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A Small Molecule Transcriptional Activation Domain

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Transcriptional activators play an essential role in the regulatory network that controls gene-specific transcription (Figure 1).[1] The misregulation of this complex event cascade is correlated with a growing number of human diseases,[2] and thus interest in developing artificial transcriptional activators has intensified.[3] Endogenous activators contain two key functional domains: a DNA binding domain (DBD) that interacts sequence-specifically with DNA and an activation domain that mediates a variety of protein−protein interactions that lead to specific levels of gene activation (Figure 1b).[1] These domains are exchangeable, and artificial activators that target novel DNA binding sites have successfully been generated by replacing endogenous DBDs with nonnatural equivalents.[3] In contrast, it has proven a far greater challenge to replace the activation domain with a small molecule counterpart; despite the enormous potential of such compounds, no examples have been reported. We describe here the design and synthesis of isoxazolidines that activate transcription at levels comparable to those of a natural activation domain. These heterocycles are the first examples of small molecules with such function and thus represent an important addition to the repertoire of small molecules that control complex biological processes.

Contributing to the difficulty in identifying small molecule transcriptional activation domains is the lack of a detailed picture of the structure and function of endogenous activation domains. In eukaryotes the most well-studied examples are amphipathic sequences containing interspersed hydrophobic and polar amino acid residues that interact with the shallow binding surfaces of target proteins in the transcriptional machinery.[1] These activation domains often contain surreptitious repeats of 6−14 amino acids,[4,5] and the minimal repeat unit of a natural transcriptional activator can itself function as an activator when attached to a DBD.[6,7] Evidently even these relatively short sequences are capable of interacting with the various transcriptional protein targets with sufficient affinity for activation to occur. For example, the peptide ATF14 (Figure 1c) is a minimal functional module of the potent viral activator VP16 and up-regulates transcription effectively both in vitro and in cell culture.[8] This minimal activation module is thus an excellent guidepost for the design of small molecule functional counterparts.

To identify a minimal functional unit for a small molecule-based activation domain, a series of isoxazolidines containing functional groups typically found in endogenous activation domains were designed (Figure 2a). This heterocyclic scaffold was chosen due to the relative ease with which diverse functional groups could be incorporated in a stereocontrolled manner onto the conformationally constrained ring,[9] thus displaying those groups in a three-dimensional array. Key functional groups found in ATF14 and related activation domains include phenyl, hydroxyl, carboxylic acid, and isobutyl groups. Isoxazolidine **2** has three hydrophobic groups appended at the N2 and C3 positions, while isoxazolidines **3**−**6** each have a combination of polar (hydroxyl and carboxylic acid) and hydrophobic groups (phenyl, isobutyl) at N2, C3, and

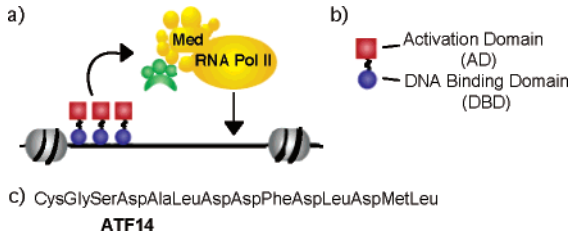

Figure 1. (a) Transcriptional activators bind to specific sites on DNA and participate in the recruitment of RNA polymerase II holoenzyme and general transcription factors to initiate transcription.[1] (b) The DNA binding domain and activation domain can be covalently linked as shown or assembled through noncovalent interactions.[3] (c) Sequence of ATF14.

C5. The relative stereochemistry at C3 and C5 in key intermediate **7** was set via addition of an allyl Grignard to isoxazoline **8**,[9c] and alkylation of N2 served to introduce the benzyl or acid group at that position (see Supporting Information for details). The final step of the syntheses of **2**−**6** was hydrazone formation with methotrexate hydrazide (**1**), used to localize the isoxazolidines to DNA in functional assays. ATF14 was also synthesized and coupled to methotrexate to enable a direct functional comparison with the small molecules.

