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Nanomolar binding affinity of quinine-based antimalarial compounds by the cocaine-binding aptamer

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ABSTRACT

An unusual feature of the cocaine-binding aptamer is that it binds quinine much tighter than the ligand it was selected for, cocaine. Here we expand the repertoire of ligands that this aptamer binds to include the quinine-based antimalarial compounds amodiaquine, mefloquine, chloroquine and primaquine. Using isothermal titration calorimetry (ITC) we show that amodiaquine is bound by the cocaine-binding aptamer with an affinity of (7 ± 4) nM, one of the tightest aptamer-small molecule affinities currently known. Amodiaquine, mefloquine and chloroquine binding are driven by both a favorable entropy and enthalpy of binding, while primaquine, quinine and cocaine binding are enthalpy driven with unfavorable binding entropy. Using nuclear magnetic resonance (NMR) and ITC methods we show that these ligands compete for the same binding sites in the aptamer. Our identification of such a tight binding ligand for this aptamer should prove useful in developing new biosensor techniques and applications using the cocaine-binding aptamer as a model system.

1. Introduction

Aptamers are usefully employed in many biosensor and biotechnology applications for a number of reasons including their stability compared to protein-based antibodies, the cost effectiveness of their manufacture and the fact that, once selected, they are usually highly specific for a particular ligand.[1–5] The advantage of binding specificity is only partly true for the cocaine-binding aptamer. While this aptamer binds cocaine, it only binds very weakly the common cocaine metabolites ecgonine, benzoyl ecgonine and ecgonine methyl ester.[6–11] The cocaine-binding aptamer also displays high affinity for quinine and several quinine analogues.[9,11–14] This "off-target" binding is unusual as it is approximately 50 fold tighter than for cocaine, the ligand used in selection of the aptamer. The ability of this aptamer to bind quinine has recently been exploited to develop a biosensor for quinine in wastewater.[15]

The cocaine-binding aptamer was originally reported by Stojanovic et al. in 2000[6] and has subsequently become a model system for both developing new applications of aptamers in biotechnology and for aptamer-focused functional studies. The secondary structure of the aptamer contains three stems arranged around a three-way junction that contains a tandem AG mismatch (Fig. 1).[16] This AG mismatch is essential for binding, as making any sequence change, even to a GA mismatch eliminates ligand binding.[14,17] The aptamer contains two binding sites for its ligand, a high-affinity and a low-affinity site.[18] Ligand binding at the low-affinity site is often not observed, as its affinity decreases as NaCl concentration increases. Two variations of the cocaine-binding aptamer are often studied. In the first, stem 1 (Fig. 1) has 6-7 base pairs and the secondary structure is formed in the ligand-free state. In the second form, stem 1 is shortened to have 3 base pairs and the ligand-free form is dynamic or unstructured and the aptamer becomes structured upon ligand binding.[16,19–21] It is this second "structure switching" version of the cocaine-binding aptamer that is most often used in biosensor development.[22–26]

Quinine is found in the bark of some trees of the genus *Cinchona*, and extracts from the bark have been traditionally used in South America to stop shivering, which lead to its use as a treatment for malaria. The use of quinine as an antimalarial agent has led to the development of a series of compounds derived from quinine such as amodiaquine, mefloquine and chloroquine. The mode of action of this set of compounds is not yet fully understood, but it is thought to inhibit heme polymerization through an association with heme.[27] Often, one of these quinine-based compounds is used in conjunction with artemisinin in antimalarial combination treatment.[28]

In this manuscript we further expanded the range of ligands bound by the cocaine-binding aptamer to include a number of quinine-based

Abbreviations: bp, base pair; DMSO, dimethyl sulfoxide; ITC, isothermal titration calorimetry; NMR, nuclear magnetic resonance; Tris, 2-amino-2-hydroxymethyl-propane-1,3-diol

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antimalarial compounds. We use isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) spectroscopy to show that all of the quinine-based compounds are bound by the cocaine-binding aptamer, that these compounds share common high and low-affinity binding sites and that some compounds, such as amodiaquine, are bound by the cocaine-binding aptamer in the single-digit nanomolar range. This affinity is much tighter than previously observed for quinine.

2. Materials and methods

2.1. Materials

Aptamer samples were obtained from Integrated DNA Technologies (IDT, Coralville, Iowa) with standard desalting and used without further purification. The veracity of the DNA was verified by mass spectrometry performed by the manufacturer. DNA samples were dissolved in distilled, deionized water and then exchanged three times in a 3-kDa molecular weight cutoff concentrator with 1 M NaCl and washed at least three times with distilled deionized water. All DNA samples were exchanged with the appropriate buffer three times before use. Aptamer concentrations were determined by absorbance spectroscopy using the calculated extinction coefficients. All small molecule ligands were obtained from Sigma Aldrich. Stock solutions of compounds for binding experiments were prepared by dissolving the appropriate weight of each analogue into either 100% DMSO or 20 mM Tris pH 7.4 140 mM NaCl 5 mM KCl.

