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Towards the discovery of drug-like RNA ligands?

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Targeting RNA with small molecule drugs is an area of great potential for therapeutic treatment of infections and possibly genetic and autoimmune diseases. However, a mature set of precedents and established methodology is lacking. The physicochemical properties of RNA raise specific issues and obstacles to development, and contribute to explain the distinct characteristics of natural RNA ligands, including antibiotics. Yet, RNA-targeting strategies are being implemented to reinvigorate antibacterial discovery by using the ribosomal X-ray structures to modify known antibiotics. To exploit further these structures, we suggest the use of existing protein kinase-directed libraries of drug-like compounds to target the A-site of the bacterial ribosome, on the basis of a specific structural hypothesis.

The targeting of RNA opens up a largely unexploited category of therapeutic targets for small molecule drug discovery [1]. Macrolide, aminoglycoside and other antibiotics target RNA in the bacterial ribosome. Their widespread clinical use demonstrates that RNA is an important potential drug target [2,3]. Oxazolidinones also target the ribosome and became the first novel class of antibiotics to enter the market in about 30 years [4]. Thus, the development of novel inhibitors of the bacterial ribosome is an important response to the threat represented by increasing bacterial resistance. The X-ray structures of ribosomal subunits [5] revealed that the clinically used antibiotics bind to RNA rather than protein regions, reinforcing the importance of RNA as a drug target. Yet, targeting RNA for drug design is perceived as problematic. Some companies that had focused in this area, such as RiboTargets (now Vernalis, Cambridge, UK) and Anadys (San Diego), have shifted towards protein targets, which are considered as 'more druggable'. These moves reflect a broader trend in the pharmaceutical industry, where antibiotic development is being scaled back or discontinued [6].

Recent reviews have described the status of drug candidates derived from ribosome-binding natural products [7] and surveyed X-ray structures of antibiotics bound to the ribosome [8,9]. Many of these antibiotics are natural products with physicochemical properties at odds with the prevailing views of drug-likeness, raising questions regarding the druggability of RNA. Are the properties of the RNA-targeting antibiotics irrevocably dictated by the nature of RNA, or are we only beginning to learn how to design more drug-like RNA ligands? Here, we discuss strategies that adapt established medicinal chemistries to the development of novel antibiotics on the basis of structure-based drug design (SBDD) that targets the bacterial ribosome. We also discuss prospects for targeting a broader range of RNA with small molecules.

Special issues facing medicinal chemistry targeting RNA

Drug-likeness and RNA-binding ligands

Much RNA-targeting effort exploits large molecules (antisense, protein nucleic acid, ribozyme, and RNA interference). Ribosome-binding antibiotics demonstrate that small molecule RNA ligands can be drugs. Yet, many of these antibiotics raise questions regarding properties typically associated with clinically relevant drugs [10].

Drugs need to be more than ligands, especially when oral bioavailability is desired. For example, they require a balanced polar character to be water soluble and cross biological membranes. They must be readily produced chemically, biosynthetically or by some hybrid protocol. The empirical observation of physicochemical

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1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2006.09.001

