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Rational ligand design for RNA: the role of static structure and conformational flexibility in target recognition

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Abstract

The role of static structure and conformational flexibility in the recognition of RNA targets by small molecule ligands is discussed with emphasis on the natural aminoglycoside antibiotics and their promiscuity in RNA target binding. A brief overview is given of previous efforts to design simplified aminoglycoside derivatives targeted at the bacterial decoding site RNA. © 2002 Société française de biochimie et biologie moléculaire / Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Drug design; RNA; Three-dimensional structure; X-ray crystallography

1. Introduction

Following the wide acknowledgment of RNA as a valid molecular target for therapeutic intervention [1–5], the repertoire of contemporary drug discovery tools has been deployed to develop novel RNA-targeted ligands [6–8]. Among these tools, structure-based drug design stands out as perhaps the most ambitious approach, fueled by a plethora of three-dimensional structure information emerging from X-ray crystallographic studies of the ribosome [9–13] and fragments thereof [14,15]. While structure-based ligand discovery for RNA targets may be the potentially most rewarding method, its success will critically depend on a precise understanding of the conformational flexibility of RNA domains and its role in adaptive ligand binding. The importance of adaptive processes is well acknowledged for ligand binding to in vitro-selected RNA aptamers [16], peptide– [17] and protein–RNA [18] interactions. Whereas the extent of structural adaptation of aptamer ligand pockets upon binding of their cognate substrates has readily been attributed to the evolution of an RNA scaffold around an immobilized target molecule [16], conformational capture and adaptation in molecular recognition of natural RNAs might be more widespread than previously anticipated. In a recent insightful review, Leulliot and Varani [18] have pointed out that both induced fit and conformational capture are key determinants for the control of specificity and biological function in RNA–protein complexes.

2. Conformational flexibility in aminoglycoside–RNA interactions

It is tempting to speculate that mechanisms involving conformational adaptation might also play a role for small molecule interactions with natural RNAs. Michael and Tor [19] have suggested earlier that a flexible recognition model may best describe the interaction of aminoglycosides with RNA. Conformational capture and adaptation may contribute to the bewildering promiscuity observed in aminoglycoside binding to a variety of RNA targets [20–22] (Fig. 1), notwithstanding their highly specific biological function, which is elicited by precise molecular recognition of the decoding site in 16S bacterial rRNA [11,23–25]. Although the use of natural aminoglycoside antibiotics in antibacterial therapy has fallen out of favor in all but few indications due to compound toxicity and widespread bacterial resistance [26], aminoglycosides provide so far the best validated paradigm in RNA target recognition [1–3]. The potential of aminoglycosides for high affinity binding to a number of

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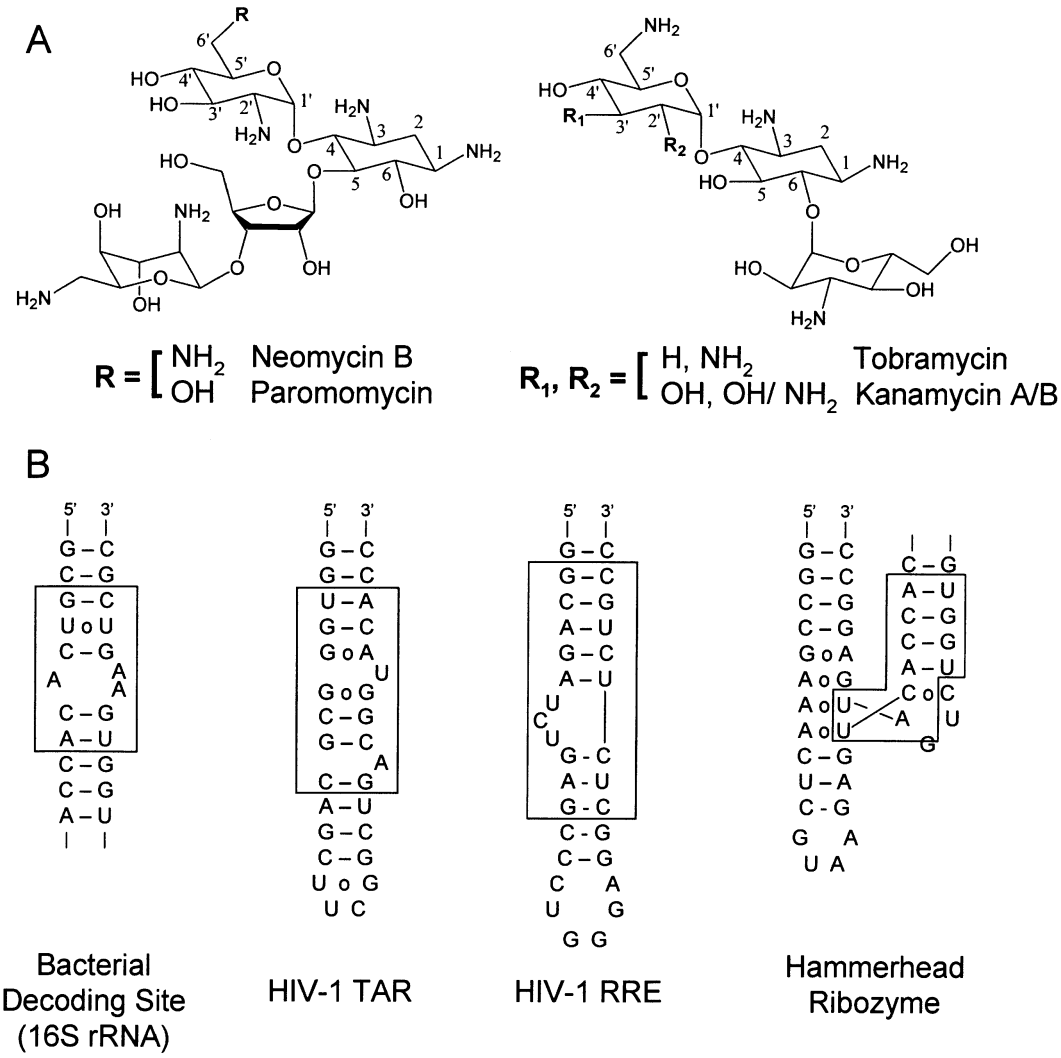