2.2. Isothermal titration calorimetry

ITC binding experiments were performed using a MicroCal VP-ITC instrument in a manner similar to what we previously described.[29] Samples were degassed before analysis with a MicroCal Thermo Vac unit. All experiments were corrected for the heat of dilution of the titrant. Titrations were performed with the aptamer samples in the cell and the ligand as the titrant, in the needle. All aptamer samples were heated in a 95 °C water bath for 3 min and cooled in an ice water bath prior to use in a binding experiment to allow the DNA aptamer to anneal in an intramolecular fashion.

The binding experiments were performed at 15 °C with the aptamer solution at 20–60 μM using an antimalarial drug concentration of 0.312 to 0.936 mM. Amodiaquine, mefloquine, pyrimethamine and dapsone were diluted to experimental concentrations with buffer to a final DMSO concentration of 3% (v/v). DMSO was added to the aptamer solution and the reference cell at the same concentration to avoid buffer mismatch. All binding experiments were performed at 15 °C and consisted of an initial delay of 60 s, first injection of 2 μL and 300 s delay. Subsequent 34 injections were 8 μL, spaced every 300 s. The first point was removed from all data sets due to the different injection volume and delay parameters.

ITC data following a one-site binding model were fit using the manufacturer provided Origin 7 software. Two-site binding data were fit to a two-site independent binding model developed by Freiburger et al.[30] using Matlab 14 software.

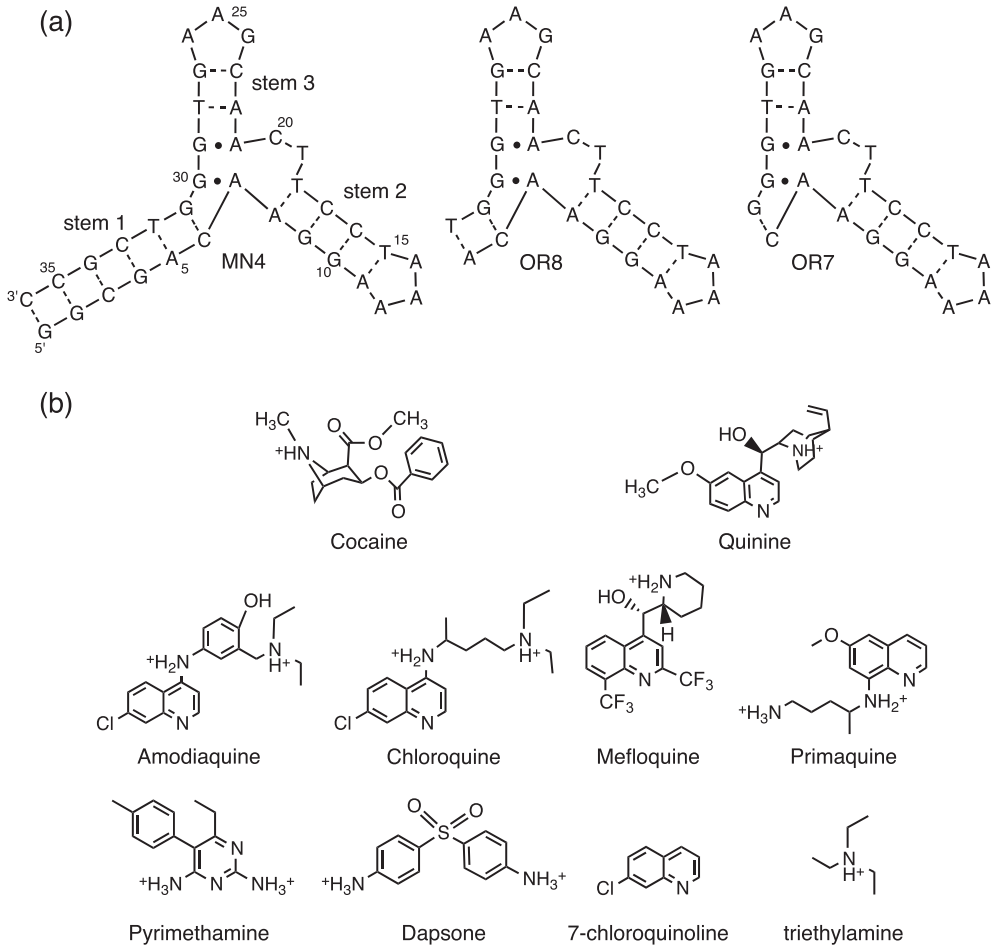

Fig. 1. (a) Secondary structure of the different cocaine-binding aptamer constructs used in this study. Dashes between nucleotides indicate Watson − Crick base pairs and dots indicate the location of non-Watson − Crick base pairs. (b) Chemical structures of the compounds investigated in this study.