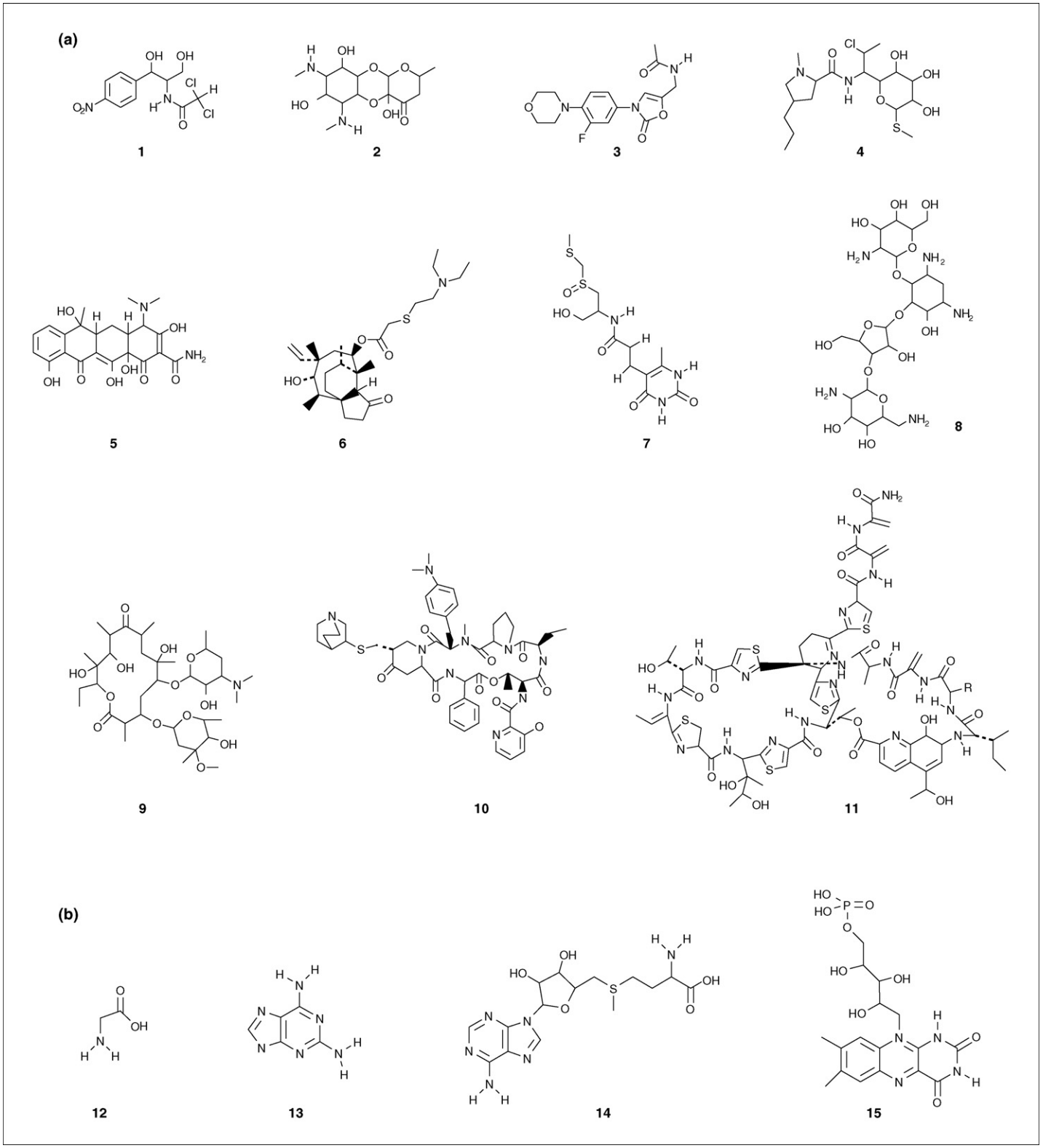

FIGURE 1

(a) Antibiotics that are believed to target rRNA. Relatively drug-like antibiotics: chloramphenicol (**1**, MW = 323, K_d = 2 μM [71]), spectinomycin (**2**, MW = 332, K_d = 0.2 μM [72]), linezolid (**3**, MW = 337, an oxazolidinone, K_d of the closely related compound eperezolid is reported as 40 μM or weaker [73]), clindamycin (**4**, MW = 425, a lincosamide, K_d = 8 μM [74]), tetracycline (**5**, MW = 444, K_d = 1 μM or weaker [75]), tiamulin (**6**, MW = 494, a pleuromutilin in development, K_d = 0.14 μM [76]), sparsomycin (**7**, MW = 363, K_d = 0.66 μM for yeast ribosomes with polyphenylalanine [77]). rRNA-binding antibiotics that are not drug-like according to the Lipinski criteria: paromomycin (**8**, MW = 616, an aminoglycoside, K_d = 12.3 μM [78]), erythromycin (**9**, MW = 734, a macrolide, K_d = 0.99 μM [79]), quinupristin (**10**, MW = 1022, a streptogramin, K_d for related streptogramins virginiamycin M and S are reported as 0.32 and 2.5 μM, respectively, with synergestic effects when the compounds are bound together [80]) and thiostrepton (**11**, MW = 1652, a thiazole, K_d = 0.1 μM [81]). Chloramphenicol, tetracycline, linezolid, clindamycin, paromomycin, erythromycin and quinopristin illustrate the most important chemical classes of clinically used ribosome-binding antibiotic.

BOX 1

The Lipinski rules.

- Molecular weight <500 Da;
- c log $P < 5$;
- <10 acceptor groups (O + N);
- <5 donor groups (O–H, N–H).

The Lipinski rules are typical of principles used to guide medicinal chemistry towards orally bioavailable 'drug-like' molecules [10]. Exceptions might occur by active transport.

properties typically associated with drugs has led to the notion of 'drug-likeness'. The so-called Lipinski rules (Box 1), derived from compounds in clinical use [10], provide popular criteria for drug-likeness. Compounds that fit these rules are small, their balanced polarity is reflected by a limited number of hydrogen-bond donors or acceptors, which reflects their water:octanol partition coefficient. Lipinski suggested that exceptions to these rules might be circumventing the membrane barrier by entering the cell through active transport.

Many ribosomal antibiotics (Figure 1a) violate these rules (Box 1). Some exceed the limits for molecular weight, hydrogen-bond donors and acceptors. Some are overly polar (e.g. aminoglycosides such as **8**, Figure 1a) or hydrophobic (e.g. thiazoles, such as **11**). Aminoglycosides and streptogramins (**10**) (reviewed in [4]) are given intravenously [11]. Thiazoles [12] are restricted to veterinary applications, as were the original streptogramins and pleuromutilins [7]. If only these types of molecules can effectively target RNA, it might be necessary to tackle difficult issues, such as targeting active transport or devising biosynthetic means of producing hard-to-synthesize molecules. The prospects of finding classically drug-like RNA ligands would, then, appear daunting, and one might aim to produce only injected drugs.