Fig. 1. Aminoglycosides and some of their RNA targets. (A) The therapeutically relevant aminoglycoside antibiotics fall into two classes, comprising the 4,5-linked 2-deoxystreptamines (2-DOS), such as neomycin B and paromomycin, and the 4,6-linked 2-DOS, including tobramycin and the kanamycins. (B) Natural aminoglycosides elicit their antibacterial activity by binding to the decoding site in 16S rRNA [23–25]. In addition, aminoglycosides have been shown to bind with affinities in the low micromolar range and interfere with the functions of the HIV TAR [40] and RRE [41] regulatory RNA elements, the hammerhead [42] and hepatitis delta virus [43] ribozymes, self-splicing group-I introns [44], bacterial RNaseP [45], and tRNA [29,46]. The approximate binding regions of aminoglycosides in the secondary structures of the decoding site, HIV TAR, RRE, and the hammerhead ribozyme are indicated by boxes.

unrelated RNA motifs, not accounted for by merely unspecific electrostatic interactions of the positively charged compounds, has been attributed earlier to their unique structure, which provides a rigid scaffold for the directed spatial presentation of hydrogen bond donor groups [27,28]. The initial recognition of different RNA targets by aminoglycosides might thus involve interactions of distinct subsets of amino and hydroxyl groups, combined with displacement of target-bound metal cations [27,28] and stacking of sugar and carbocyclic moieties. These interactions might provide sufficient affinity to allow aminoglycosides to select in various unrelated RNAs conformational states that are favorable for binding (Fig. 2). It has been suggested, for example, that many aminoglycosides can bind specifically to the hammerhead ribozyme, yet in a number of different orientations, directed by the position of metal binding sites in the RNA [27]. The initial phase of target recognition might involve conformational capture of a state that is a minor population in the ligand-free RNA, not necessarily related to the conformation required for its biological function. A striking example of conformational capture of a non-native RNA state has been described by Westhof and coworkers who found that the aminoglycoside tobramycin binds specifically to $tRNA^{Asp}$ and thereby locks it in a conformation that is no longer recognized by its cognate tRNA synthetase [29]. In certain RNA targets, such as the bacterial decoding site rRNA, the initial conformational capture by an aminoglycoside ligand might be followed by a structural rearrangement, leading to adaptation of the binding site and formation of additional intermolecular contacts that contribute to both binding affinity and specificity (Fig. 2). With their ability to recognize a variety

