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Small Molecule Microarrays of RNA-Focused Peptoids Help Identify Inhibitors of a Pathogenic Group I Intron

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ABSTRACT Peptoids that inhibit the group I intron RNA from *Candida albicans*, an opportunistic pathogen that kills immunocompromised hosts, have been identified using microarrays. The arrayed peptoid library was constructed using submonomers with moieties similar to ones found in small molecules known to bind RNA. Library members that passed quality control analysis were spotted onto a microarray and screened for binding to the *C. albicans* group I intron ribozyme. Each ligand binder identified from microarray-based screening inhibited self-splicing in the presence of 1 mM nucleotide concentration of bulk yeast tRNA with IC_{50}'s between 150 and 2200 μM. The binding signals and the corresponding IC_{50}'s were used to identify features in the peptoids that predispose them for RNA binding. After statistical analysis of the peptoids' structures that bind, a second generation of inhibitors was constructed using these important features; all second generation inhibitors have improved potencies with IC_{50}'s of <100 μM. The most potent inhibitor is composed of one phenylguanidine and three tryptamine submonomers and has an IC_{50} of 31 μM. This compound is 6-fold more potent than pentamidine, a clinically used drug that inhibits self-splicing. These results show that (i) modulators of RNA function can be identified by designing RNA-focused chemical libraries and screening them *via* microarray; (ii) statistical analysis of ligand binders can identify features in leads that predispose them for binding to their targets; and (iii) features can then be programmed into second generation inhibitors to design ligands with improved potencies.

RNA plays a variety of roles in biological systems that cement its status as a therapeutic target (*1–3*). Therefore, developing compounds that modulate RNA activity is important. Microarrays have proven to be an effective platform to identify small molecules (*4–7*) that bind protein or RNA. They have also been used to study the interactions between aminoglycosides and mimics of rRNA A-sites and to identify RNA internal loops that bind aminoglycoside derivatives when the loops are presented as members of an RNA motif library (*8–10*).

To better understand how small molecules interact with RNA targets, we designed and studied a library of compounds that are predisposed for binding RNA. Library members were designed by taking cues from small molecules that are known to bind RNA and displaying portions of them on a peptoid scaffold. Peptoids are ideal because chemically diverse compounds can be easily synthesized from amine-containing submonomers that are modularly installed onto polymeric chains (*11–13*). Furthermore, a chemical handle for surface immobilization can be installed at the end of each peptoid to enable high throughput screening *via* microarray.

Many potential RNA drug targets are available to screen for binding to array-immobilized small molecules; however, few can be directly assayed *in vitro* for modulation of activity. One such class of RNAs to which an activity assay can be easily applied is group I introns, which are catalytically active in the absence of protein (*14*). The group I intron from *Candida albicans* was selected for microarray-based screening of peptoids because its activity is essential for the assembly of active ribosomes since the intron is embedded in the large subunit ribosomal RNA (rRNA) precursor (*15–17*). More-

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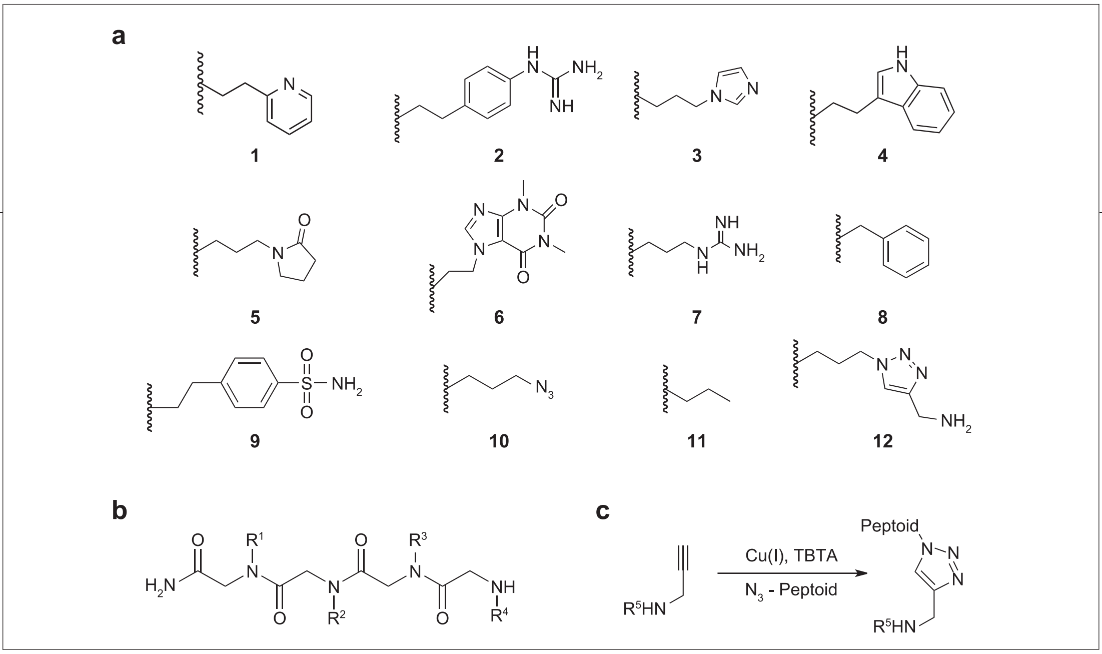

