiple chemical entities** [10–12,22]. The latter approach can generate a direct map between RNA structural motifs and ligand chemotypes. The 2D combinatorial screening framework described in the source material analyzed more than 61 million theoretical RNA–ligand interactions and experimentally identified hundreds of RNA-binding compounds, including numerous previously unrecognized scaffolds [22]. The resulting interaction map was enriched in internal loops and bulges and provided a systematic foundation for motif-based small-molecule design. R-BIND and related databases complement these screening approaches by consolidating physicochemical descriptors, RNA structural contexts, and biological activities of experimentally validated RNA-targeting compounds [10–

12]. The emergence of RNA-focused libraries has subsequently become an important field of its own, with increasing emphasis on diversity, structural complementarity, tractability, and compatibility with medicinal-chemistry optimization [56].

5. Sequence-Selective Recognition of RNA

5.1 Motif recognition rather than simple electrostatic binding

Sequence-selective RNA targeting depends on more than recognizing the negatively charged phosphate backbone. High-quality ligands must discriminate between related local structures using combinations of base stacking, hydrogen bonding, steric fit, electrostatic interactions, and recognition of noncanonical nucleobase patterns [1–8]. Inforna exemplifies a sequence-to-structure design strategy in which experimentally established RNA motif–ligand relationships are used to identify small molecules for predetermined RNA sequences [1,2,22,24]. Rather than screening a target blindly, the sequence is first analyzed to identify ligandable structural motifs. This converts RNA sequence information into a chemical hypothesis.

5.2 The functional-site problem

An important emerging distinction is between **binding selectivity** and **functional selectivity**. High-affinity binding does not automatically produce a measurable phenotype. In a genome-wide RNA context, a small molecule may recognize many structurally similar motifs, yet only a subset may be functionally relevant [22]. This challenge was illustrated by motif-level analyses showing that numerous predicted RNA interactions may occur at sites that do not regulate processing or translation. Consequently, target selection should include the biological position of the motif, RNA abundance, structural accessibility, RNA-binding proteins, and functional consequences of perturbation [1–3,22].

5.3 Multivalent and host–guest recognition

One strategy to enhance specificity is to combine multiple recognition elements. In a multivalent design, two or more weak interactions can cooperate when they engage adjacent RNA motifs. Host–guest systems provide an additional approach by using a larger RNA-recognition scaffold to deliver and solubilize a smaller guest molecule [20]. The Cbl-riboswitch work is particularly informative because cobalamin can function as a host that positions synthetic substituents within a newly accessible RNA environment. This approach may be generalized to other structured RNAs in which a sequence-selective host can provide both localization and additional binding energy [20,21].

6. RNA-Focused Chemical Libraries and Fragment-Based Discovery

RNA ligands have traditionally been dominated by polycationic, heteroaromatic, or aminoglycoside-like scaffolds. However, systematic analysis suggests that biologically active RNA ligands can occupy a broader chemical space characterized by suitable shape, polarity, rigidity, and spatial distribution of functional groups [10–12,43]. Focused libraries can be designed to enrich for scaffolds capable of stacking with nucleobases, filling narrow RNA grooves, or making selective contacts with loops and bulges. Aminoglycosides illustrate both the potential and limitation of this strategy: they have strong RNA affinity but frequently lack the specificity required for selective therapeutic applications [43].

Fragment-based discovery is particularly attractive for cryptic pockets because small fragments can access narrow or transient cavities more readily than larger molecules [15].

Fragment hits can then be merged, grown, or linked into compounds with improved affinity and selectivity. Importantly, fragment discovery should be combined with structural methods because weak affinity alone does not establish a productive or functional RNA-binding mode (Fig. 2).