There are counterexamples. Chloramphenicol and linezolid (Figure 1a, **1** and **3**) are drug-like. Tetracycline (**4**) fits most of the Lipinski criteria. These compounds (and some macrolides) are administered orally.

In addition, nature provides examples of small RNA ligands as regulators of biological functions (Figure 1b). Bacterial mRNA regulatory elements control biosynthesis of small molecule metabolites by binding to them [13]. Often, these molecules are more drug-like than most of the natural ribosomal antibiotics. These findings were foreshadowed by studies of RNA aptamers [14] generated through *in vitro* evolution to recognize a specific ligand. These compounds achieve potency and can discriminate remarkably among small molecules [15]. The compounds in Figure 1b, together with the few drug-like examples in Figure 1a, suggest that small RNA ligands with classically drug-like properties can be found.

The physicochemical properties of RNA: implications

The unique physicochemical properties of RNA help explain the properties of its ligands, as well as some difficulties in targeting it. Because RNA is highly negatively charged, many of its ligands are polycationic, potentially inhibiting cell penetration. The highly charged nature of RNA means that it is strongly solvated. Displacement of water molecules and counterions introduces extra complications compared with targeting more easily desolvated hydrophobic protein pockets.

DNA is also polyanionic. It is targeted by drugs that show weak sequence selectivity, and often act either by direct incorporation in the double-helix or through covalent modification or cleavage of DNA. These mechanisms are very different from the specific recognition of pockets via non-covalent binding, which is addressed in the review.

The components of RNA (and DNA) are not as chemically varied as those of proteins. One might therefore question how often RNA folding will produce unique 3D pockets for specific binding by small ligands. However, available 3D RNA structures demonstrate that tertiary interactions in RNA can produce remarkably specific binding to surfaces, rarely seen with DNA. Combinations of sequences and folds in large cellular RNAs have even greater potential to form diverse pockets.

Overall, RNA properties will influence the chemical space of RNA ligands to include areas not covered by protein ligands. Hence, generic compound libraries might not be optimal for RNA ligands.

Another problem might arise when designing ligands to disrupt protein–RNA interactions. Protein–RNA interfaces, like protein–protein interfaces, are often large 'flat' surfaces [16] without well-defined binding pockets. Therefore, a protein–RNA complex might be most effectively disrupted by blocking a large part of this surface, which in turn could require a 'large' molecule.

Binding constants (K_d) reported for RNA-binding drugs illustrate another peculiar property (Figure 1). A surprising number of these drugs bind RNA with relatively weak (μM) affinity. The fact that such drugs are nonetheless used as antibiotics shows that (at least ribosomal) RNA targets can be relevant even without strong affinities typically sought in drug discovery. Weak affinity might still elicit sufficient potency if a drug interferes with conformational changes, without competing with another endogenous ligand. This might contribute to explain the efficacy of some RNA-binding drugs. Some proteins also bind RNA with affinities in the μM range [17]. For some RNA targets, these characteristics might have implications for setting potency hurdles when implementing primary screens.

The relative rarity of potent RNA ligands might be partly explained by the fact that binding of charged groups contributes relatively little to potency [18]. The interaction between ligand and receptor charges favours binding, but this is compensated by a

Sparsomycin, an antitumor agent that preferentially binds eukaryotic ribosomes, tiamulin and thiotrepton, a natural product used in veterinary applications, are also included. **(b)** Small molecules that bind to and manipulate mRNA secondary structures (called riboswitches) in gram-positive bacteria, thereby regulating their own biosynthesis: glycine (**12**, MW = 75, K_d = 30 μM for the first of two cooperative binding sites, 3–30 nM for the second), diaminopurine (**13**, MW = 150, K_d = 5–50 nM), *S*-adenosyl methionine (**14**, MW = 400, K_d = 5 nM), flavin mononucleotide (**15**, MW = 458, K_d = 5 nM). These are naturally occurring drug-like molecules manipulating function through specific binding to RNA. Riboswitch binding K_ds are from [82] and references therein. Antibiotic K_ds (b) for ribosome binding are from various sources, methods and to varying precisions. Quantitative comparisons must therefore be made cautiously, but there is a clear trend towards relatively weak binding affinities for ribosomal-binding antibiotics, as compared with some riboswitch effectors and/or the low nanomolar affinities that remain the goal of most drug discovery projects.

comparable cost of desolvating these charges. There is good evidence that hydrophobic effects, rather than polar interactions, drive ligand–receptor associations [18]. Yet, there are potent RNA ligands, such as those binding natural and artificial aptamers with low nM affinity or stronger. Most of these ligands are not highly polar (Figure 1a,b), and this might explain why RNA-binding sites are normally in, or adjacent to, non-duplex regions. Unpaired bases are available for hydrophobic and dispersion–attraction interactions, which would otherwise participate in helix stabilization. The availability of these unpaired bases contrasts with DNA and provides opportunities for achieving potency.