Figure 1. General structure of peptoid library members and the submonomers used to construct them. a) Structures of the peptoid submonomers that were used to construct the library. Submonomers 2 and 4 were protected during synthesis with *tert*-butoxycarbonyl, and 7 was protected with 2,2,5,7,8-pentamethyl-chromane-6-sulfonyl; protecting groups were removed upon cleavage from the resin. b) General structure of the peptoid library that was synthesized. c) Huisgen dipolar cycloaddition reaction that was used to anchor azide-displaying peptoids onto alkyne-functionalized agarose (R^5 = agarose) or to conjugate to propargylamine (R^5 = H) for testing inhibition of self-splicing. Unless indicated, the compounds tested for inhibition of self-splicing contained a triazole (12) at position R4.

over, *C. albicans* is a pathogenic fungus that infects and kills AIDS patients and is acquiring resistance to current treatments (*18–20*).

Results show that all binders identified from microarray screening inhibit self-splicing. In addition, several features in the peptoids that are important for RNA binding and inhibition of self-splicing were identified, including the presence of N-1-alkylated imidazole, indole, and phenylguanidine side chains (Figure 1). Interestingly, these studies afforded six compounds that are as potent or more potent than a clinically used drug for inhibition of group I intron self-splicing, pentamidine. These studies should allow for more effective design and screening of ligands that bind to and modulate the activity of RNAs to serve as therapeutics or chemical genetics probes of function. Furthermore, these studies improve our understanding of how the results from microarray screening correlate to modulation of a target's activity.

RESULTS AND DISCUSSION

Many important RNAs are being discovered in genomic sequences. Most of these represent untapped potential as targets for therapeutics or chemical genetic probes. The present study seeks to develop a general method to identify lead ligands that bind to highly structured RNA targets and modulate their activities. Critical to this study were the selection of ligands and the use of microarray-based screening to score members of the ligand library for binding.

Small molecule microarrays allow for facile screening for binding of target biomolecules including proteins and RNAs (*5, 6, 21–25*). Though screening is relatively easy, it is often difficult to correlate this information to modulation of activity or as a general design tool for other biomolecules. One way microarrays could facilitate the elucidation of general design principles is to screen a diverse chemical landscape to pinpoint features in small molecules that allow them to bind RNA. These studies are important given the low hit rates for RNA binders (compared to their protein counterparts) from high throughput screening assays (*26*). We designed a peptoid library biased for binding RNA by selecting peptoid submonomers that have similarities to known RNA-binding compounds or that have the potential to stack and/or hydrogen bond with RNA. Other methods that could be used to introduce bias include conjugation of small molecules to aminoglycosides and using information about aminoglycoside binding modes to design compounds that display functional groups in a similar manner (*27, 28*).

Selection of Library Members To Be Synthesized and Arrayed. A small peptoid library was synthesized using solid-phase methods and arrayed onto alkyne-functionalized agarose to create small molecule microarrays (*5–7, 10, 25*). The submonomers for construction of the library were chosen because they have a high probability of interacting with RNA due to similarities in their structure to known RNA binders or their potential to interact with RNA by stacking or hydrogen bonding

(Figure 1, panel a). For example, **1** was chosen because pyridines bind to a mimic of the bacterial rRNA A-site (*29*). The other submonomers have similar structural features present in other known RNA binders: **2** (4′,6-diamidino-2-phenylindole, DAPI) (*30*); **3** (4-heterocyclic 2-deoxystreptamine derivatives) (*31*); **4** (DAPI and peptides that bind RNA) (*30, 32, 33*); **5** (similar to the oxazolidinone ring in linezolid) (*34*), **6** (xanthinol, which binds TAR RNA) (*32, 35*); and **7** (pentamidine and arginine-rich peptides that bind RNA) (*36, 37*). The benzene submonomer (**8**) was chosen because it may stack with RNA bases and is a common group in known drugs (*38*), whereas the benzenesulfonaminde (**9**) may stack with and hydrogen bond to RNA. The last submonomer installed in each synthesis was 3-azidopropylamine (**10**) to allow for surface conjugation onto alkyne-agarose *via* a Huisgen dipolar cycloaddition reaction (HDCR) (*39*).

