Published in final edited form as:

Bioorg Med Chem Lett. 2011 August 1; 21(15): 4524–4527. doi:10.1016/j.bmcl.2011.05.130.

Structure activity studies of RNA-binding oxazolidinone derivatives

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Abstract

The structure activity relationship of a series of oxazolidinones binding to T-box riboswitch antiterminator RNA has been investigated. Oxazolidinones differentially substituted at C-5 were prepared and the ligand-induced fluorescence resonance energy transfer (FRET) changes in FRET-labeled antiterminator model RNA were assayed. Both qualitative and quantitative analysis of the structure-activity relationship indicate that hydrogen bonding and hydrophobic properties play a significant role in ligand binding.

Keywords

RNA; Oxazolidinone; Antiterminator; T-box Riboswitch; FRET

Targeting RNA with small molecules is an emerging field for which much remains to be explored.¹⁻⁷ In this paper we report on key ligand-RNA recognition features as part of our comprehensive drug discovery investigation for targeting and disrupting the function of T-box antiterminator RNA. The T-box antiterminator is a key component of the T-box transcription antitermination riboswitch.^{8,9} Riboswitches control RNA transcription (or translation) by binding and structurally responding to metabolic effector molecules to control whether transcription (or translation) proceeds.¹⁰ In the T-box transcription antitermination riboswitch, the 5'-untranslated mRNA region (5'-UTR) binds and structurally responds to uncharged (non-aminoacylated) tRNA. The anticodon of the tRNA binds to a complementary specifier sequence in Stem 1 of the 5'-UTR and the four nucleotides at the tRNA acceptor end base pair with four nucleotides in the bulge of the antiterminator structural element (Figure 1).⁸ This latter base pairing does not occur with charged tRNA and is required for transcription antitermination to occur. In the absence of uncharged tRNA, an alternative, more stable, secondary structural element (the terminator) is formed and leads to termination of transcription.⁸ The antiterminator and terminator structural elements share common nucleotides and are therefore mutually exclusive. Thus, the entire 5'-UTR functions as a riboswitch that responds to the charging ratio of cognate tRNA (i.e., uncharged, cognate tRNA is the metabolic effector molecule).⁸

Given the prevalence of T-box riboswitches in controlling the transcription of vital genes in Gram-positive bacteria (including many pathogenic examples)⁹ and the high sequence

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Supplementary Material Multiple linear regression data as well as alternate versions of Table 1 containing the data organized by functional group are provided in the supplementary material.

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conservation of the antiterminator element,^{9,11} the T-box antiterminator is an important target for RNA drug discovery. Previous work in our labs has identified 4,5 oxazolidinones as ligands with high affinity and specificity for the antiterminator element.^{12,13} In this paper we report a structure activity relationship study of a series of oxazolidinones binding to functionally relevant antiterminator model RNA (AM1A, Figure 1B)^{14,15} and a reduced function variant (C11U).¹⁴ We previously observed that oxazolidinones with an N-phenyl piperazine group at the C-4 position had excellent binding properties with the antiterminator model RNA.¹² In those previous studies we had examined only a limited number of acyl groups at the C-5 position. Given that the acyl group is introduced in the final synthetic step and the ready availability of a large number of acyl donors, we decided to leave the phenyl piperazine moiety intact and examine the effect of changing acyl groups.

The oxazolidinones used in this study were prepared as previously reported.¹² The aziridine **1** was treated with N-phenyl piperazine to provide **2** in 85% yield. We had previously converted **2** directly to **3** by reaction with an excess of an acid chloride.¹⁶ As we needed to use a larger variety of acyl donors such a reaction sequence would not be generally useful. The trityl group was thus removed with HCl to provide the corresponding alcohol. The alcohol could then be acylated with a wide variety of acylating agents and reaction conditions. Depending upon the availability and reactivity acid chlorides, carboxylic acid/DCC, carboxylic acid/polystyrene supported DCC (PS-DCC), carboxylic acid/silica gel supported DCC (Si-DCC), or an isocyanate could be used for this reaction. The yields and identities of the oxazolidinones are shown in Table 1. Our choices for acylating agents were guided by the identity of previously identified molecules showing good binding to the antiterminator RNA. Two R groups that had stood out were R = CH₂Ph (**ANB-22**) and R = 4-(CH₃CO)-C₆H₄NH (**ANB-40**). We planned to examine substitution on the aromatic ring of both the ester and carbamate derivatives, the position of the aromatic ring relative to the carbonyl, isosteric replacements for the benzene ring, and a few non-aromatic R groups.