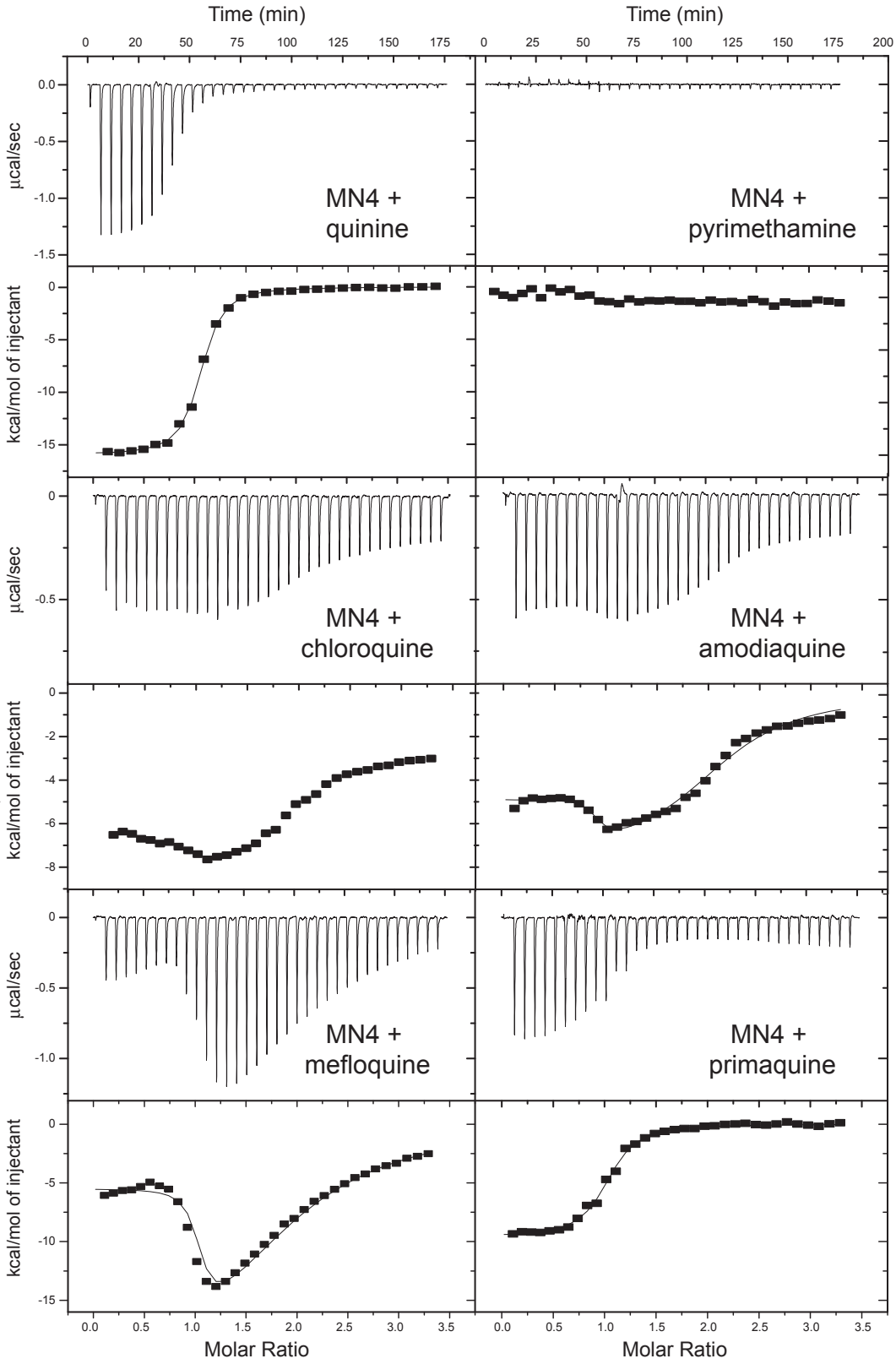

Fig. 2. ITC analysis of ligand binding by the MN4 cocaine-binding aptamer. On top is the titration data showing the heat resulting from each injection of ligand into an aptamer solution. On bottom are the integrated heats corrected for the heat of dilution. Binding experiments were performed at 15 °C in 20 mM Tris (pH 7.4), 140 mM NaCl, 5 mM KCl.

Table 1
Binding affinities and thermodynamic values for the binding between the MN4 cocaine-binding aptamer and ligands in high salt buffer (140 mM NaCl, 5 mM KCl, 20 mM Tris, pH 7.4).

Ligand	K_{d1} (μM)	ΔH_1 (kcal mol^{-1})	$-T\Delta S_1$ (kcal mol^{-1})	K_{d2} (μM)	ΔH_2 (kcal mol^{-1})	$-T\Delta S_2$ (kcal mol^{-1})
Cocaine[a]	5.5 ± 0.4	−11 ± 1	2.4 ± 0.8	–	–	–
Quinine[a]	0.20 ± 0.05	−14 ± 1	5.7 ± 0.7	–	–	–
Primaquine	0.5 ± 0.1	−9.5 ± 0.1	1.2 ± 0.2	–	–	–
Amodiaquine[b]	0.007 ± 0.004	−2.8 ± 0.9	−8 ± 1	2.1 ± 0.5	−5 ± 2	−2 ± 2
Chloroquine	0.037 ± 0.002	−4 ± 2	−6 ± 2	1.6 ± 0.4	−7 ± 1	−0.2 ± 0.1
Mefloquine[b]	0.035 ± 0.005	−6 ± 2	−4 ± 2	15 ± 3	−29 ± 1	22 ± 1
Dapsone[b]	NB					
Pyrimethamine[b]	NB					

The values reported are averages of 2 or 3 individual experiments.The error reported is the standard deviation. NB denotes no binding detected at these experimental conditions. Data acquired at 15 °C.