Altogether, it is worth investing in long-term efforts towards drug design against RNA targets, despite difficulties specific to RNA. The discovery of druggable RNA sites (i.e. pockets with a balanced polarity) will be important, and obtaining and analyzing the 3D structure of RNAs is relevant in this respect.

Structure-based tools must be adapted to RNA targets

RNA-directed medicinal chemistry lacks the large body of historical precedents available for the targeting of proteins. As a result, computational methodologies used for SBDD are more difficult to implement. For example, we are not aware of a program dedicated to detect and analyze the pockets and clefts in large folded RNA molecules, such as ribosomes. The sheer size of these structures precludes the equivalent manual analysis still frequently performed with proteins. Ideally, such software would rank cavities according to their druggability, possibly for virtual screening. Of course, the targeted sites need to be functionally relevant.

Most *in silico* docking packages rely on scoring functions that use parameters derived solely from protein data. Because of the empirical nature of these functions, it is unclear how this bias affects scoring with RNA. A recent study has compared the output of two docking packages to experimental ligand-RNA structures [19]. The paucity of ligand–RNA structures with known binding affinities hinders the calibration and validation of scoring functions for use with RNA. At least two scoring functions parameterized for RNA binding were reported [20,21], by which receptor-based docking could distinguish known RNA binders from other compounds [20,21], and new RNA binders were discovered [20,22]. Another study aimed to reproduce X-ray structures of aminoglycosides bound to their RNA target by incorporating displaceable solvent molecules and RNA flexibility with the program AutoDock [23]. Large-scale conformational changes upon ligand binding, as observed with the aptamers, present an additional challenge for theoretical methods.

Given the charged character of RNA, computational methods treating the electrostatics more rigorously, especially with respect to polar desolvation (i.e. dehydration of charges), have special importance. These methods typically rely on molecular mechanics force-fields to treat long-range electrostatics, combined with explicit or implicit solvation models. Because they are faster to compute, implicit solvation models, which treat the solvent as a continuum, are better suited to ligand discovery. They can rely on the Poisson-Boltzmann equation [24,25] (the non-linear formulation of which is recommended with highly charged systems [26]) or the more *ad hoc* generalized Born models. An interesting finding is that electrostatic 'hotspots' can arise in the major groove because of the juxtaposition of electronegative groups on the bases. The electrostatic potential is not strictly dominated by negatively charged phosphates, which are partly shielded by water and counterions. Thus, distinct 3D RNA folds are likely to provide sites for specific recognition via electrostatic interactions [26,27].

However, in addition to selective binding, druggable sites should offer a binding pocket with a well-balanced polarity. For that, the ribosome provides a learning opportunity.

Druggability of antibiotic binding sites in the bacterial ribosome

The wide spectrum of properties of rRNA ligands (Figure 1a) indicates that some ribosomal sites are more druggable than others. The X-ray structures of bacterial ribosomes [8,9,28,29] should help identify these druggable sites.

The structure of the decoding A-site of bacterial rRNA (Figure 2) suggests that this site meets the criteria for druggability. The binding pocket is largely lined with neutral bases. Binding of aminoglycoside antibiotics such as paromomycin (**8**) to the A-site interferes with a 'proofreading' mechanism of bacterial protein synthesis [28] and, thus, disrupts the fidelity of translation, resulting in impaired proteins detrimental to bacterial survival.

Another target is the peptidyl-transferase centre (PTC) of bacterial rRNA where peptide bonds are formed. Several antibiotics shown in Figure 1 (**1**,**4**,**6**,**7**,**9**,**10**, possibly **3**) interact with rRNA near the growing peptide exit tunnel of the PTC. These drugs block the tunnel, thus aborting translation [30].

Many of the ribosomal antibiotics have been observed bound to, or near, the two above-mentioned regions in X-ray structures [8,9]. Other sites might be more problematic. The binding site of the L11 protein in rRNA is the site of action of thiazole antibiotics such as thiostrepton (Figure 1, **11**). There is no X-ray structure of a thiazole bound to the ribosome and these compounds are not drug-like. Yet the 'greasiness' of the thiazoles suggests that their binding site is not dominated by a polar surface, and thiostrepton's affinity for the ribosome is relatively strong. Drug-like compounds which inhibit *in vitro* translation and thiostrepton binding have been produced [31]. A model for thiazole binding has been proposed, consistent with biochemical, X-ray [32,33] and NMR data (reviewed in [3]), suggesting that this class of drugs binds a protein–RNA interface [34]. The L11 binding site thus represents an intriguing and possibly druggable target.

Modifying existing ribosomal antibiotics

Modifying known antibiotics provides a testing ground for the idea of RNA-directed drug design, using the ribosomal X-ray structures to guide the process.