The series of oxazolidinones were screened for binding to antiterminator model RNA using a previously described Fluorescence Resonance Energy Transfer (FRET)-based assay.^{12,17} Data are reported as the relative percentage change in the fluorescence $\%F_{rel} = [(F - F_0) / F_0] * 100$, where F₀ represents the fluorescence at 585 nm of the FRET-labeled RNA in the absence of ligand and F represents the fluorescence in the presence of the ligand.

A comparison of ligand-induced FRET changes in the antiterminator RNA for each compound is detailed in Table 1 and relative RNA specificity is summarized in Figure 2. The ligand-induced effects were, on average, 23% lower for AM1A compared to C11U. This is consistent with the known small structural differences between AM1A and C11U^{14,15} and likely indicates slight ligand binding mode differences between the two RNA.

Interestingly, two compounds led to a negative %F_{rel} (**IMB-15** and **IMB-21**). Both **IMB-15** and **IMB-21** contain a strongly electron-withdrawing nitro group on the phenyl carbamate. The fact that related nitro-substituted phenyl esters (**IMB-52**, **IMB-58**, **IMB-68**) do not show this effect suggests that the decrease in the FRET may be due to ligand-specific binding interactions resulting from the enhanced acidity of the carbamate N-H rather than a non-specific electrostatic repulsion with the phosphodiester backbone of the RNA helices. The hypothesis that H-bonding may play a role in binding is further supported by the fact that in the series of 4-substituted phenyl carbamates the %F_{rel} increases as the electronegativity of the substituent decreases.

While the relative magnitude of ligand-induced %F_{rel} change does not necessarily correlate with RNA affinity, previous studies have demonstrated that FRET changes are indicative of

ligand binding.^{12,13,17} Consequently, the oxazolidinone %F_{rel} data were correlated with ligand descriptors in order to begin to develop a quantitative structure-activity relationship (QSAR) for ligands binding the T-box antiterminator RNA. Physico-chemical descriptors of the compounds were computed using QikProp 2.1 (Schrödinger) and utilized in a multiple linear regression analysis of the data with QikFit 2.1 (Schrödinger). The correlation of experimental vs. calculated %F_{rel} data are shown in Figure 3 and the fit parameters are listed in the Supplementary Material. Previous studies indicated the significance of protonated amines and ionic interactions in RNA ligand binding.^{12,17,18} In this study, the library was specifically designed to investigate non-ionic interactions at the C-5 position.

In the multiple linear regression fit for AM1A ($R^2 = 0.8$) and the fit for C11U ($R^2 = 0.8$), the descriptors with greatest contribution to the fit that were the most statistically significant ($P < 0.01$) were dipole (computed dipole moment), FISA (hydrophilic component of total solvent accessible surface area), donorHB (number of hydrogen bond donors), QPlogPo/w (predicted octanol/water partition coefficient), and index of cohesive interaction in solids.^{19,20} That the dipole moment and hydrogen bonding were significant is consistent with the observations discussed above with respect to the acidity of the carbamate nitrogen in **IMB-15** and **IMB-21**. Substitution of the phenyl of benzoate esters showed a clear impact of this with halogen, and methoxy substitution (**IMB-53**, **IMB-55**, **IMB-64**, and **IMB-65**) leading to higher %F_{rel} values. The significance of the QPlogPo/w descriptor highlights the importance of hydrophobic groups. For example, ligands with higher QPlogPo/w values typically correlated with higher %F_{rel} values, including the two compounds with the greatest extent of ligand-induced FRET changes (**IMB-73** and **IMB-80**). Other compounds with high QPlogPo/w values included **IMB-61**, **IMB-67**, **IMB-69**, **IMB-76**, and **IMB-81**. This trend was overshadowed by other factors in the carbamate series. For example **IMB-22**, a phenyl carbamate substituted by a t-butyl group and methyl group, did not show this higher %F_{rel} even though it has a high QPlogPo/w value. This hydrophobic trend, along with the significance of hydrogen bonding, are consistent with previous studies which indicated that non-ionic interactions, including hydrophobic interactions, likely play a role in oxazolidinone binding.^{12,13}