[a] Data taken from Neves et al.[18]

[b] Data acquired in the same buffer with 3% DMSO.

2.3. NMR spectroscopy

NMR experiments on aptamer samples were performed using a 600 MHz Bruker Avance spectrometer equipped with a ^{1}H-^{13}C-^{31}P triple-resonance probe. All NMR spectra were acquired in a 10 mM sodium phosphate (pH 7.4) buffer in $^{1}H_2O/^{2}H_2O$ (90%/10%) at 5 °C. Water suppression was achieved through the use of excitation sculpting.[31] Aptamer concentrations for NMR studies varied from 0.25 to 1.0 mM. The 2D NOESY spectrum was acquired with a mixing time (τ_m) of 200 ms and processed and analyzed using NMRPipe/NMRDraw.[32]

3. Results and discussion

3.1. Binding affinity and thermodynamics of antimalarial compounds

We performed ITC experiments to see if the quinine-based antimalarial compounds: primaquine, amodiaquine, chloroquine and mefloquine (Fig. 1) are bound by the cocaine-binding aptamer. As seen by their thermograms (Fig. 2), all of these compounds are bound by the MN4 cocaine-binding aptamer and their binding affinity and thermodynamics are given in Table 1. It is notable that, with the exception of primaquine, these compounds bind 5-29 fold tighter than quinine. Amodiaquine binds the tightest of all currently known cocaine-binding aptamer ligands, having an affinity of (7 ± 4) nM. Also reported in Table 1 are binding results for MN4 to both cocaine and quinine acquired at the same conditions.

The binding thermodynamics for amodiaquine, chloroquine and mefloquine by MN4 show that these ligands bind with both a favorable enthalpy and entropy. For cocaine, quinine and primaquine binding is enthalpically favorable, but counterbalanced by an unfavorable binding entropy (Table 1). Having both enthalpy and entropy as a driving force for binding likely contributes to the reason why amodiaquine, chloroquine and mefloquine bind so much tighter than quinine. The reason why these ligands are entropically favorable may be due to their higher hydrophobicity as their logP values (octanol/water partition coefficient) are higher than for the ligands that have an unfavorable binding entropy. However, there are other factors likely involved including molecular shape and hydration and solvation effects. For amodiaquine, the extra aromatic ring could also contribute to its tight binding through potential additional stacking interactions, a known driving force for ligand-cocaine-biding aptamer interactions.[14]

The non-sigmoidal nature of the ITC thermograms for amodiaquine, chloroquine and mefloquine are due to these three compounds binding at both the high and low-affinity sites in the cocaine-binding aptamer at 140 mM NaCl at 20 μM DNA aptamer concentration. This behaviour is in contrast to that seen with quinine and primaquine, which display no significant amount of second site binding at 140 mM NaCl at 20 μM aptamer concentration. The data for amodiaquine, chloroquine and mefloquine were fit to a two-site independent binding model in a similar fashion as described previously for cocaine and quinine two-site binding in low salt buffer.[18] The thermodynamic parameters for binding at the second, low-affinity, site are provided in Table 1.

As a control for ligand binding, we also assayed the ability of the cocaine-binding aptamer to bind dapsone and pyrimethamine. Both of these compounds have been used as antimalarial agents and both of these compounds have two 6-membered aromatic rings, like cocaine, but not the quinoline ring system (Fig. 1). Neither of these compounds displays binding by MN4 as observed by ITC (Fig. 2; Table 1).

Due to the very tight binding of amodiaquine by MN4, we analysed the binding of two structural components of amodiaquine, 7-chloroquine and triethylamine (Fig. 1). From the thermogram of 7-chloroquine titrated into MN4, we observed that this bicyclic ring is bound by MN4 with a K_d value of (0.92 ± 0.04) μM (Fig. S-1a). In contrast, we observed no binding of triethylamine by MN4 (Fig. S-1b). These results mirror what we observed previously with quinine where we looked at the binding of the aromatic (6-methoxyquinoline) and the aliphatic (quinuclidine) portions individually and binding of (0.5 ± 0.1) μM and no binding were observed, respectively.[9] We noted that even though amodiaquine binds much tighter than quinine, 6-methoxyquinoline binds tighter than 7-chloroquine. Similarly, for this and our previous study,[9] we observed no binding for the aliphatic portion of the ligand even though the triethylamine would be positively charged at the pH value studied. These results are consistent with our proposed mechanism of binding being driven by stacking interactions between the aromatic portion of the ligand and a base or base pair(s) in the aptamer.[14]

3.2. Competition binding experiments

In order to see if quinine and these quinine-based compounds share a common binding site on the cocaine-binding aptamer, we performed an ITC-based competition binding experiment with amodiaquine and quinine. In the first experiment, we bound quinine (3:1 molar ratio, ligand:MN4) to the cocaine-binding aptamer and then titrated in amodiaquine (Fig. 3a). In this case, the tighter binding ligand, amodiaquine, displaces quinine and two-site binding is observed. Next, we performed a second experiment where amodiaquine (3:1 molar ratio, ligand:MN4) was bound to the cocaine-binding aptamer and quinine was titrated. As seen in Fig. 3b, no binding was observed for the addition of quinine into amodiaquine-bound aptamer. We conclude that both ligands share the same high-affinity binding site. Previously, we showed with both ITC and NMR-based competition experiments that cocaine and quinine compete for the same binding site in MN4.[13] Therefore, we propose that all the quinine-based antimalarial compounds here compete for the same high affinity binding site in the cocaine-binding aptamer.