Rib-X Pharmaceuticals (USA) has taken this approach. Several modifications of oxazolidinones and macrolides have been the subject of patent applications. RX-A_{667} originated from the linkage of sparsomycin to the fluoro-substituted ring in linezolid [35]. Minimum inhibitory concentrations for this compound compared favourably to those of linezolid and other antibiotics for a range of community and hospital strain pathogens. These findings demonstrate the usefulness of the X-ray structures used for postulating juxtaposed binding sites for the two parent compounds [35]. Clinical studies for compounds from this series started in December 2005.

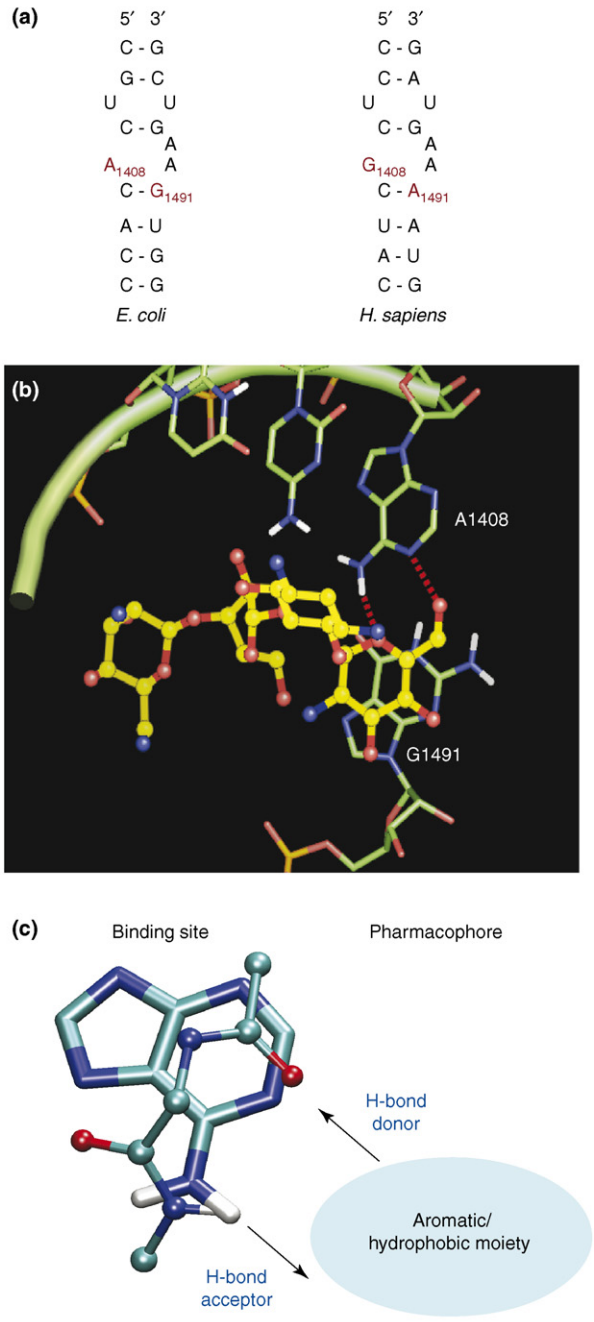

FIGURE 2

(a) Secondary structure of decoding A-site from the small ribosomal subunit. The bacterial A-site is of particular interest from a practical point of view because oligonucleotide model systems of this site are available for crystallography, NMR and compound binding assays. The prokaryotic (left) and eukaryotic (right) sequences are shown, both using the *E. coli* sequence numbering. Two key bases for selectivity between prokaryotes and eukaryotes (A1408 and G1491 in *E. coli*) are highlighted in a different colour. **(b)** X-ray structure of paromomycin bound to the *E. coli* A-site RNA (PDB entry 1J7T [83]). The hydrogen bonds mimicking a base pairing with the 'Watson–Crick face' of A1408 are highlighted by red dashes. **(c)** The similarity between the bacterial A-site RNA and kinase active site pharmacophores is illustrated. On the left is an overlay of the key hydrogen-bond donors and acceptors of the bacterial ribosomal A1408 (PDB entry 1IBK [84]) and of the kinase backbone motif of protein kinase A (PDB entry 1ATP [85]). Protein kinase A was chosen somewhat arbitrarily to represent the kinase backbone motif which has a highly conserved structure across kinases. Hydrogens were added to the hydrogen-bond donors of interest, which are the backbone nitrogen of Val123 in protein kinase A and the N6 of A1408. The two hydrogen-bond acceptors of interest are the backbone oxygen of Glu121 in protein kinase A and the N1 of A1408. On the right, the binding pharmacophore common to both active sites is illustrated schematically. A hydrophobic/aromatic moiety would stack upon G1491 in the A-site. Arrows represent hydrogen-bonding interactions, pointing to the hydrogen-bond acceptor.

Tiamulin, now in development, represents another derivative of a previously existing natural product series (the pleuromutilins). Biochemical data were used to guide the optimization of this compound, now seen bound in the PTC [36].