With regards to RNA specificity, the globularity descriptor²⁰ was significant in the AM1A fit ($0.01 < P < 0.05$), but not in the C11U fit. This is consistent with the known structural differences between the two antiterminator model RNA.^{14,15} The ligand globularity may contribute to the observed ligand-induced FRET differences between AM1A and C11U.

In summary, we have investigated the structure-activity relationship for a series of oxazolidinone ligands binding to T-box antiterminator model RNA. The overall QSAR highlighted the importance of hydrogen bonding and hydrophobic properties in ligand binding. Further studies on binding affinity, effects of chirality, 3D descriptors and binding location will be reported in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the National Institutes of Health (GM073188) for support of this work. We also thank Ohio University for support of the BioMolecular Innovation and Technology project.

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Figure 1. T-box riboswitch (A) and antiterminator model RNA AM1A (B) with sequence variant C11U indicated by an arrow.

Figure 2.
Comparison of Experimental %F_{rel} for AM1A vs. C11U

Figure 3. Comparison of experimental vs. calculated %F_{rel} for AM1A and C11U with 90% prediction bands shown as dashed lines.

Scheme 1.

Table 1

Oxazolidinones ligands: Synthetic yield and ligand induced FRET RNA changes

Compound	Yield % ^a	R	AMIA %F _{rel} c	CI1U %F _{rel} c	Compound	Yield % ^a	R	AMIA %F _{rel} c	CI1U %F _{rel} c
ANB-22	NA	C ₆ H ₄ CH ₂ -	8.5	15.9	IMB-55	70	3-(MeO)-C ₆ H ₄ -	23.8	24.7
ANB-40	NA	4-(CH ₃ CO)-C ₆ H ₄ NH-	7.7	13.4	IMB-56	56	3,4,5-(PhCH ₂ O) ₃ -C ₆ H ₂ -	14.3	18.6
IMB-7	53	4-(MeO)-C ₆ H ₄ CH ₂ -	13.7	18.8	IMB-57	76	2-furan-	6.4	14.7
IMB-8	27	<i>n</i> -C ₇ H ₁₅ -	18.1	25.4	IMB-58	35 ^a	4-(NO ₂)-C ₆ H ₄ CH ₂ -	9.1	16.5
IMB-9	52	(Me) ₂ CHCH ₂ -	9.5	14.0	IMB-59	64 ^b	3,4-Cl ₂ -C ₆ H ₃ CH ₂ -	29.1	28.9
IMB-10	65	(Me) ₂ CH(CH ₂) ₂ -	15.0	20.2	IMB-60	60 ^b	3-(MeO)-C ₆ H ₄ -CH ₂ -	18.9	19.6
IMB-11	26	<i>n</i> -C ₅ H ₁₁ -	15.5	21.0	IMB-61	48 ^b	4-Ph-C ₆ H ₄ -CH ₂ -	25.3	22.3
IMB-12	18	Ph(CH ₂) ₂ -	20.6	26.1	IMB-62	63 ^b	3-(Me ₂ N)-C ₆ H ₄ -	24.6	27.1
IMB-13	52	PhOCH ₂ -	9.5	9.4	IMB-63	68 ^b	2-F-C ₆ H ₄ -	18.5	20.4