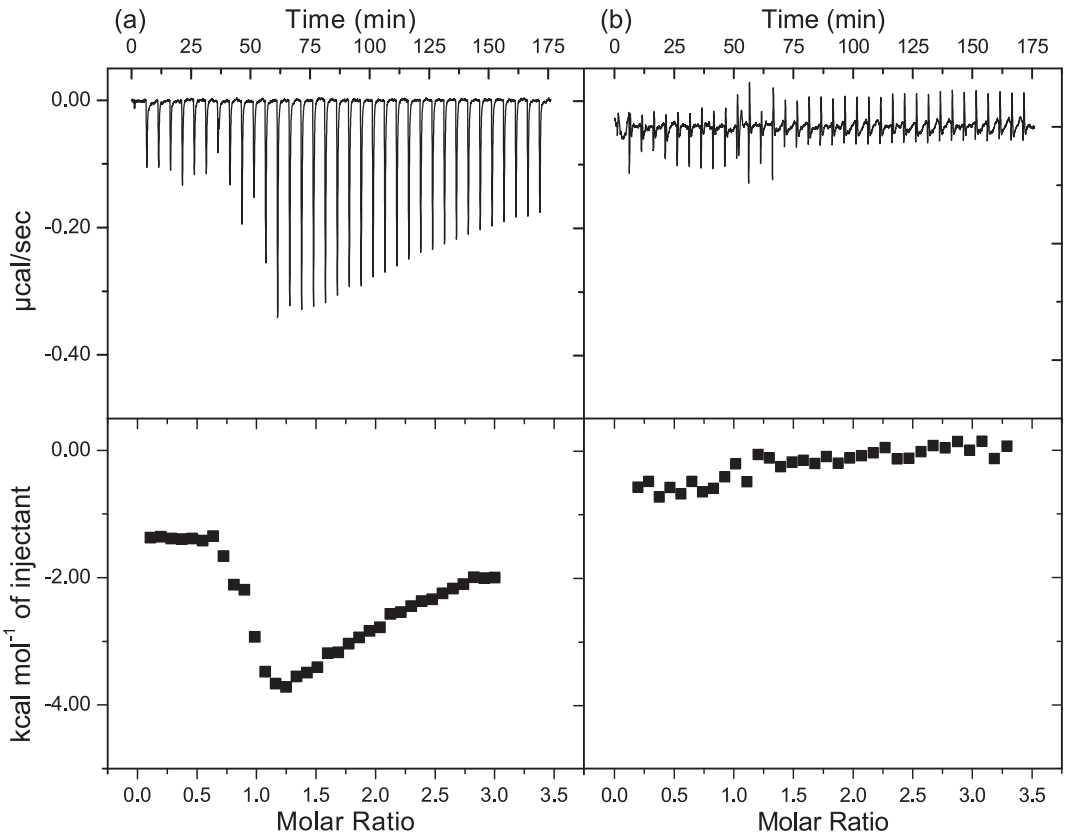

Fig. 3. ITC-based competition binding experiments. Shown are the interaction of (a) amodiaquine with quinine-bound MN4 and (b) quinine into amodiaquine-bound MN4. On top of each part is the raw titration data showing the heat resulting from each injection of ligand into aptamer solution. On the bottom is the integrated heat plot after correcting for the heat of dilution. All binding experiments were performed at 15 °C in a buffer of 20 mM Tris (pH 7.4), 140 mM NaCl, 5 mM KCl.

Table 2
Binding affinities and thermodynamic values for the binding between the MN4 cocaine-binding aptamer and ligands in low salt buffer (20 mM Tris, pH 7.4).

ligand	K_{d1} (µM)	ΔH_1 (kcal mol^{-1})	$-T\,\Delta S_1$ (kcal mol^{-1})	K_{d2} (µM)	ΔH_2 (kcal mol^{-1})	$-T\,\Delta S_2$ (kcal mol^{-1})
Cocaine[a]	1.3 ± 0.4	−11 ± 1	3 ± 1	16 ± 8	−20 ± 2	13 ± 2
Quinine[a]	0.17 ± 0.07	−10 ± 1	1.9 ± 0.8	1.2 ± 0.6	−26 ± 2	19 ± 9
Primaquine	0.30 ± 0.02	−7 ± 2	−1.7 ± 0.9	8 ± 2	−20 ± 3	13 ± 3
Amodiaquine[b]	0.21 ± 0.05	−0.9 ± 0.1	−7.9 ± 0.3	5.6 ± 0.9	−4 ± 2	-3 ± 2
Chloroquine	0.4 ± 0.1	−5 ± 1	−3 ± 1	19 ± 7	−20 ± 2	14 ± 2
Mefloquine[b]	1.7 ± 0.7	−10 ± 1	3 ± 2	vwb		

The values reported are averages between 2 and 3 individual experiments. The error reported is the standard deviation. vwb denotes very weak binding observed that was not quantifiable. Data acquired at 15 °C.