A library of 109 peptoids containing three positions of diversity was arrayed; compounds were arrayed only after they passed a mass spectral quality control analysis (*40*) in order to ensure that each compound was successfully synthesized. This is in contrast to arrays of compounds synthesized from a split-and-mix approach where there is no quality control step. By arraying quality-tested compounds, binding patterns can be used to infer features in ligands that confer or inhibit binding to RNA without having to be concerned if these results are obscured due to inefficient synthesis. Such features can then be further studied or used to design more potent inhibitors.

Serial dilutions of each azide-containing peptoid were conjugated onto alkyne-agarose arrays *via* a HDCR (*10, 39, 41*). Agarose arrays were used because they provide a three-dimensional surface for high ligand loading that resists nonspecific binding (*42, 43*). In addition, the HDCR provides each compound with a triazole group upon immobilization. Triazoles are present in many small molecules that bind RNA (*31, 44*), and therefore the immobilization chemistry could also contribute favorably to RNA binding.

TABLE 1. Data for inhibition of precursor self-splicing by peptoids that bind to the ribozyme when displayed on a microarray

Sequence R^1-R^2-R^3-R^4	IC$_{50}$ (μM)[a]	Relative array binding signal[b,c]
4-4-3-12	158 ± 14	1.00
4-6-3-12	150 ± 40	0.35
4-3-3-12	157 ± 40	0.66
6-4-3-12	209 ± 90	0.71
3-3-2-12	399 ± 140	0.56
7-6-2-12	500 ± 110	0.42
8-3-2-12	633 ± 153	0.48
3-4-3-12	817 ± 76	0.24
3-8-2-12	1217 ± 301	0.79
1-3-2-12	1320 ± 814	0.27
3-1-2-12	1733 ± 681	0.34
3-6-3-12	2200 ± 1304	0.35
1-5-8-12[d]	$>$5,000	0.00
Pentamidine	200 ± 35	

[a] All reactions were completed in the presence of 1 mM nucleotide concentration of bulk yeast tRNA competitor. [b] In these assays, peptoids are conjugated to alkyne-functionalized agarose microarrays *via* HDCRs. [c] Values are determined by dividing the signal from the compound by the signal from **4-4-3-12** at the highest loading spot from Figure 2. [d] Compound **1-5-8-12** is a negative control that did not give signal for binding to the ribozyme on the array.

Array-Based Screening Identifies Peptoids That Bind to the *C. albicans* Ribozyme. Once constructed, arrays were hybridized with a ^{32}P-labeled ribozyme construct that was derived from the large subunit rRNA precursor from the fungal pathogen *C. albicans* (*16*). These experiments were completed in the presence of unlabeled bulk yeast tRNA that is in excess over the total amount of ligand delivered to the array surface (Figure 2, panel a). The signal-to-noise ratio on the array for binding to labeled ribozyme is quite good ($>$50:1), and 12 of the 109 peptoids bind (Figure 2, panel a); in order to be considered a binder, a ligand had to give at least 20% of the highest signal observed on the array, from **4-4-3-12** (Table 1). For all peptoids that bind the ribozyme, a dose–response is observed (Figure 2, panels b and c). In general, the binders have similar structural features, such as side chains from the phenylguanidine (**2**), N-1-alkylated imidazole (**3**), indole (**4**), or theophylline (**6**) submonomers. Interestingly, each of the binders contained either **2** or **3** at the third position. It should be noted that due to synthetic challenges with submonomer **2**, it was installed only at the third position (Figure 1, panel b). Attempts to install this submonomer at other positions yielded impure peptoids that were discarded.