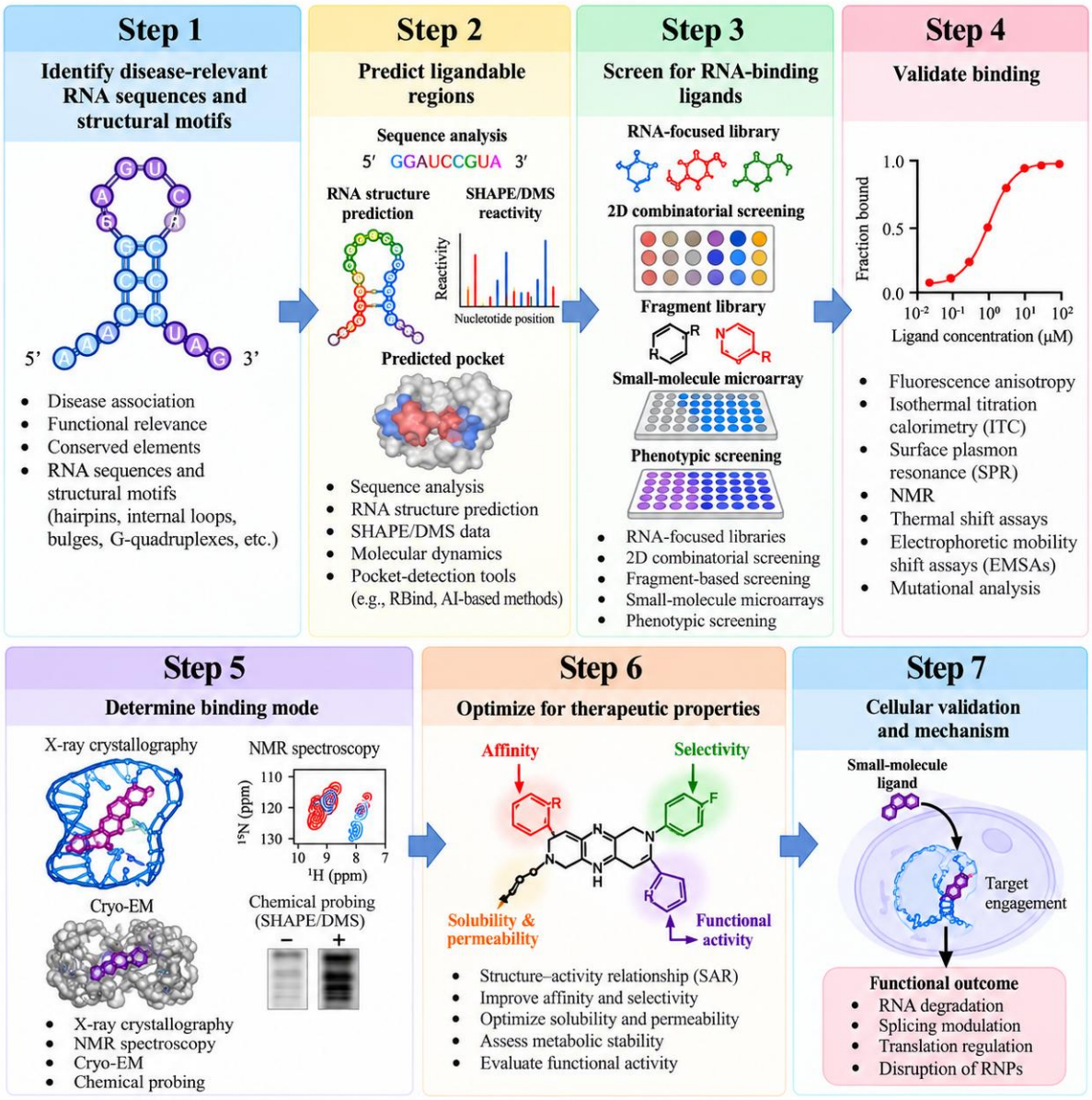

Figure 2. Integrated discovery workflow for cryptic and sequence-selective RNA ligands.