[a] Data taken from Neves et al.[18]

[b] Data acquired in the same buffer with 3% DMSO.

3.3. Binding at different NaCl concentrations

The two-site binding of amodiaquine, chloroquine and mefloquine by MN4 at 20 µM aptamer and 140 mM NaCl distinguishes the binding of these ligands from the observation of one-site binding of cocaine, quinine and primaquine at the same NaCl concentration (Fig. 2). We acquired binding data at different NaCl concentrations to further investigate this difference. First, we acquired binding data in 20 mM Tris buffer with no added NaCl (Table 2). Primaquine behaves similarly to cocaine and quinine, where binding becomes slightly tighter and binding at the low-affinity site is observed in the absence of added NaCl (Fig. S-2). In contrast, amodiaquine, chloroquine and mefloquine are bound weaker at both the high-affinity and low-affinity site by MN4 with no NaCl added.

We then increased the NaCl concentration and measured amodiaquine binding at 500 mM and 750 mM NaCl (Table 3; Fig. S-3). At both of these concentrations, binding affinity at both the high and low affinity sites decreased as the NaCl concentration increased.

3.4. NMR analysis of amodiaquine binding

In order to gain insight into the structure of the amodiaquine-bound MN4 aptamer and the location of the amodiaquine-binding site, we added amodiaquine into the MN4 aptamer and monitored the titration by 1D-^{1}H NMR spectroscopy (Fig. 4). The imino region of the ^{1}H NMR spectrum of unbound MN4 observed here is identical to that we previously assigned.[13,16,20] We observe during the titration that the imino resonances of the free and bound aptamer are in slow exchange on the NMR timescale. This is consistent with the tight affinity observed by ITC between the MN4 aptamer and amodiaquine.

Assignment of the imino protons of the 1:1 molar ratio complex of amodiaquine-MN4 was obtained by following the titration as ligand was added (Fig. 4) and confirmed with a 2D NOESY spectrum of the complex. Past a 1:1 M ratio, the second ligand binding site becomes

Table 3
Binding affinities and thermodynamic values for the interaction between the MN4 cocaine-binding aptamer and amodiaquine at different NaCl concentrations.

[NaCl] (mM)	K_{d1} (μM)	ΔH_1 (kcal mol^{-1})	$-T\,\Delta S_1$ (kcal mol^{-1})	K_{d2} (μM)	ΔH_2 (kcal mol^{-1})	$-T\,\Delta S_2$ (kcal mol^{-1})
0	0.21 ± 0.05	−0.9 ± 0.1	−7.9 ± 0.3	5.6 ± 0.9	−4 ± 2	−3 ± 2
140	0.007 ± 0.004	−2.8 ± 0.9	−8 ± 1	2.1 ± 0.5	−5 ± 2	−2 ± 2
500	0.2 ± 0.1	−2.3 ± 0.1	−6.4 ± 0.5	18 ± 2	−6.8 ± 0.6	0.5 ± 0.2
750[a]	1.5 ± 0.4	−3.2 ± 0.2	−4.5 ± 0.3		vwb	

Data acquired at 15 °C in 20 mM Tris (pH 7.4), 3% DMSO at NaCl concentrations indicated with an aptamer concentration of 60 μM. The values reported are averages of between 2 and 3 individual experiments. The error reported is the standard deviation. vwb denotes very weak binding observed but not quantified.
[a] Aptamer concentration used was 20 μM in order to best define binding at high-affinity site.