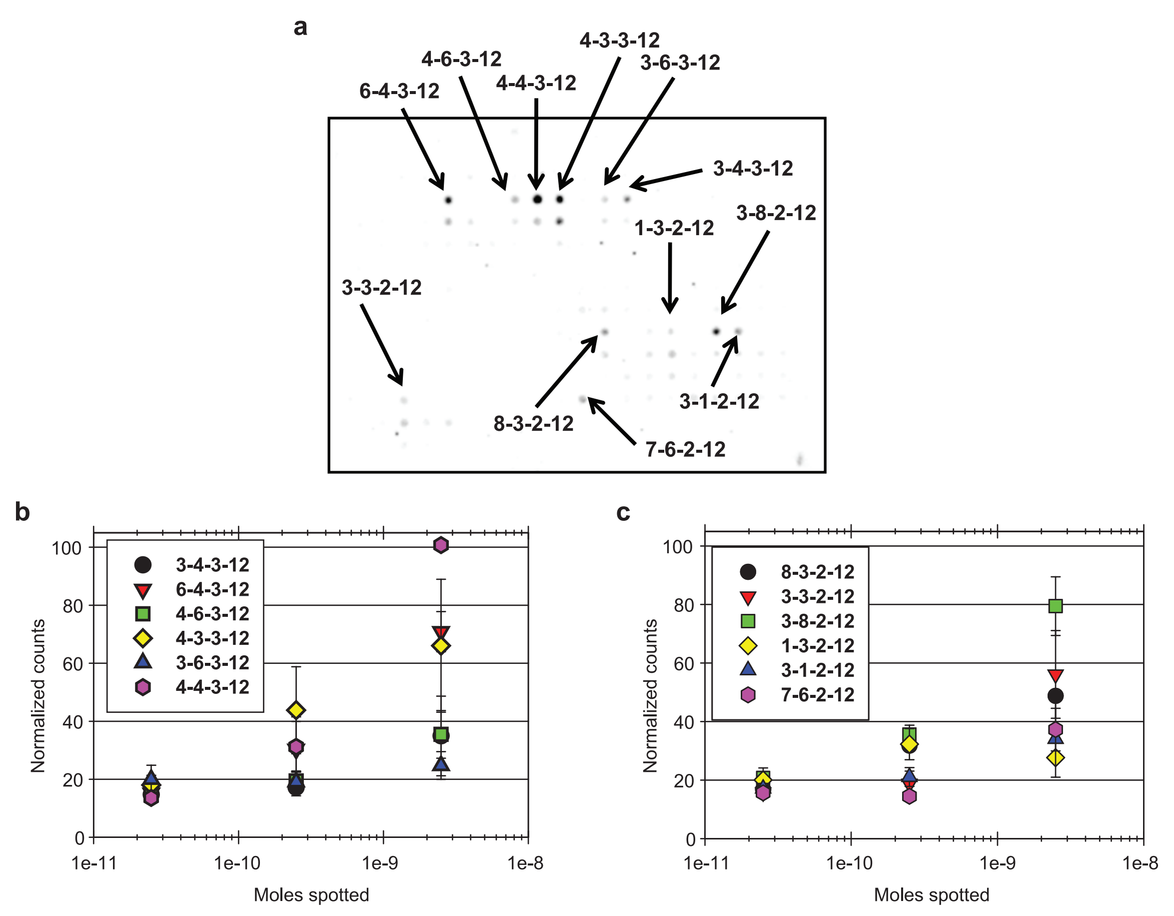

Figure 2. Results from microarray-based screening of the peptoid library for binding the *C. albicans* ribozyme. a) Image of a peptoid microarray after hybridization with the *C. albicans* ribozyme, with the arrows pointing to the spots from the highest loading of the compounds determined to be binders. b, c) Plots of radioactive signal for each peptoid binder as a function of the moles of peptoid delivered to the array surface; errors are standard deviations from three measurements. Panel b contains the peptoids without the phenylguanidine in the third position, and panel c contains the peptoids with phenylguanidine in the third position. Data were normalized by comparison to the highest loading spot of compound 4-4-3-12, which gave the highest signal. Only compounds that gave at least 20% of the normalized signal of 4-4-3-12 at the highest ligand loading were scored as binders.

Statistical Analysis of Binders Identified from Array-Based Screening. Structural features of the binders were then subjected to statistical analysis to quantify their significance relative to the entire peptoid library. Briefly, similarities and trends in the binders were searched for, and the percentage of binders with the trend of interest was then calculated. This percentage was compared to the percentage of peptoids in the entire library that display the same trend. If the percentage was greater for the binders, a *Z*-test was used to determine if the over-representation in the binders was not due to random chance. The generated *Z*-value was then converted to a confidence level, or a two-tailed *p*-value, indicative of statistical significance. Please see Supporting Information for example calculations.

The first set of *p*-values was generated by simply considering the representation of each submonomer in the binders relative to their representation in the whole library. This analysis yielded *p*-values of 0.0168 for the imidazole (**3**) and 0.0135 for the indole (**4**) features. The positional dependence of the features was then computed. This analysis showed that phenylguanidine (**2**) was preferred at the third position, with a *p*-value of 0.0039. There was no other statistically significant positional dependence for single submonomers in the analysis. A final analysis was completed by determining if the relative positioning of two or more submonomers was statistically significant. This calculation revealed that peptoids with **3** in the second and **2** in the third position had a *p*-value of 0.0027.