Step 1: identify disease-relevant RNA sequences and structural motifs. **Step 2:** predict conserved, dynamic, or ligandable regions using sequence analysis, RNA structure prediction, SHAPE/DMS data, and pocket-detection tools. **Step 3:** interrogate the target with RNA-focused libraries, 2D combinatorial screening, fragments, small-molecule microarrays, or phenotypic screening. **Step 4:** validate binding by orthogonal biophysical and biochemical assays. **Step 5:** resolve the binding mode using X-ray crystallography, NMR, cryo-EM, chemical probing, or molecular modeling. **Step 6:** optimize affinity, selectivity, solubility, permeability, and functional activity. **Step 7:** determine cellular target engagement and pharmacological mechanism [10–15,29–37,56].

7. Representative RNA Targets and Therapeutic Strategies

7.1 Riboswitches

Riboswitches remain among the most chemically validated RNA targets because their native ligand-binding mechanisms provide direct evidence that RNA can form selective drug-like pockets [15–19]. Ribocil-derived compounds targeting the FMN riboswitch demonstrate that metabolite-recognition sites can be repurposed for antibacterial discovery [16]. Likewise, fragment screening against the thiM thiamine pyrophosphate riboswitch established that weak ligand interactions can be elaborated into more substantial RNA-targeting chemical matter [15]. The Cbl-riboswitch findings extend this paradigm by demonstrating that the most useful RNA pocket may not be visible until after ligand binding [20,21].

7.2 Group II introns

The group II intron represents another example of de novo targeting of a complex RNA tertiary structure. Fedorova and colleagues used high-throughput screening, SAR, and lead optimization to identify compounds capable of inhibiting group II intron splicing and suppressing growth of pathogenic fungi [38]. This work was important because it demonstrated that RNA tertiary structures other than riboswitches can yield pharmacologically useful small-molecule leads.

7.3 HIV-1 TAR and viral RNAs

Viral RNAs have long served as model targets because regulatory elements such as HIV-1 TAR and other internal structural motifs are essential for viral replication or gene expression [40–42,47]. Structural and chemical studies have identified unconventional binding modes in TAR, illustrating that sequence selectivity may derive from interactions with local RNA architecture rather than classical Watson–Crick base recognition [40]. The broader viral RNA field provides an important test case for RNA-targeted chemistry because rapid sequence evolution can create resistance while simultaneously generating strain- or genotype-specific structural differences [42,47].

7.4 Repeat expansions

Disease-associated repeat expansions generate recurring structural motifs that can be recognized by modularly assembled small molecules. Myotonic dystrophy type 1 and fragile-X-associated RNA repeat disorders have therefore served as important model systems for sequence- and structure-selective ligand design [44,45]. For example, modular recognition of r(CUG) repeats demonstrates how RNA motif recognition can be coupled to biological function. Such strategies are particularly relevant when repeat length, local structure, and ligand multivalency can collectively contribute to selectivity.

7.5 SMN2 and splicing modulation

The most clinically advanced example of non-ribosomal RNA-targeted small-molecule pharmacology is the SMN2 splicing pathway. Risdiplam and related compounds modulate SMN2 pre-mRNA splicing through structural recognition of an RNA regulatory element and interaction with the spliceosomal machinery [25–28]. Experimental studies showed that small-molecule splicing modulators can enhance productive spliceosome assembly and rescue disease-associated phenotypes [26,27]. Structural and medicinal-chemistry studies subsequently established the molecular requirements for high selectivity and bioactivity [25–28]. The clinical significance of risdiplam illustrates a central point of RNA drug discovery: the therapeutic mechanism does not have to be simple competitive inhibition of an RNA-binding pocket. An RNA-targeted small molecule can function as a molecular glue-like

splicing modulator, stabilizing productive interactions between RNA and endogenous cellular machinery [3].

7.6 G-quadruplexes and structured untranslated regions

Structured 5′- and 3′-untranslated regions can regulate translation and mRNA stability. G-quadruplex structures are particularly attractive targets because they contain stacked guanine tetrads and loop regions that can support aromatic and electrostatic interactions. Small molecules recognizing a G-quadruplex in the 5′ UTR of ADAM10 mRNA have been investigated as a means of altering translation [39]. These examples demonstrate that RNA-targeted small molecules can be used not only to silence gene expression but also to increase or redirect it (Table 1).