Aminoglycosides, which bind the bacterial A-site, have been the other main target for modification. Aminoglycosides are not orally bioavailable, providing impetus for identifying replacements.

Ring 2 of neamine (a small aminoglycoside derived from neomycin) was modified by attaching linear substituents [37]. This approach produced strong antibacterial activity against *Escherichia coli* strains expressing aminoglycoside modifying enzymes [38]. Anadys took a similar approach but later replaced the aminoglycoside 2-deoxy-streptamine scaffolds with azepane-glycosides [39]. These aminoglycoside mimetics led to a 3,5-diamino-piperidinyl triazine series with >50% protection against a lethal *E. coli* infection in mice [40]. This series shows a mechanistic profile in several assays parallel to that of aminoglycosides, including bactericidal activity, supporting the hypothesis that this series targets the A-site.

The move from aminoglycoside 'derivatives' to 'mimetics' is a major step towards improving the bioavailability of A-site inhibitors. However, options for modification of existing antibiotics are finite and resistance mechanisms provide an imperative for identifying novel classes of drugs [41]. Previous ligand discovery studies support the notion that the A-site can be targeted with drug-like compounds [2,22,42].

X-ray crystallography has played a major role in the developments described in this section. Aminoglycosides were observed in X-ray structures of 30S subunits [43–45], and in the structures of an oligonucleotide model system of the A-site [46,47]. New X-ray structures have been reported for a model fragment containing a human A-site sequence [48], and for the 70S *E. coli* ribosome [49].

Structure-based discovery of ligands is becoming a realistic strategy, for example with the targeting of kinases. We propose a specific strategy to transfer some of the experience with kinases to target RNA for the development of drug-like antibacterials.

Could kinase inhibitors target the bacterial ribosomal decoding A-site?

Kinases control many regulation pathways in eukaryotic cells by transferring a phosphate from ATP to substrate proteins. Many inhibitors of kinases, which target their ATP binding site, have been devised to influence several disease states [50]. Kinases are attractive drug targets because their ATP site can be inhibited with drug-like molecules [51]. Entire libraries of drug-like compounds specifically designed for kinases have been elaborated. We present specific reasons of why these compounds should be of interest to target the bacterial ribosomal A-site towards the design of novel antibiotics.

Key differences between the A-site in bacterial and human cytosolic ribosomes help to explain the effectiveness of antibiotics

targeted to this site [48,52]. The experimental structures of aminoglycosides bound to the A-site [44,53,54] reveal that they stack on G1491 (*E. coli* numbering) and hydrogen-bond the Watson–Crick face of A1408 (Figure 2b). G1491 and A1408 are conserved in bacteria, but are replaced by adenine and guanine, respectively, in human cytosolic ribosomes (Figure 2a) [48,52]. Consistent with the structures, biochemical and mutation data indicate that these sequence differences largely account for the bacterial selectivity of aminoglycosides [55]. Novel A-site ligands should maximize interactions with G1491 and A1408 to become clinically relevant antibiotics. Ligands targeting G1491 and A1408 are expected to contain a specific pharmacophore (Figure 2c). The pharmacophore would include a ring stacking on G1491, together with a hydrogen-bond donor to N1 of A1408 and a hydrogen-bond acceptor complementing N6 of A1408. There is a striking correspondence between these A-site pharmacophoric features and those commonly found in kinase inhibitors (Figure 2c). We propose that this analogy could be exploited for the discovery of novel drug-like A-site ligands.

For ligands binding to kinase ATP sites, a typical pharmacophore also includes a hydrogen-bond donor and acceptor combined with an aromatic ring (Figure 2c). The hydrogen-bond donor and acceptor pair allows the ligands to bind to their counterpart in the conserved kinase backbone motif in the ATP-binding site [51]. The aromatic ring reflects the shape of the binding site, which has evolved to recognize adenine as part of the nucleotide. It is empirically known that the vast majority of kinase inhibitors fit this pattern. Figure 2c shows a superposition of the hydrogen-bond donor and acceptor from the backbone kinase motif of protein kinase A with the Watson–Crick face of A1408. In both cases, the same relative spacing and orientation is observed between the donor and acceptor groups. In addition, the heteroaromatic ring typically bearing these donors and acceptors in kinase ligands could stack on G1491. In doing so, they would mimic a base-pairing with the Watson–Crick face of A1408, providing selectivity versus eukaryotic ribosomes [48,55]. This is why chemical scaffolds designed to fit the kinase active site pharmacophore also match the bacterial A-site pharmacophore.

An illustration of a strategy to exploit this parallel between the kinase and A-site pharmacophores, including specific compounds identified through virtual screening, is presented in the accompanying Box 2 and in Figure 3.

Wider applications of kinase-directed libraries?