Inhibition of *C. albicans* Precursor Self-Splicing by the Arrayed Peptoids. The peptoids that bound the ribozyme were studied for their ability to inhibit self-splicing of the *C. albicans* group I intron precursor (Table 1). To mimic the arrayed structure, each peptoid was synthesized to contain a triazole by conjugation to propargylamine (Figure 1, panel c). The IC_{50}'s for inhibition of self-splicing were determined at 2 mM Mg^{2+} in the presence of competing 1 mM nucleotide concentration of bulk yeast tRNA. Compound **1-5-8-12** was used as a negative control as it did not bind the ribozyme above background when displayed on the microarray surface. As expected, it did not inhibit precursor self-splicing up to the highest concentration tested, 4000 μM.

All peptoids that bind to the ribozyme when displayed on the microarray surface inhibit group I intron self-splicing, with IC_{50}'s ranging from 150 to 2200 μM (Table 1). Compounds **6-4-3-12**, **4-6-3-12**, **4-3-3-12**, and **4-4-3-12** were the most potent inhibitors and also gave some of the highest signals from the microarray screening assay. Compound **3-8-2-12** is an outlier, however, since it has the second highest signal for binding on the array, with 79% of the signal from **4-4-3-12**, but is 8-fold less potent at inhibiting precursor self-splicing. On the basis of its binding signal, the IC_{50} of **3-8-2-12** was expected to be more in line with that of compound **6-4-3-12**. Therefore, microarray screening identifies inhibitors of self-splicing; however, in order to gain clearer insight into the features in the peptoid that lead to enhanced potency, each peptoid was tested for inhibition of self-splicing.

Design of Potent Second Generation Inhibitors Using Insights from Statistical Analysis of Microarray Binding Data. Using the data from statistical analysis of binders identified from the array and their corresponding IC_{50}'s, two subsets of second generation inhibitors were designed and tested. The first subset contained submonomer **2** in the third position since statistical analysis identified a preference for **2** in this position. Compounds **4-4-3-12** and **4-6-3-12** gave excellent binding signals on the array and were the two most potent inhibitors of self-splicing (Figure 2, panel a and Table 1). The initial library screened by microarray, however, did not contain the corresponding compounds with **2** in the third position. Therefore, **4-4-3-12** and **4-6-3-12** were modified to contain submonomer **2** in the third position, yielding compounds **4-4-2-12** and **4-6-2-12**. Both peptoids showed ~1.5-fold improvements in potency compared to that of their parent compounds, with IC_{50}'s of 97 and 109 μM for **4-4-2-12** and **4-6-2-12**, respectively. Evidently, having submonomer **2** in the third position provides more potent inhibitors (Table 2).

TABLE 2. Structure-based analyses of 4-4-3-12 and 4-4-2-12 for inhibition of precursor self-splicing[a]

Sequence R^1-R^2-R^3-R^4	IC_{50} (μM)
4-4-3-12	158 ± 14
4-4-3-10	533 ± 58
4-4-3	633 ± 153
4-4-2-12	97 ± 49
4-4-2-10	338 ± 88
4-4-2	125 ± 35
4-4	733 ± 115
3	>5,000
11-4-3-12	>5,000
4-11-3-12	1,333 ± 144
11-4-2-12	1,083 ± 227
4-11-2-12	1,233 ± 252
4-4-11-12	767 ± 153
tris(benzyltriazolylmethyl)amine	>5,000

[a] Submonomers were either sequentially deleted or replaced with **11**.

Structure-Based Analysis of High Potency Peptoids. Peptoids **4-4-3-12** and **4-4-2-12** were further analyzed to detemine the contribution of each feature to inhibition potency (Table 2). In the first method, a fragmentation approach was taken in which submonomers in the peptoids were sequentially removed and the resulting fragment was tested for inhibition. First, the triazole functionality was removed, leaving a free azide corresponding to compounds **4-4-3-10** and **4-4-2-10**. Both were ~3-fold less potent than the corresponding parent compound. Removal of the azide to afford compounds **4-4-3** and **4-4-2** also decreased potency. When the submonomer in the third position was removed from either peptoid to afford compound **4-4**, the IC_{50} increased to 733 μM (an ~4-fold increase in IC_{50} compared to that of **4-4-3-12** and an ~7-fold increase compared to that of **4-4-2-12**). As expected monomeric **3** is a poor inhibitor of self-splicing with no inhibition observed up to the highest concentration tested (5000 μM). Monomer **4** was not soluble in water, so accurate measurements of its potency could not be completed.