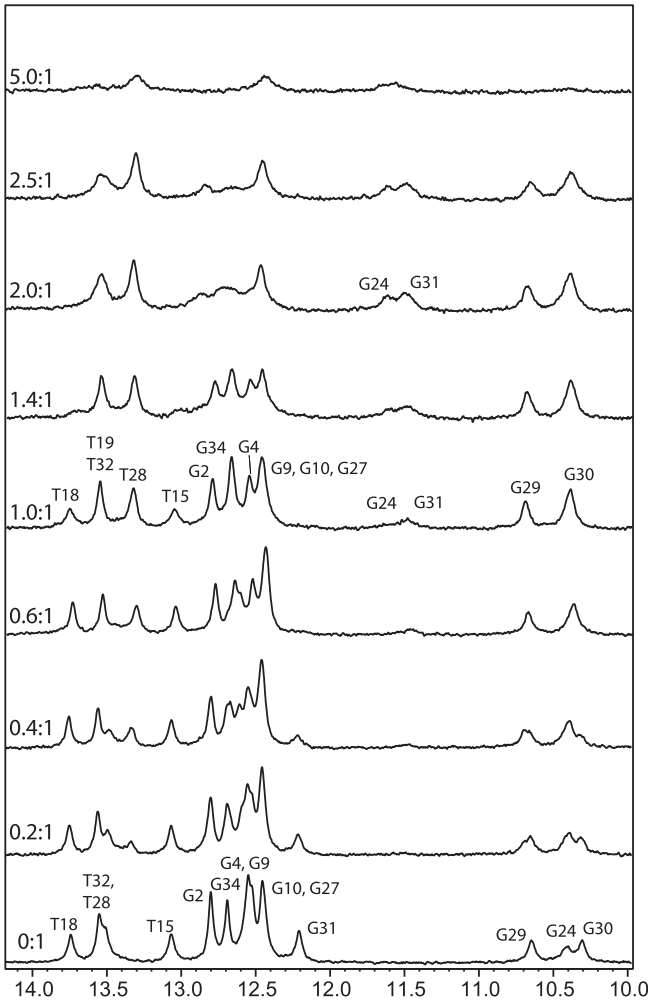

Fig. 4. Amodiaquine binding by MN4 monitored by 1D ^{1}H NMR. Displayed is the imino resonance region of the NMR spectrum as a function of increasing amodiaquine concentration. All spectra were acquired in 90% H_2O/10% ^2H_2O in a 10 mM sodium phosphate (pH 7.4) buffer at 5 °C at the molar ratio of ligand to aptamer indicated.

populated and the resonances in the ^{1}H NMR spectrum from T18 and T15 soon disappear due to line broadening. Then, additional signals become severely line broadened (Fig. 4). This disappearance of T18 and T15 resonances, then additional peaks becoming line broadened is the same as we observed when quinine was bound at the second binding site in MN4.[18] This provides further evidence that the two binding sites for quinine and amodiaquine are at the same location.

With the exception of G24, the resonances in MN4 that most change chemical shift with amodiaquine binding are the same as those that most change chemical shift with cocaine and quinine binding (Fig. S-4). In particular, the G31 imino resonance remains an excellent indicator of ligand binding in MN4. The similarity in chemical shift patterns between the three ligands is consistent with the competitive ITC binding experiments that indicate cocaine, quinine and amodiaquine compete for the same high affinity-binding site.

The most distinctive difference between the NMR spectrum of amodiaquine-MN4 and the changes we observed previously for the cocaine and quinine complexes is that the imino proton of G24 shifts dramatically downfield with amodiaquine binding. G24 is the resonance that changes chemical shift the most with amodiaquine binding (Fig. 4, Fig. S-4). This nucleotide is in the loop in stem 3 (Fig. 1) and was the resonance that experienced the third greatest chemical shift change with cocaine and quinine, but not to the large extent seen with amodiaquine. It is likely that the larger size of amodiaquine means that the bound ligand extends further toward the loop in stem 3 than quinine or cocaine, possibly making contact with the loop in stem 3. This potential additional site of contact could give rise to the greater affinity seen with amodiaquine. Consistent with G24 shifting more with amodiaquine, the imino of T28 also in stem 3 close to G24, shifts more with amodiaquine than seen previously (Fig. 4; Fig. S-5). Alternately, the shift in G24 could be associated with binding at the low-affinity site that may be populated to a greater extent with amodiaquine than for quinine at the same ligand concentration, as seen in the ITC thermograms. Evidence for G24 being a resonance marker for binding at the second site is that its resonance appears sharper at a 2:1 than 1:1 ligand:MN4 ratio.

3.5. Binding of short stem 1 constructs

The affinity of two different cocaine-binding aptamer constructs (OR7, OR8; Fig. 1) with stem 1 lengths of 1 and 2 base pairs for amodiaquine and chloroquine were analysed using ITC. Chloroquine was bound at both the high and low-affinity site by OR8 but no binding of chloroquine was observed by the OR7 aptamer (Fig. 5; Table 3). In contrast, the tighter binding ligand, amodiaquine, is bound by OR7 at both the high and low-affinity sites (Fig. 5; Table 4).

These experiments were performed to expand our previous studies where we destabilized the folded structure of the cocaine-binding aptamer by shortening stem 1 and using these destabilized aptamers to improve ligand selectivity for tight binding ligand.[21] We previously showed that OR8 (2 bp stem 1) could bind quinine, but not cocaine. The idea behind this difference is that the weaker binding ligands do not supply enough free energy (ΔG) from binding to both fold and bind the aptamer while tighter binding ligands supply enough free energy to both fold the aptamer and use the remaining ΔG from binding as an "effective" binding affinity.

In this study, we used the high affinity of amodiaquine to bind and fold the OR7, a cocaine-binding aptamer that contains only one base pair in stem 1 (Fig. 1). The affinity of amodiaquine to OR7 is reduced compared to binding by MN4 with this reduction in affinity reflecting the contribution from the binding free energy to the folding of OR7. The next tightest binding ligand, chloroquine, was not bound by OR7 and binding by OR7 reflects a way to distinguish between these two ligands. Similarly, chloroquine is bound by OR8 (Fig. 1; 2 bp stem 1), as this aptamer needs less free energy from binding in order to fold compared with OR7.