There might be a wider range of applications to the idea of adapting abundant kinase-directed chemistry to the targeting of RNA. It is likely that there are other binding sites in RNAs, where a ligand would stack on a base while hydrogen-bonding the edge of another base. The so-called 'purine riboswitch' elements are a good example, with at least three of them being identified and characterized structurally in complex with their small molecule partners [56–58]. Although riboswitches have so far been found largely in gram-positive bacteria, they could represent a useful test case for the development of RNA-targeting ligands.

Other RNA targets

Viral RNAs

In contrast to antibacterials, the search for RNA-directed antivirals starts without proof of principle. Issues of drug-likeness and target flexibility loom larger. The first candidates might be delivered intravenously. However, any cursory inspection of the virology literature reveals the central role played by numerous RNA regulatory signals and, therefore, the immense potential of RNA-targeting therapies.

The binding site for the HIV-1 regulatory protein Tat has historically been a major focus of RNA-targeting drug design (reviewed in [59]). NMR studies of small molecules binding to the transactivation response element (TAR, the binding site for Tat) revealed a large degree of flexibility in the RNA conformation [60,61]. Small molecule ligands induce a variety of TAR–RNA structures when bound [27,62,63]. These results demonstrate the relevance of iterative 3D structural characterization for the interpretation of structure–activity relationships when targeting RNA. They also illustrate the limitations of theoretical predictions. This might be a serious issue for several RNAs. Indeed, many natural and artificial aptamers couple small molecule binding to global rearrangements in tertiary or even secondary structure [64], and function is regulated at a kinetic as well as thermodynamic level. Therefore, the coupling between small binding molecule and RNA conformational dynamics should become a crucial area of investigation.

Another attractive viral RNA target is the internal ribosomal entry site (IRES) from hepatitis C virus [65]. Although the native ligands for this RNA are large complexes of host proteins (including the 40S human ribosomal subunit), a few small natural product ligands have been identified [66,67], as well as a novel compound derived through fragment screening [68].

BOX 2

Exploiting kinase–focused libraries to inhibit the bacterial ribosomal A-site.

Many drug-like compounds were designed to target kinase ATP-binding sites, by incorporating a kinase pharmacophore (Figure 2c). This pharmacophore is similar to the A-site pharmacophore, therefore compounds designed against kinases are also interesting candidates as A-site ligands. The A-site structure at the periphery of G1491 and A1408 differs sufficiently from kinase ATP sites that A-site binders would lose their potential affinity for kinases after optimization for the A-site.

Computational docking of a kinase-directed library to the A-site can prioritize these compounds to identify the best candidate A-site ligands. Such calculations assess the overall structural complementarity of these compounds to the site, beyond a basic pharmacophore. For example, compounds with a positive charge interacting with the RNA phosphates in the major groove (Figure 3b) might favour binding to the A-site.

For illustration, we have docked a kinase library of 3500 compounds to the A-site with the program rDock (an extension of RiboDock [22]). These compounds contain the pharmacophore of interest. Figure 3a shows selected compounds with favourable docking scores and forming sensible interactions with the RNA. A common feature in these compounds is a heteroaromatic core stacking on G1491 and hydrogen-bonding A1408. These interactions are particularly important for discrimination between bacterial and eukaryotic ribosomes. The aminopyrazine moiety of **16** illustrates this interaction (Figure 3). Compound **16** also illustrates how additional favourable interactions might be formed in the RNA major groove. Thus, docking and ranking of a kinase library can identify compounds with an overall sensible fit to the decoding A-site.