Table 1. Representative RNA targets, structural features, ligands, and functional mechanisms.

RNA target	Structural feature / pocket	Representative small-molecule strategy	Functional mechanism	Development context
FMN riboswitch	Preformed metabolite pocket	Ribocil-like metabolite mimics	Riboswitch inhibition	Antibacterial lead [16,17]
TPP thiM riboswitch	Ligand-binding tertiary pocket	Fragment screening and optimization	Riboswitch modulation	Discovery platform [15]
Cobalamin riboswitch	Cryptic site exposed by nucleotide displacement	Cbl host–guest and biphenyl-like ligands	Pocket occupation / riboswitch antagonism	Preclinical chemical biology [20,21]
Group II intron	Large RNA tertiary structure	HTS + SAR	Inhibition of self-splicing	Antifungal leads [38,51]
HIV-1 TAR	Hairpin with structured bulge/loop	Noncanonical RNA binders	Disruption of Tat/TAR function	Antiviral research [40,42]
ADAM10 5′-UTR	G-quadruplex	G-quadruplex-binding small molecules	Increased translation	Preclinical concept [39]
r(CUG) repeat	Repeating hairpin motifs	Modular RNA binders	Functional repeat recognition / cleavage	Myotonic dystrophy research [44,45,50]
pre-miR-96	Hairpin precursor	RNA binder + bleomycin A5	Targeted RNA cleavage	Cancer chemical biology [48]
pre-miR-155	Hairpin / internal structural motif	Inforna-derived binder; RIBOTAC	RNase-mediated degradation	Preclinical [22,24]
JUN IRES	Internal loop / bulge	Inforna-derived RIBOTAC	RNase L recruitment	Preclinical oncology [22]
MYC IRES	Internal loop / bulge	Inforna-derived RIBOTAC	Translation suppression via RNA degradation	Preclinical oncology [22]
SMN2 pre-mRNA	Exonic/intronic regulatory structure	Risdiplam-class splicing modulators	Productive spliceosome assembly	Clinical / approved medicine [25–28]

Note: The examples are representative rather than exhaustive. The uploaded source similarly identifies FMN and Cbl riboswitches, SMN2, pre-miR-155, JUN IRES, and MYC IRES as key examples of structured RNA targeting.

8. From RNA Binding to Functional Therapeutics

RNA occupancy alone is often insufficient to create a strong phenotype. This limitation has stimulated the development of functionalization strategies that convert a passive RNA binder into an active chemical modality [1–4,22,23].

8.1 RIBOTACs

Ribonuclease-targeting chimeras (RIBOTACs) couple an RNA-binding ligand to an RNA-cleaving effector. The RNA-binding module provides target recognition, whereas the

effector recruits an endogenous or engineered nuclease [22,23]. This strategy addresses the problem of biologically silent binding by turning occupancy into catalytic RNA destruction.

Tong and colleagues demonstrated this principle for multiple disease-relevant RNAs, including precursor microRNAs and oncogenic IRES-containing RNAs [22]. In the JUN IRES system, an Inforna-derived small molecule recognized an RNA structural element and enabled recruitment of RNase L to promote RNA cleavage. Similar logic was applied to MYC-associated RNA structures.

8.2 Directly cleaving conjugates

An alternative is to attach a catalytic or cleaving group directly to an RNA-binding small molecule. The resulting molecule combines molecular recognition with localized chemical damage. DeglycoCugamycin, for example, coupled a selective r(CUG)-binding element to a deglycosylated bleomycin derivative and produced allele-selective RNA cleavage while reducing some unwanted DNA damage associated with the parent cleaving agent [50].