IMB-14	68	2,6-Cl ₂ -C ₆ H ₂ -	15.3	16.2	IMB-64	64 ^b	2-Cl-C ₆ H ₄ -	30.4	28.8
IMB-15	58	4-(NO ₂)-C ₆ H ₄ NH-	-1.8	-4.9	IMB-65	45 ^b	2-I-C ₆ H ₄ -	28.6	22.4
IMB-16	82	3,4-Cl ₂ -C ₆ H ₄ NH-	8.9	14.7	IMB-66	25 ^b	2-CN-C ₆ H ₄ -	7.3	16.7
IMB-17	58	4-(MeO)-C ₆ H ₄ NH-	7.8	7.7	IMB-67	60 ^b	2-(PhO)-C ₆ H ₄ -	30.8	26.5
IMB-18	68	4-(<i>n</i> -C ₅ H ₁₁)-C ₆ H ₄ NH-	10.6	11.1	IMB-68	31 ^b	2-(Me)-3-(NO ₂)-C ₆ H ₄ -	23.3	24.5
IMB-19	14	4-(CH ₃ CH ₂)-C ₆ H ₄ NH-	18.3	19.1	IMB-69	51 ^b	(2-norbornyl)-CH ₂ -	29.5	27.4
IMB-20	40	<i>n</i> -C ₆ H ₁₃ NH-	17.4	23.7	IMB-71	72 ^b	<i>n</i> -C ₃ H ₇ -	10.1	19.7
IMB-21	90	3-(NO ₂)-C ₆ H ₄ NH-	-1.1	0.2	IMB-72	75 ^b	(<i>c</i> -C ₆ H ₁₁)(CH ₂) ₃ -	31.8	29.0
IMB-22	87	2-(<i>n</i> -Bu)-6-Me-C ₆ H ₃ NH-	14.8	15.4	IMB-73	53 ^b	(Me) ₂ CCH(CH ₂) ₂ CH(Me)CH ₂ -	38.9	42.4
IMB-23	73	PhNH-	8.4	11.2	IMB-74	16	(Me) ₃ CNH-	8.9	8.7
IMB-24	25	<i>n</i> -C ₄ H ₉ NH-	10.5	14.0	IMB-75	22	<i>n</i> -C ₃ H ₇ NH-	10.3	9.6
IMB-25	39	PhCH ₂ NH-	6.1	11.7	IMB-76	48 ^b	(Ph)(CH ₂) ₅ -	32.6	30.9
IMB-49	76	3-(Me)-C ₆ H ₄ NH-	16.8	26.7	IMB-77	83	<i>c</i> -C ₆ H ₁₁ -	19.5	20.7
IMB-50	16	(Me) ₃ C-	7.1	13.2	IMB-78	32	PhO-	12.4	21.3
IMB-51	57	Ph-	14.8	18.6	IMB-79	53 ^b	(BocNH)-(CH ₂) ₄ -	29.4	27.7

Compound	Yield % ^a	R	AMIA %F _{rel} c	C11U %F _{rel} c	Compound	Yield % ^a	R	AMIA %F _{rel} c	C11U %F _{rel} c
IMB-52	79	4-(NO ₂)-C ₆ H ₄ -	13.1	15.2	IMB-80	43	(R)-PhCH(BocNH)-	39.6	42.6
IMB-53	76	4-Cl-C ₆ H ₄ -	29.1	22.8	IMB-81	25	(S)-PhCH(BocNH)-	26.7	19.4
IMB-54	78	4-(Me)-C ₆ H ₄ -	27.4	27.5					

^aThe reported yield is based on the two-step sequence of deprotection followed by acylation.

^bThe coupling was carried out using Si-DCC or PS-DCC and the corresponding carboxylic acid.

^cEach ligand binding reaction contained 100 nM 3'-Fl-AM1A-18-Rhd¹⁷ and 10 μM of ligand (from DMSO stock solution) in 100 μL binding buffer (50 mM sodium phosphate pH 6.5, 50 mM NaCl, 5 mM MgCl₂ and 0.01 mM EDTA). Following excitation at 467 nm, the normalized percent change in fluorescence was calculated as %F_{rel} = [(F/F₀)-1]*100 where F is the fluorescence at 585 nm in the presence of ligand and F₀ in the absence of ligand (i.e., DMSO control). Data represent the average of duplicate experiments.