In a second method, submonomers were replaced with propylamine (*11*) to determine the contribution of each heterocycle displayed at the corresponding position (Table 2 and Figure 3). For compound **4-4-3-12**, the first position contributes the most to potency as replacement with submonomer **11**, affording **11-4-3-12**, results in an inactive compound ($IC_{50} > 5000$ μM). Replacement of the second and third positions with **11** increased the IC_{50}'s by ~8-fold and ~5-fold, respec-

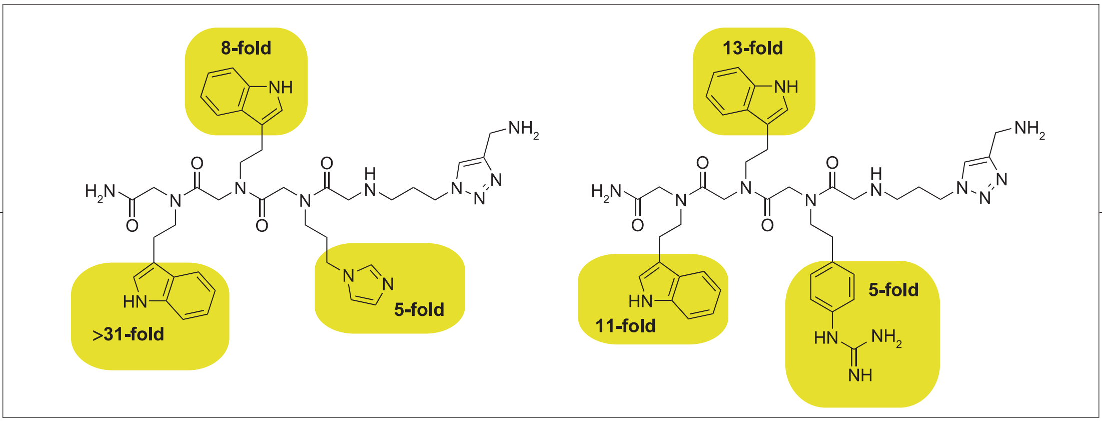

Figure 3. Studies to determine the contribution of each submonomer to the potency of compounds 4-4-3-12 **and** 4-4-2-12**. The values above each heterocycle are the increase in IC_{50} for inhibition of self-splicing when the highlighted position is replaced with a propylamine submonomer (*11*).**

tively. The results for replacing the submonomers of **4-4-2-12** with **11** are similar. Replacement of the first, second, or third position with **11** caused an ~11-fold, ~13-fold, and ~8-fold increase in IC_{50}, respectively. Therefore, each submonomer contributes to the ability of the ligands to inhibit self-splicing.

Combining Multiple Trends from Statistical Analysis Affords the Most Potent Compounds. Another subset of second generation inhibitors incorporated other trends identified by statistical analysis (Table 3). The first trend is the importance of having **3** in the second position and **2** in the third position (two-tailed *p*-value of 0.0027). A peptoid with four points of diversity similar to **4-4-3-12** and **4-4-2-12**, compound **4-4-3-2-12**, was synthesized and tested for its ability to inhibit self-splicing. Addition of the **2** side chain improved the IC_{50} from 158 μM (**4-4-3-12**) to 88 μM. Another trend is the presence of the tryptamine submonomer (**4**). Therefore, two statistically significant trends, having **4** submonomers and the **2** submonomer in the last position, were used to design **4-4-4-2-12**. This afforded the most potent peptoid with an IC_{50} of 31 μM. Thus, incorporating multiple trends from statistical analysis can provide inhibitors with increased potency relative to those identified from microarray screening. In fact, **4-4-4-2-12** is an ~5-fold better inhibitor than the three most potent inhibitors identified from the microarray screen.

TABLE 3. Data for inhibition of precursor self-splicing by second generation peptoids designed by using statistical analysis[a]

Sequence R^1-R^2-R^3-R^4	IC_{50} (μM)
4-4-2-12	97 ± 49
4-6-2-12	109 ± 19
4-4-3-2-12	88 ± 4
4-4-4-2-12	31 ± 6

[a] All IC_{50}'s are lower than the most potent first generation peptoid.

Effect of Mg^{2+} and Bulk Yeast tRNA on Inhibitor Potency. Identification of compounds that are active and specific for the target of interest is essential for rational drug design. Inclusion of competing bulk yeast tRNA in microarray screening yielded compounds that were specific to our target by suppressing signals from nonspecific binders. To further address the specificity of the most potent inhibitor of self-splicing identified in this study, **4-4-4-2-12**, the effects of increasing concentrations of Mg^{2+} and bulk yeast tRNA on the potency of **4-4-4-2-12** were determined (Table 4).