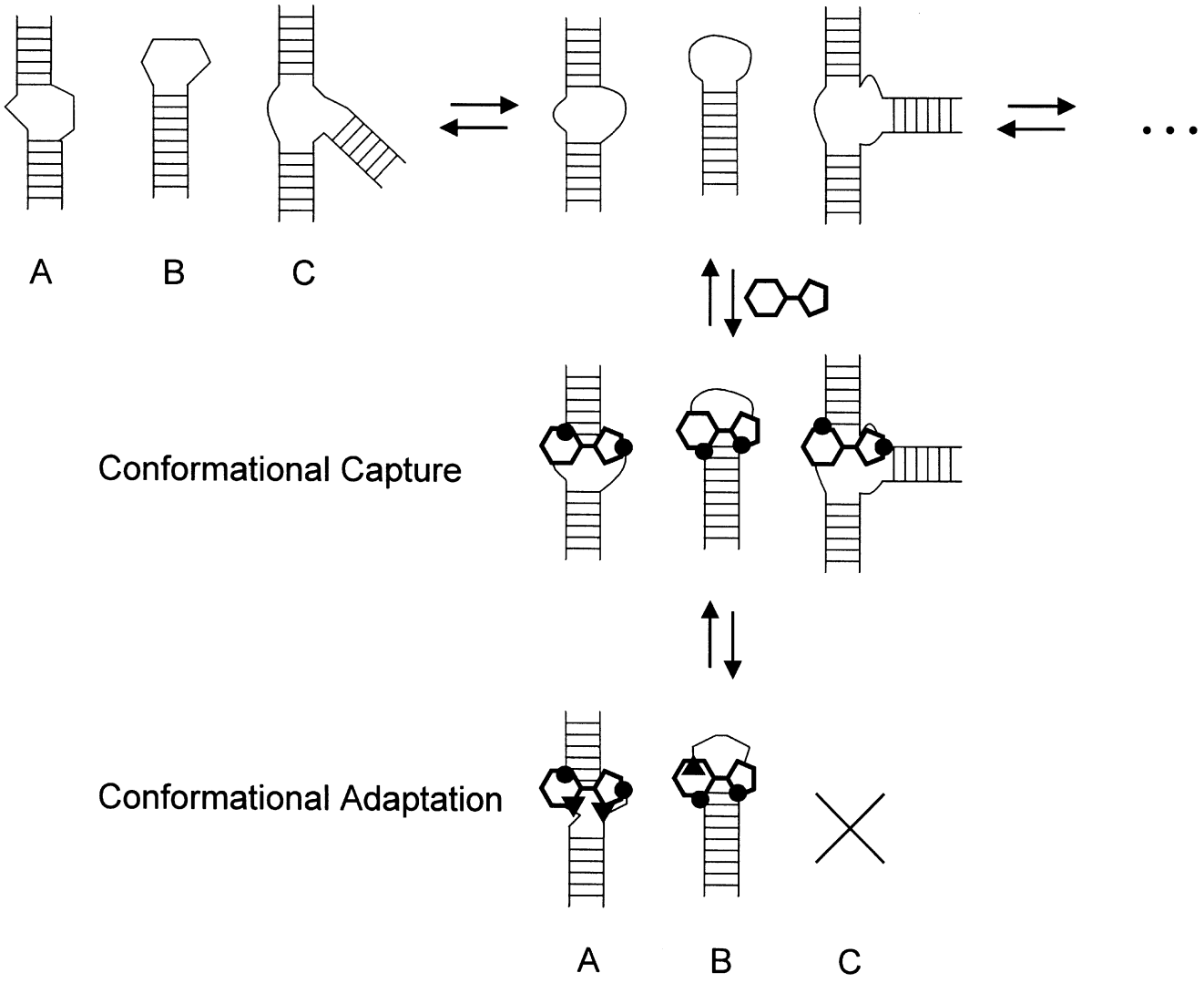

Fig. 2. Conformational capture and adaptation in ligand binding to RNA. The targets A, B, and C contain conformationally flexible structure elements that, in the ligand-free RNAs, account for an equilibrium of states, one among which allows sufficiently strong molecular interactions (filled circles) with a ligand leading to complex formation. For some of the RNAs (A, B), the conformation captured upon complexation may undergo an adaptative structural transition that gives rise to additional intermolecular contacts (filled triangles) and even stronger ligand binding. Other targets (C), lacking this conformational adaptation, might display lower specificity and affinity for the ligand. Following this scheme, RNA-binding ligands, like the aminoglycosides, might be promiscuous in binding to a range of different targets, yet display high specificity in eliciting biological function by interaction with a particular RNA structure, such as the bacterial decoding site RNA. For a model describing conformational capture of a biologically inactive state of an RNA by ligand binding, see also Fig. 10 in the publication by Westhof and coworkers [29].

of unrelated RNA targets, aminoglycosides may thus be considered as the ultimate “RNA-friendly” small molecule ligands, evolved in nature as modulators of RNA functions [21].