8.3 Chem-CLIP and cellular target validation

Cellular target identification is essential because biochemical affinity does not establish intracellular engagement. Chem-CLIP-Map-Seq uses a chemically tagged ligand to capture and map RNA-binding sites in cells [49]. These methods can establish whether an RNA interaction occurs at the intended target and can help distinguish primary from indirect pharmacological effects.

9. Computational Design and Artificial Intelligence

Computational tools increasingly complement experimental RNA structure determination. RBind and related algorithms have been developed to predict potential RNA-binding regions from structural and sequence-derived descriptors [37]. Benchmarking resources such as RNA-Puzzles have simultaneously improved the evaluation of RNA three-dimensional structure prediction [33,34]. Recent protein–nucleic-acid prediction systems, including RoseTTAFoldNA and AlphaFold 3, extend machine-learning-based structural modeling to RNA-containing molecular complexes [35,36]. Although these systems should not yet be regarded as replacements for experimental validation, they can support hypothesis generation, conformational sampling, docking, and prioritization of candidate binding sites.

Deep-learning approaches dedicated specifically to RNA small-molecule pockets are also emerging. SMARTPocket represents an example of geometric deep learning developed to identify ligandable and cryptic RNA pockets from three-dimensional structures [58]. This direction is particularly relevant because conventional geometric pocket-detection methods may overlook transient or shallow RNA cavities. Machine learning is additionally becoming important for predicting ligand–motif interactions, ranking focused libraries, recognizing physicochemical patterns, and integrating large RNA–small-molecule datasets [52–56]. Nevertheless, model performance remains constrained by dataset size, structural diversity, assay heterogeneity, and limited representation of cellular context (Table 2).

Table 2. Experimental and computational toolbox for RNA-targeted small-molecule discovery.

Stage	Method/tool	Primary information obtained	Major strength	Major limitation
RNA structure	X-ray crystallography	Atomic structure of RNA/ligand complex	Precise binding-site definition	Requires suitable crystals

RNA dynamics	NMR spectroscopy	Conformational exchange and ligand interactions	Captures solution behavior	Size and sensitivity constraints
Large RNA complexes	Cryo-EM	Near-native structural information	Suitable for large RNP systems	Resolution can vary across regions
In-cell structure	SHAPE-MaP	Nucleotide-level structural reactivity	Cellular structural context	Indirect structural readout [29]
In-cell structure	DMS-MaPseq	Reactivity of accessible bases	Genome-scale/targeted analysis	Chemical accessibility ≠ complete structure [30]
Library screening	2DCS / library-versus-library	RNA motif–ligand relationships	Large interaction space	Requires follow-up validation [22]
Fragment discovery	NMR/X-ray fragment screening	Weak binding fragments	Access to compact pockets	Weak affinity and high validation burden [15]
Databases	R-BIND / Inforna	Known RNA–ligand relationships	Enables motif-based design [11,12,24]	Database bias and incomplete coverage
Pocket prediction	RBind / related algorithms	Candidate ligandable regions	Rapid prioritization [37]	False positives possible
Structure prediction	RNA-Puzzles / modern AI	3D RNA conformations	Enables computational triage [33–36]	Experimental validation remains necessary
Deep learning	SMARTPocket	Cryptic/ligandable pocket prediction	RNA-specific geometric learning	Early-stage validation [58]
Cellular target mapping	Chem-CLIP-Map-Seq	Direct RNA target/site identification	Cellular mechanistic evidence [49]	Requires chemically modified probes
Functional degradation	RIBOTAC	Targeted RNA depletion	Converts occupancy into activity [22,23]	Effector recruitment and pharmacology
Direct cleavage	Small-molecule–cleaving conjugates	Site-selective RNA cleavage	Catalytic functionalization [48,50]	Potential collateral reactivity

10. Challenges in Developing Sequence-Selective RNA Therapeutics

10.1 Selectivity against the transcriptome

The human transcriptome contains many repeated or related structural motifs. A ligand that recognizes one internal loop or bulge may therefore interact with other RNAs carrying similar motifs [1–4,10–12]. Selectivity cannot be evaluated solely with a single unrelated RNA competitor; transcriptome-wide profiling is increasingly necessary.