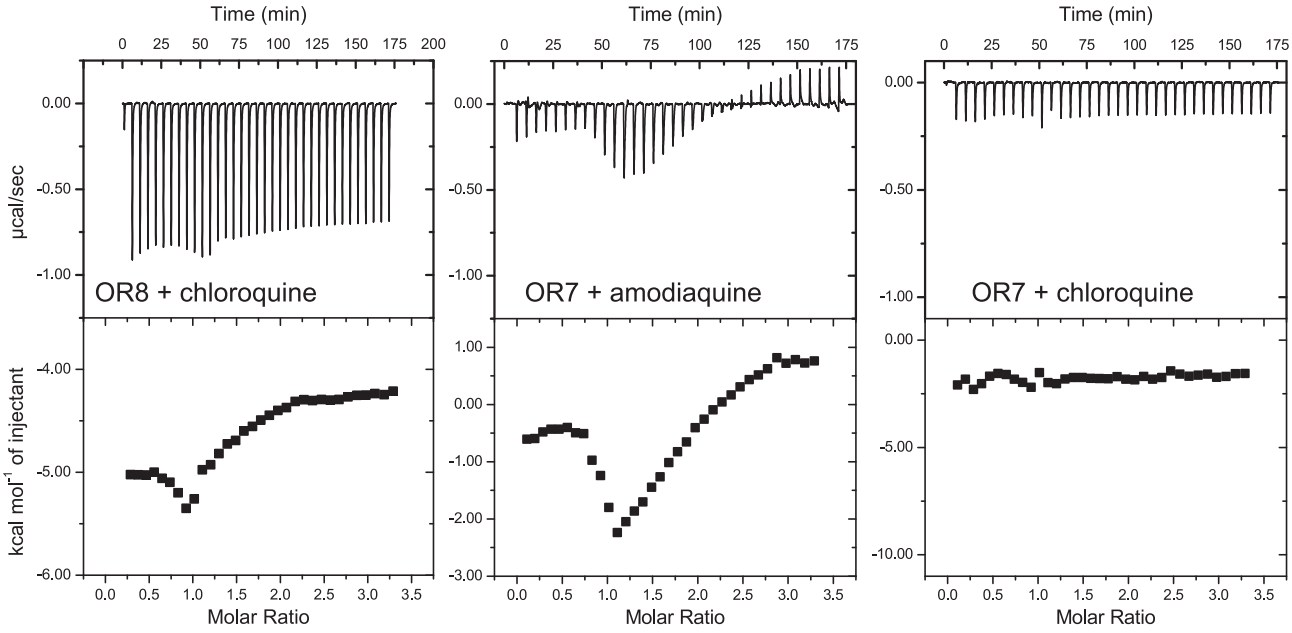

Fig. 5. ITC data showing the interaction of two different stem 1 length aptamers with chloroquine and amodiaquine. On top is the raw titration data showing the heat resulting from each injection of ligand into aptamer solution. The bottom shows the integrated heat plot after correcting for the heat of dilution. Data were acquired in 20 mM Tris (pH 7.4), 140 mM NaCl, 5 mM KCl.

Table 4
Binding affinities and thermodynamic values for the interaction of amodiaquine and chloroquine with the short stem 1 aptamers OR7 and OR8.

	K_{d1} (µM)	ΔH_1 (kcal mol^{-1})	$-T\Delta S_1$ (kcal mol^{-1})	K_{d2} (µM)	ΔH_2 (kcal mol^{-1})	$-T\Delta S_2$ (kcal mol^{-1})
Amodiaquine						
OR7	0.08 ± 0.04	−1.8 ± 0.2	−7.6 ± 0.5	20 ± 7	−6 ± 1	−0.6 ± 0.3
OR8	N/A					
Chloroquine						
OR7	NB					
OR8	0.12 ± 0.09	−0.9 ± 0.1	−10 ± 1	18 ± 9	−4 ± 2	−3 ± 2

Data acquired at 15 °C in 20 mM Tris (pH 7.4), 140 mM NaCl, 5 mM KCl, 3% DMSO. The values reported are averages of 2 or 3 individual experiments. The error reported is the standard deviation. N/A denotes data not acquired. NB denotes no binding detected at these experimental conditions.

4. Conclusions

In conclusion, we show that the cocaine-binding aptamer tightly binds an even wider range of ligands than previously known, with amodiaquine the tightest binding ligand currently known. Binding of the three tightest ligands (amodiaquine, chloroquine and mefloquine) is both entropically and enthalpically favorable while the binding of cocaine, quinine and primaquine are enthalpically driven, with unfavorable entropy at the conditions studied. NMR chemical shift perturbation experiments and ITC competition experiments indicate that these quinine-based ligands share the same high affinity and low affinity binding sites.

The binding of these antimalarial compounds by the MN4 aptamer may have practical uses in drug delivery by using an aptamer-based DNA delivery mechanism. Also, it may be possible to use aptamer binding as a way of stabilizing or preserving a quinine-based antimalarial compound. Another use could be through the use of cocaine-binding aptamers with different lengths of stem 1. It should be possible, given the variation in affinity, to distinguish the binding of quinine (by MN19)[21] from chloroquine/mefloquine (by MN19 and OR8) and amodiaquine (by MN19, OR7 and OR8) (Fig. 5).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2018.09.017.

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