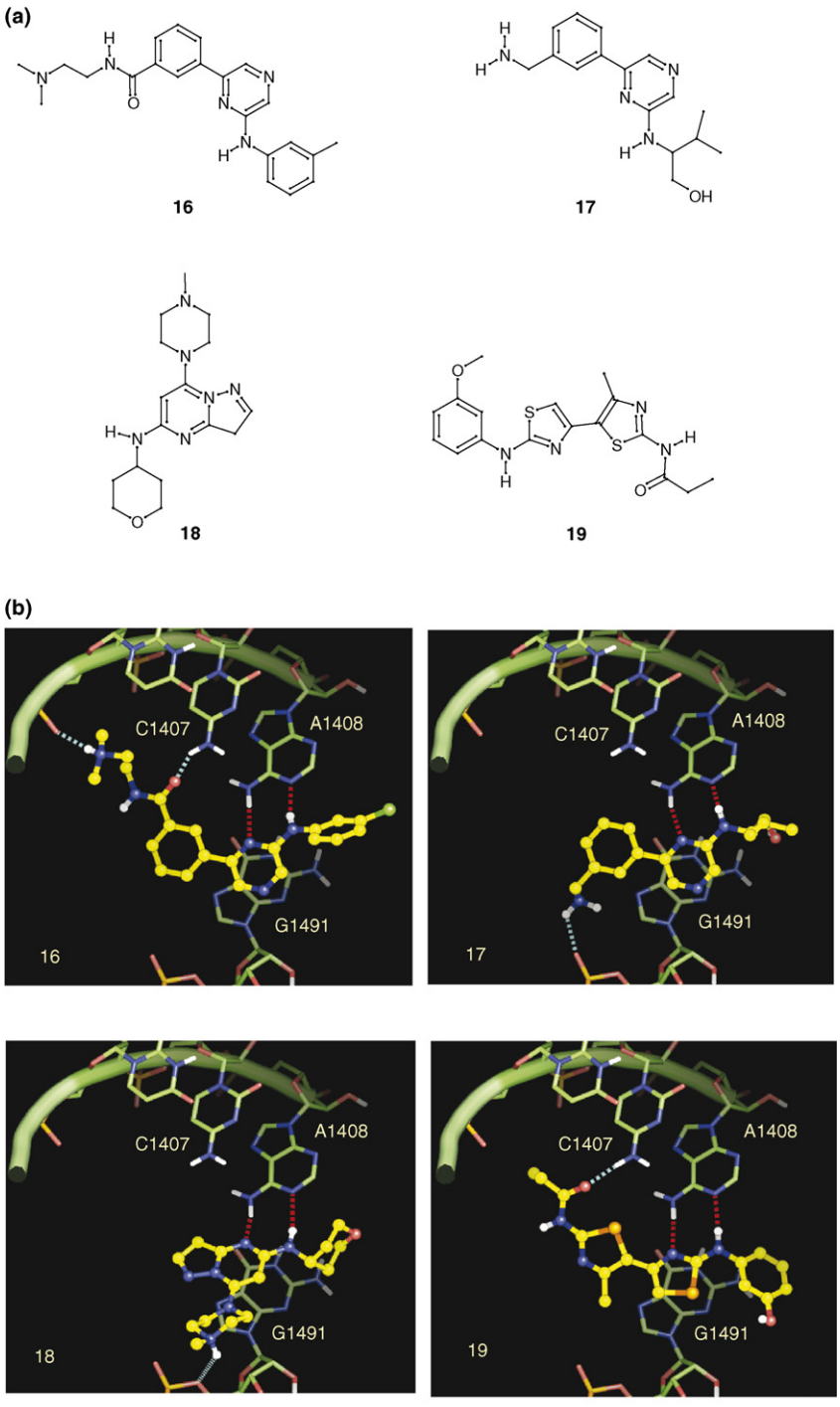

FIGURE 3

(a) Compounds **16–19** identified from an *in silico* screen of a kinase-directed library, which were identified as potential binders to the bacterial A-site RNA. **(b)** Docked orientation and positioning obtained for the compounds shown in (a), which illustrates a good structural complementarity between the compounds and the bacterial A-site. Compounds have been docked to A-site RNA coordinates derived from PDB entry 1IBK [86]. The compound number [referring to the numbering in (a)] is given in each panel. A1408 contains the hydrogen-bond donor and acceptor pharmacophore presented in Figure 2, and G1491 represents a stacking partner for the compounds. Hydrogen bonds between the compounds and A1408 are highlighted by red dashed lines. Additional hydrogen bonds are highlighted by cyan dashed lines. The docking suggests that some compounds could form ionic contacts with RNA phosphates in the major groove. To note, for example, the ionic interaction between **16** and the phosphate of U1406.

Non-coding RNAs and human genetic diseases

There is increasing awareness of the importance of parts of the genome not translated into proteins [69], including noncoding RNAs. There might be potential RNA therapeutic targets involved in signalling, alternative splicing, regulation through RNA silencing, or other as yet unimagined functions. The discovery of 'toxic RNA' as a factor in genetic diseases, such as Fragile X-associated tremor/ataxia syndrome [70], might open up yet another class of targets for RNA-directed small molecule drugs.

Conclusions

The targeting of RNA for the design of novel, small therapeutic molecules is an exciting new area of pharmacology. However, the physicochemical properties of RNA, in particular its polyanionic nature, contribute to make it a problematic target for the discovery of drug-like molecules. The success of drug design against RNA will depend on the identification of druggable sites on RNA molecules. It will also benefit from detailed investigation of the physics of ligand–RNA interactions. Molecular recognition principles derived therefrom can help focus medicinal chemistry and computational efforts towards those regions of drug-like chemical space compatible with these principles. In the meantime, much can be achieved with antibacterials by modifying known drugs.

In recent years, many industrial programs for the discovery of anti-infectives have been scaled down or terminated. The associated resources have been frequently redirected towards projects targeting kinases. The issue of bacterial resistance to existing antibiotics will, however, not disappear, and is expected to become more pressing with time. Therefore, the discovery of new antibiotics remains scientifically, therapeutically and commercially relevant. Here, we have presented how libraries of drug-like compounds targeted to kinases could also be exploited at a minimal cost to discover novel inhibitors of bacterial translation by targeting the prokaryotic ribosomal A-site. Because compounds in modern kinase-targeted libraries are drug-like, they would have a realistic chance to become clinically relevant antibiotics.

Acknowledgements

We would like to thank Dr. Roscoe Klinck (Université de Sherbrooke) for stimulating discussions.

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