10.2 Cell permeability and physicochemical properties

Many high-affinity RNA-binding molecules contain multiple basic or polar groups that can impair membrane permeability, passive diffusion, metabolic stability, and oral drug-like behavior [10–12,43]. The optimization problem is therefore multidimensional: increasing affinity should not simply be pursued at the cost of permeability or selectivity.

10.3 Structural heterogeneity

RNA structures can differ substantially between purified biochemical systems and living cells. RNA-binding proteins, ion concentrations, cotranscriptional folding, translation, molecular crowding, and interactions with other RNAs can alter the accessible conformational ensemble [29–32]. A pocket discovered in vitro may therefore be inaccessible in its physiological state.

10.4 Functional versus nonfunctional binding

A central challenge is that many RNA-binding interactions may be biologically silent [22]. This means that ligand discovery must eventually incorporate functional-site annotation. A structurally attractive pocket is more valuable when it lies within a regulatory element controlling splicing, translation, degradation, processing, localization, or RNA–protein recognition.

10.5 Resistance

RNA mutations can modify or eliminate a ligand-binding motif, especially in viruses and rapidly evolving organisms. RNA-targeted therapeutics may therefore benefit from compounds recognizing multiple structural determinants or from strategies that exploit conserved RNA architecture rather than a single nucleotide [42,47]. Degrader strategies may add an additional layer of complexity because resistance can arise through altered nuclease recruitment, RNA sequence variation, or changes in cellular RNA metabolism.

11. Strategies for Improving RNA Ligand Selectivity

Several complementary principles are emerging. First, exploit higher-order structure rather than sequence alone. A ligand that recognizes a three-dimensional motif generated by several noncontiguous nucleotides can achieve better discrimination than a ligand dependent on a short linear sequence [1–8]. Second, exploit induced fit. Cryptic-pocket ligands can take advantage of local conformational rearrangements that are less favorable for unrelated RNAs [14,20,21]. Third, use multivalency. A pair of weak interactions can become highly selective when both motifs occur in the correct spatial relationship within the target RNA [20,22,44]. Fourth, optimize cellular function rather than affinity alone. Compounds should be ranked according to a composite profile involving affinity, selectivity, cell entry, target engagement, and functional response [2,3]. Fifth, integrate orthogonal validation. Biophysical binding, mutational analysis, chemical probing, structural determination, competition experiments, and cellular target mapping should ideally converge on the same mechanism [29,37,49].

12. Emerging Discovery-to-Translation Framework

The future of RNA small-molecule discovery will likely depend on integration rather than reliance on a single screening technology. A practical workflow can be divided into five interconnected stages, shown in Fig. 3.

Disease-associated RNA sequences should first be prioritized on the basis of biological importance, conservation, abundance, cellular compartment, and functional structure. Secondary- and tertiary-structure prediction should be integrated with SHAPE/DMS measurements, comparative genomics, molecular dynamics, and pocket-detection algorithms. Candidate pockets can then be screened using RNA-focused libraries, fragments, combinatorial motif libraries, virtual screening, or phenotypic approaches. The binding site should be confirmed using structural biology, mutational analysis, orthogonal biophysics, chemical probing, and cellular target-engagement assays. Lead compounds should be optimized not only for affinity but also for selectivity, pharmacokinetics, intracellular target occupancy, and desired biological mechanism. Functionalization with degraders or cleavage groups may be considered when simple occupancy is insufficient [20–24,46–50].