3. Consequences for structure-based ligand design

The dynamic structure of many RNA targets renders structure-based ligand design a formidable challenge. Specifically, the balance between sites of promiscuous interactions and highly selective sites depends highly on the energy landscape of the ligand and RNA interfaces in the absence of the interacting partners. Thus, the energetics of complex formation is determined not only by the intermolecular contacts of the bound state but also by the energetic penalties associated with conformational capture and adaptation in either binding partner. As a consequence, while constructing binding site hypotheses for use in rational drug design, one has to consider that residues critical for binding may not make direct contact with the ligand, yet they either define the correct structure of the binding site, or facilitate a favorable conformational change induced by initial binding of the ligand, or even disfavor conformations of the ligand-free state. From the perspective of the ligand, specificity for recognition of a flexible RNA target may be obtained by precisely defined intermolecular interactions involving functional groups on a rigid small molecule

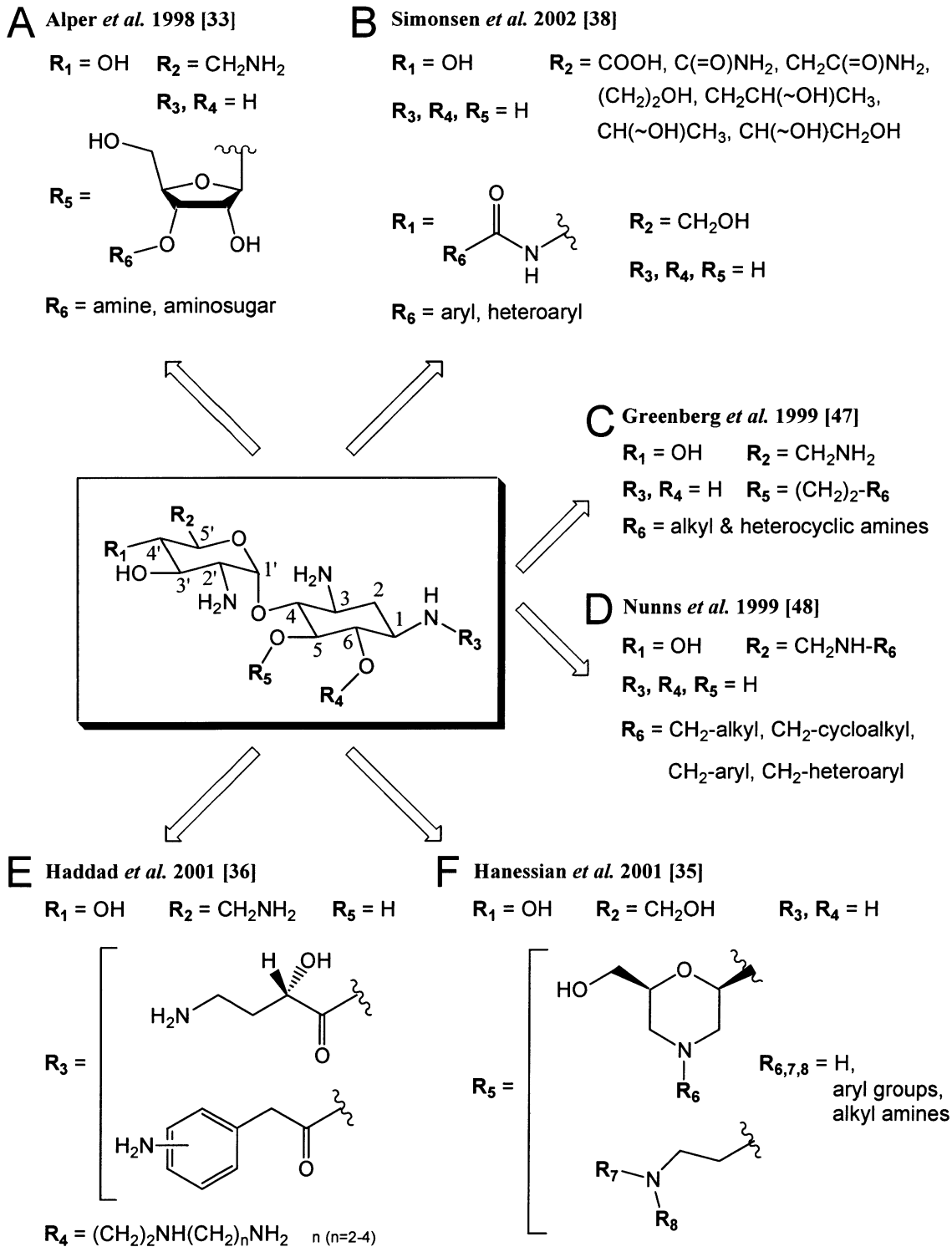

Fig. 3. Neamine and paromamine libraries that have been synthesized for testing against the bacterial decoding site RNA. The ribostamycine compounds [33] (A), and the neamine [36] (E) and paromamine [35] derivatives (F) were outlined based on an NMR model of a complex between paromomycin and a decoding site oligonucleotide [31]. The paromamine library [38] (B) was designed using structural data from a crystal structure of paromomycin bound to the 30S ribosomal subunit [11]. The neamine derivatives (C) [47] and (D) [48] were described without explicit reference to a structure-based design strategy.

scaffold. However, the entropic cost of fixing certain degrees of freedom in the ligand may ideally be moderated by allowing for residual flexibility within the small molecule scaffold. Again, the natural aminoglycoside antibiotics provide exemplary models illustrating this principle, as is attested by their restricted conformational flexibility, which