The results show that increasing the Mg^{2+} concentration from 2 to 10 mM in the presence of 1 mM nucleotide bulk yeast tRNA resulted in an increase in the IC_{50} from 31 to 57 μM. Previous studies on oligonucleotide inhibition of *C. albicans* intron self-splicing have also observed a decrease in potency as the concentration of Mg^{2+} is increased (*45*). The effect of increasing the

TABLE 4. Effect of bulk yeast tRNA and Mg^{2+} on the potency of 4-4-4-2-12 **to inhibit precursor self-splicing**

[Mg^{2+}] (mM)[a]	IC_{50} (μM)
2	31 ± 6
5	56 ± 23
10	57 ± 26

[bulk yeast tRNA] (mM nucleotides)[b]	IC_{50} (μM)
1	31 ± 6
2	21 ± 5
4	28 ± 4
8	26 ± 2

[a] Assays were completed with 1 mM nucleotide concentration of bulk yeast tRNA. [b] Assays were completed in H2Mg buffer supplemented with the indicated nucleotide concentration of bulk yeast tRNA.

amount of tRNA was also studied at 2 mM Mg^{2+}. Bulk yeast tRNA was added to splicing reactions at 1, 2, 4, and 8 mM nucleotide concentrations. Even when 8 mM nucleotide concentration of competitor is added, the IC_{50} is unaffected (Table 4). If this compound was a nonspecific inhibitor, then a significant decrease in potency would be expected. Thus, **4-4-4-2-12** is specific for the *C. albicans* group I intron over bulk yeast tRNA.

Testing 4-4-4-2-12 **for Inhibition of the Group I Intron Precursors from *Tetrahymena thermophilla* (*46*) and *Pneumocystis carinii*.** (*47*) To determine if **4-4-4-2-12** is selective for the *C. albicans* precursor relative to other group I introns, it was tested for inhibition of the self-splicing of the *T. thermophila* and *P. carinii* group I intron precursors, which both reside in the large subunit pre-rRNA. Experiments were completed in 10 mM Mg^{2+} and 1 mM nucleotide concentration of bulk yeast tRNA. The IC_{50}'s were >200 and >500 μM for the *T. thermophila* and the *P. carinii* precursors, respectively. Thus, **4-4-4-2-12** is specific for the *C. albicans* group I intron over other group I introns.

Comparison of the Most Potent Compound to Other Inhibitors of Self-Splicing. Compound **4-4-4-2-12** is similar in structure to several compounds that have been found to inhibit group I intron self-splicing, including similarities between the indole side chain and the benzimidazole in Hoechst 33258 (*48*). It is also similar to pentamidine in that both compounds contain a phenyl ring capped by a positively charged group, a benzenecarboximidamide (pentamidine) or phenylguanidine (submonomer **2**). The array structure–activity relationship data identified that all inhibitors had either a phenylguanidine or imidazole group at the third position and each likely provides critical interactions to stabilize the ligand-target complex.

Conclusions. Using cues from small molecules that target RNA, a peptoid library was designed and screened for binding to the *C. albicans* group I ribozyme. Of the 109 arrayed peptoids, 12 bound the RNA and were tested for their abilities to inhibit self-splicing. We combined the data from microarray screening, statistical analysis of ligand binders, and compound potency, and identified modules **2** and **4** as important determinants in the molecular recognition of the *C. albicans* group I intron. These analyses guided the rational design of **4-4-4-2-12**, which is the most potent inhibitor identified from this study. This compound is a 5-fold more potent inhibitor of self-splicing compared to the best ligand identified from the microarray screen, **4-6-3-12** (Table 1 and Table 3). It is also a 6-fold more potent inhibitor than pentamidine, a drug used clinically to treat *Pneumocystis carinii* infections that has also been shown to inhibit self-splicing of the *C. albicans* intron *in vitro* and *in vivo* (*17, 37, 49*). In fact, all second generation inhibitors were more potent than any hit from the original library and pentamidine.

Many features that can predispose ligands for specific recognition of RNA may lie outside of presently known scaffolds that bind RNA. By expanding the results disclosed herein and probing diverse chemical space for binding of RNA *via* microarray, these scaffolds are likely to be identified. Future developments using microarray-based screening of peptoids or other small molecules would benefit from using chemoinformatics approaches to compute chemical diversity to rationally design libraries with broad chemical landscapes. By using statistical analysis of binders, general ligand features for binding RNA can be elucidated quickly.

METHODS

General Methods. All chemicals used were from Acros, Sigma-Aldrich, or MP Biochemicals and were used without purification unless noted otherwise. Please see Supporting Information for detailed procedures for the syntheses of peptoid submonomers **2** and **6** and all peptoids.