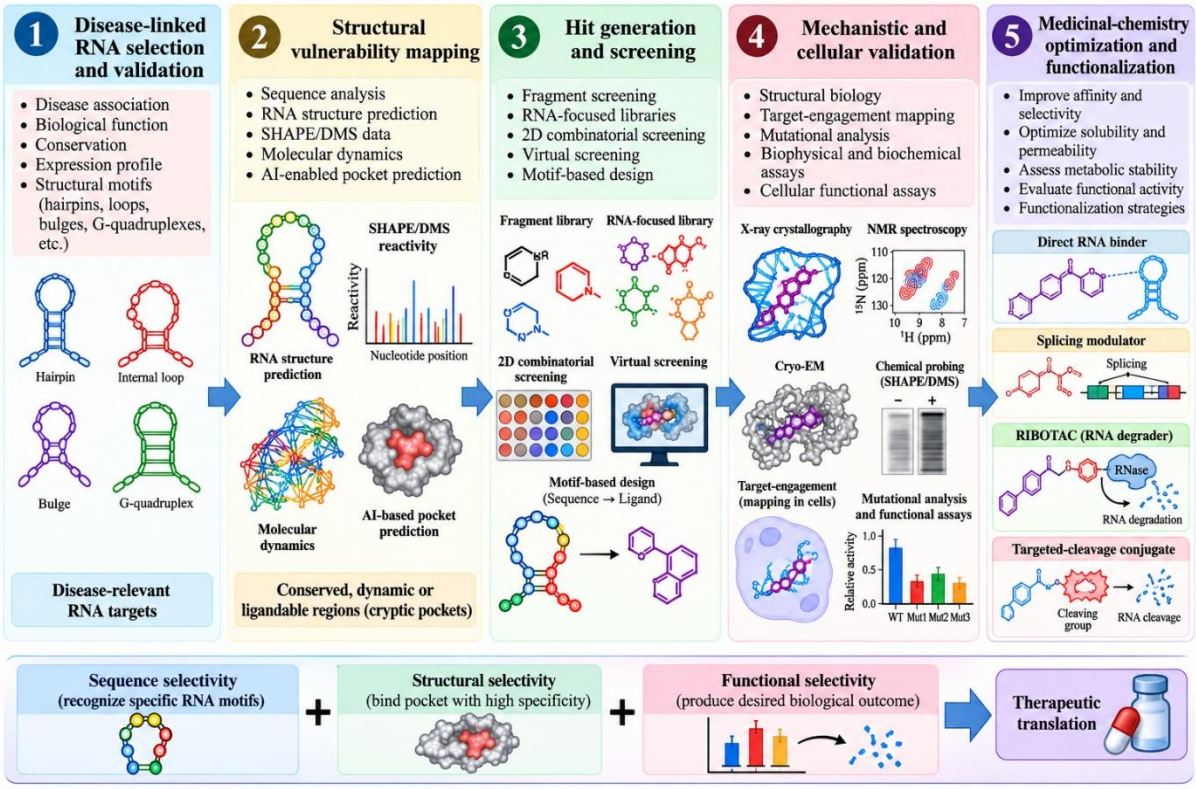

Figure 3. From cryptic RNA pocket to sequence-selective therapeutic.

A five-stage translational schematic: **(1)** disease-linked RNA selection and validation; **(2)** structural vulnerability mapping using sequence analysis, SHAPE/DMS, molecular dynamics, and AI-enabled pocket prediction; **(3)** hit generation using fragment screening, RNA-focused libraries, 2D combinatorial screening, virtual screening, and motif-based design; **(4)** mechanistic and cellular validation through structural biology, target-engagement mapping, mutational analysis, and functional assays; and **(5)** medicinal-chemistry optimization and functionalization as direct binders, splicing modulators, RIBOTACs, or targeted-cleavage conjugates. The overall concept emphasizes that sequence selectivity, structural selectivity, and functional selectivity must converge before therapeutic translation.

13. Future Perspectives

RNA-targeted small-molecule discovery is moving toward an integrated **structure–chemistry–function** paradigm. The next generation of approaches is unlikely to depend on discovering a single exceptionally high-affinity ligand. Instead, successful therapeutics will need to combine adequate affinity with selective recognition of disease-relevant structures, appropriate intracellular exposure, productive target engagement, and a mechanism that generates a sufficiently large biological response [3,4,54,55]. The growing number of RNA–ligand data points provides an important foundation for machine learning. R-BIND and related datasets have already revealed recurring physicochemical patterns, while newer RNA-focused libraries are generating larger collections of structurally annotated compounds [10–12,52,56].