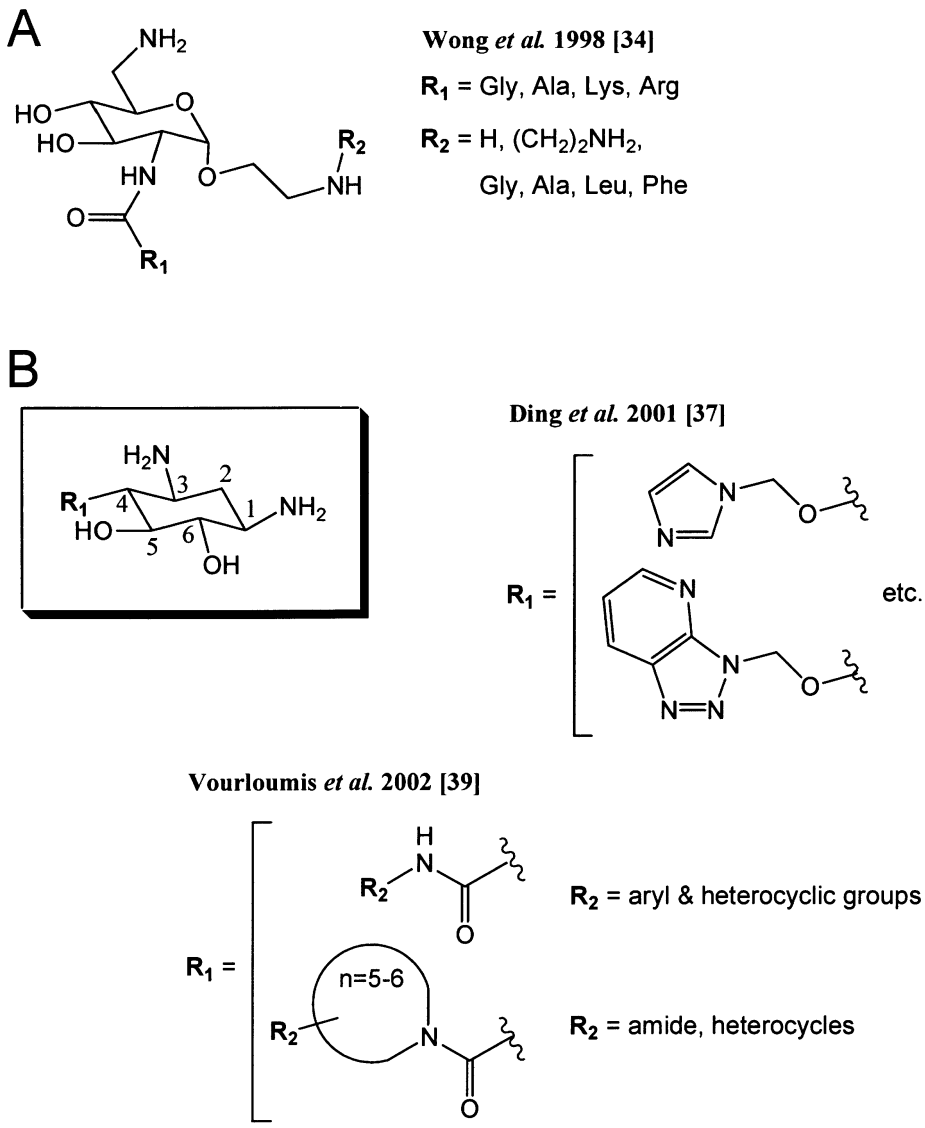

Fig. 4. Amino-glucosamine (A) and 2-deoxystreptamine (2-DOS) (B) libraries that have been synthesized for testing against the bacterial decoding site RNA. The amino-sugar compounds [34] (A), and the heterocyclic 2-DOS derivatives (B) described by Ding et al. [37] were designed based on an NMR model of a complex between paromomycin and a decoding site oligonucleotide [32]. The library of 2-DOS amides (B) outlined by Vourloumis and coworkers [39] was designed using structural data from a crystal structure of paromomycin bound to the 30S ribosomal subunit [11].

is considered instrumental for their RNA recognition capacity [28].

4. The role of water

In addition to restricted flexibility in functional groups that are not key determinants for specificity, RNA-targeted ligands may exploit the plasticity of interaction surfaces mediated by water molecules. Large parts of the first hydration shell, in particular electrostatically confined water molecules bound at sites structurally conserved among RNA architectural motifs [30], have to be considered as integral parts of RNA tertiary structure [31]. While the presence of structural water molecules in RNA targets may allow for additional ligand contacts, rational ligand design is further complicated by the partial rearrangement of the hydration shell during conformational transitions in flexible RNA.

5. Ligand design for the ribosomal decoding site RNA

In order to successfully design ligands for RNA targets, the above outlined implications of conformational flexibility in RNA call for a detailed analysis of structural data, including both three-dimensional structures of the free RNA and of the RNA target in complex with model compounds. Apart from in vitro-selected aptamers [16], the bacterial decoding site of 16S rRNA is the first target for which such information is available. Three-dimensional structures have

been solved for RNA oligonucleotides representing the bacterial decoding site in complex with aminoglycosides, determined by NMR [32] and X -ray crystallography [14,15], as well as for the whole 30S ribosomal subunit, free [9] and in complex with the ligands [11,12], determined by crystallography. Among these structures, an NMR-based model of paromomycin bound to the decoding site [32] has been used to design derivatives of ribostamycin [33], amino-glucosamine [34], paromamine [35], neamine [36], and 2-deoxystreptamine [37] (Figs. 