Diethyl pyrocarbonate (DEPC)-treated nanopure water was used for all buffers and in splicing assays. H*x*Mg buffer is 50 mM Na^+ HEPES, pH 7.5, 135 mM KCl, and *x* mM $MgCl_2$. Plasmids encoding the *C. albicans* group I intron precursor and ribozyme (*16*), the *T. thermophila* precursor (*46*), and the *P. carinii* precursor (*47*) were transcribed as described. RNAs were internally labeled using an Ambion MEGAscript or a Stratagene RiboMaxx transcription kit and [α-^{32}P]ATP and purified as described (*16*). RNAs were resuspended in DEPC-treated water and stored at −20 °C.

Microarray Construction. Glass slides (4″ × 5″) were cleaned in piranha solution (70:30 H_2SO_4/30% (v/v) aqueous H_2O_2) overnight at RT. Slides were functionalized with amines by submersion in a 3% (v/v) solution of 3-aminopropyltriethoxysilane in 95% ethanol for 1 h at RT (*6*). This solution was prepared prior to use by stirring for 10 min at RT. Arrays were then washed with 95% ethanol and cured by heating at 110 °C for 1 h. After cleaning (sonicated for 1 h in water), the amine-functionalized arrays were coated with ~15 mL of a 2% (w/v) agarose solution, and the agarose was allowed to dry to a thin film at RT. The agarose was then oxidized by submersion in 20 mM $NaIO_4$ for 3 h at RT. Alkyne-functionalized arrays were afforded by submerging the slides in an aqueous solution of 10 mM propargylamine and 100 mM $NaHCO_3$ overnight. Arrays were quenched in 200 mL of a solution (70 mL of ethanol and 130 mL of phosphate buffered saline) containing 200 mg of $NaBH_3CN$ for 5 min. Arrays were washed and submerged in a 20% (v/v) aqueous ethylene glycol solution for 1 h, washed with water (6 × 100 mL for 15 min each), and dried to a clear film on the benchtop.

Spotting the Library onto the Microarray Surface. Serial dilutions of each peptoid were placed into the wells of a 384-well plate at concentrations of 5 mM, 500 μM, and 50 μM in 1X spotting solution (10 mM Tris·HCl, pH 8.5, 1 mM $CuSO_4$, 100 μM ascorbic acid, 100 μM tris(benzyltriazolylmethyl)amine (*41*), and 10% (v/v) glycerol). Approximately 500 nL of each peptoid solution was delivered to the surface of an alkyne-functionalized slide using a 384-pin replicator (Boekel Scientific). The arrays were then incubated at RT for ~3 h. The arrays were washed in buffer (8 mM Na_2HPO_4, pH 7.0, 1 mM ethylenediaminetetraacetic acid, and 180 mM NaCl; 3 × 15 min each) to remove uncoupled peptoid and coupling reagents including Cu^{2+}, rinsed with nanopure water, and left to dry at RT.

Array Hybridization. Peptoid arrays were prehybridized with 3 mL of H2Mg buffer for 30 min to hydrate the surface. A 3 mL solution of ^{32}P-labeled ribozyme (~10 nM) was annealed in 1X H2Mg buffer at 60 °C for 5 min and allowed to cool slowly to RT. Bovine serum albumin and bulk yeast tRNA were then added to final concentrations of 0.162 and 0.1 mg mL^{-1}, respectively. Bulk yeast tRNA was added as a competitor and is in 10-fold nucleotide excess over the moles of ligand that were delivered to the array surface and in 250-fold nucleotide excess over the ribozyme. The comparison of the moles of tRNA and the moles of ligand are based on earlier experiments to determine the loading of ligands onto alkyne-arrays *via* a HDCR (*8, 50*). As such, ~10% of the ligand delivered to the array surface was assumed to be immobilized. It should be noted that the concentration of $MgCl_2$ does not significantly affect binding signals. The arrays were washed in 1X H2Mg buffer for 10 min, rinsed with water, and air-dried. After drying, they were exposed to a phosphor screen and imaged using a BioRad FX imager. Data were quantified using BioRad's QuantityOne software.

Splicing Assays. Splicing assays to determine the ability of the peptoids to inhibit precursor self-splicing were completed as described except that reactions were completed in the presence of 100 μM guanosine monophosphate and various concentrations of bulk yeast tRNA (*16, 45*). All data were quantified using BioRad's QuantityOne software. The resulting curves were fit to SigmaPlot's four parameter logistic curve to determine IC_{50}'s.

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Supporting Information Available: This material is available free of charge *via* the Internet at http://pubs.acs.org.

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