A particularly important future direction is dynamic pocket discovery. Conventional virtual screening typically assumes a relatively fixed receptor. For RNA, however, the relevant binding state may be sparsely populated and induced by the ligand itself. Computational strategies that sample conformational ensembles rather than a single structure may therefore outperform static docking for cryptic RNA targets. The development of dedicated deep-learning pocket models such as SMARTPocket is an early indication of this transition [58].

Another major opportunity is the integration of in-cell structure mapping with medicinal chemistry. Rather than identifying a pocket under idealized biochemical conditions and subsequently testing it in cells, future workflows may begin with physiological structural maps and prioritize pockets that are demonstrably accessible in disease-relevant cellular states [29–32]. This could help reduce the translational gap between biochemical affinity and cellular activity. The concept of programmable RNA pharmacology is also expanding. RNA binders may serve as molecular glues, degradation triggers, cleavage agents, translation modulators, splicing regulators, or recruiters of endogenous RNA-processing proteins [22–28,48,50]. Thus, chemical recognition of an RNA structure can increasingly be regarded as an entry point into a broader therapeutic mechanism.

Finally, cryptic-pocket discovery may expand the definition of the druggable transcriptome itself. The critical question is no longer simply whether an RNA has an obvious binding cavity. Instead, the more useful question is whether the RNA can transiently access a chemically exploitable conformation that can be stabilized, remodeled, or functionally coupled to an effector. This conceptual shift transforms RNA structural dynamics from a challenge into a potential source of selectivity.

14. Conclusions

Small-molecule targeting of structured RNA has progressed from isolated examples of ribosomal or metabolite recognition to a broader framework encompassing riboswitches, untranslated-region structures, repeat expansions, precursor microRNAs, viral RNAs, lncRNAs, and disease-associated pre-mRNAs. The discovery of cryptic RNA pockets further expands this landscape by showing that ligandable sites may be generated through RNA conformational rearrangement rather than existing as static cavities. Three developments are particularly important: **(i)** systematic identification of RNA–small-molecule interactions through focused and combinatorial screening, **(ii)** structure-informed and sequence-based design strategies that improve selectivity, and **(iii)** functionalization approaches that convert binding into degradation, cleavage, splicing modulation, or other cellular outcomes. The cobalamin-riboswitch paradigm illustrates how cryptic-pocket discovery can directly inform medicinal chemistry, while RIBOTACs demonstrate how RNA recognition can be transformed into catalytic biological activity. Clinically validated SMN2 splicing modulation further demonstrates that structured RNA can support therapeutically relevant small-molecule pharmacology. The field is now entering a phase in which structural biology, in-cell chemical probing, high-throughput screening, AI-assisted structure prediction, deep-learning pocket detection, and medicinal chemistry can be connected into a single workflow. The combination of these technologies offers a route toward discovering sequence-selective, structure-selective, and functionally productive RNA therapeutics. The future challenge is therefore not simply to bind RNA, but to identify the right RNA structure, the right pocket, the right cellular context, and the right mechanism of action.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Full Form
AI	Artificial Intelligence
Cbl	Cobalamin

cryo-EM	Cryo-Electron Microscopy
DMS	Dimethyl Sulfate
HTS	High-Throughput Screening
MD	Molecular Dynamics
NMR	Nuclear Magnetic Resonance
RNA	Ribonucleic Acid
RIBOTAC	Ribonuclease-Targeting Chimera
SAR	Structure–Activity Relationship
SHAPE	Selective 2′-Hydroxyl Acylation analyzed by Primer Extension
SMN2	Survival Motor Neuron 2
SPR	Surface Plasmon Resonance
UTR	Untranslated Region
WT	Wild Type
2D	Two-Dimensional
3D	Three-Dimensional

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