3 and 4). A more recent crystal structure of paromomycin bound to the decoding site RNA in the 30S ribosomal subunit [11] provided the basis for the design of paromamine libraries [38] and 2-deoxystreptamine amides [39] (Figs. 3 and 4). Most of these compounds have been tested in either one or a combination of assays, including binding to the bacterial decoding site RNA, inhibition of bacterial in vitro translation, and antimicrobial activity. Interestingly, few of the designed derivatives were similarly or more active than their parental compounds, neamine and paromamine. None of the synthetic ligands matched the more potent natural aminoglycosides such as neomycin B and paromomycin. However, the antibacterial activity of at least one series of designed antibiotics does not appear to be compromised by resistance-conferring bacterial enzymes such as acetyl- and phosphotransferases [36].

6. Lessons and conclusions

The rationale behind the ligand design approaches described above was the simplification of the complex structures of natural aminoglycosides, resulting in smaller molecules that are amenable to medicinal chemistry programs. These strategies implicitly acknowledge that it might be difficult to replace simultaneously aminosugar and deoxystreptamine building blocks which, when linked together, give rise to the unique structural features of aminoglycosides. Despite that the constitution of both aminosugars and deoxystreptamine contributes to the undesirable pharmacological profiles of natural aminoglycosides, it makes sense in a rational design exercise to keep at least one of these moieties in order to increase the probability that active RNA-binding ligands are obtained. Thus, experimentally determined binding affinities and biological activities may be used in a consecutive round of molecular design to improve the pharmacological profile of the compounds while removing unwanted functionalities. Undoubtedly the ultimate challenge in ligand design for the bacterial decoding site are such approaches that completely abandon the aminoglycoside chemistry in favor of novel “RNA-friendly” scaffolds that are uncompromised by undesirable pharmacological profiles and by bacterial resistance. The successful design of RNA target-specific ligands unrelated to aminoglycosides will critically depend on the availability of high quality structure data from X-ray crystallography.

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