An in vitro transcription assay was employed to assess the capability of each of the isoxazolidines to function as transcriptional activation domains under standard conditions (Figure 2c).[10] In this assay, the fusion protein LexA-DHFR[11] serves as the DNA binding domain, localizing the isoxazolidines to the promoter via the specific and high affinity methotrexate−DHFR binding interaction. This is a robust interaction tolerant of a range of substitution at the γ-carboxy position of methotrexate.[12] For each experiment, compound **1** (negative control), isoxazolidines **2**−**6**, or ATF14 (coupled to methotrexate) were combined with a DNA template consisting of a reporter gene under the control of two LexA binding sites within an AdML promoter, followed by addition of HeLa nuclear extracts and nucleotide triphosphates. mRNA production was directly measured and used to determine the activity of all compounds, displayed as percent activation relative to the positive control ATF14 (Figure 2c).

Remarkably, isoxazolidine **4** is nearly as active as the positive control ATF14 despite a considerable difference in size (MW 290 versus 1674). Further, it is the most potent of all of the isoxazolidines examined, with ~5- to 7-fold levels of activation over basal. This function is dependent upon a DNA binding domain, as an identically functionalized isoxazolidine *lacking* covalently linked methotrexate does not activate transcription and also competitively inhibits transcription mediated by **4** (see Supporting Information for details). Similar to natural activation domains such as ATF14, a balance of hydrophobicity and polarity is important for overall potency.[5,13] Substantially increasing the hydrophobicity (**2**) or the polarity (**3**) leads to a dramatic decrease in function. In contrast, slightly increasing the polarity at C3 by incorporation of an

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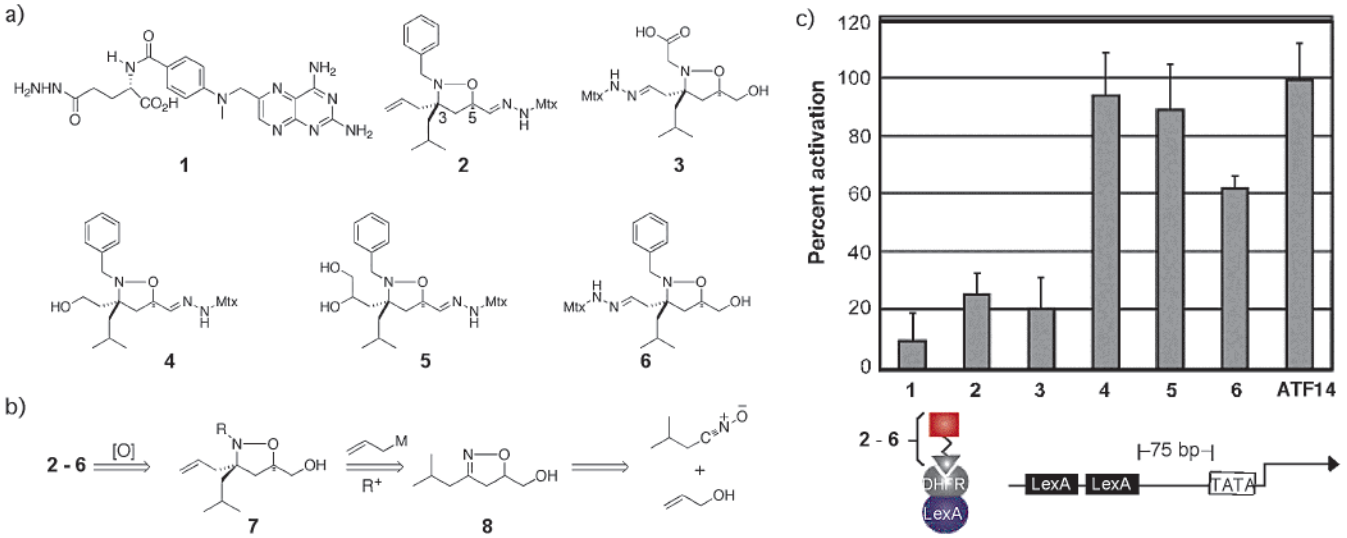

Figure 2. Isoxazolidine-based activation domains. (a) Five isoxazolidines (**2**−**6**) bearing functional groups commonly found in natural activation domains were targeted. (b) Synthetic strategy used to prepare isoxazolidines. (c) Results from in vitro transcription assays. The activity of each compound represents the average of at least three individual experiments with the indicated error (SDOM). For details see the Supporting Information.

additional hydroxyl (**5**) is well tolerated. Consistent with a preferred orientation of the hydrophobic and polar substituents, isoxazolidine **6**, containing the same functional groups as **4**, shows reduced activation potential.

Two factors likely play a role in isoxazolidine **4** being nearly as active as ATF14 despite the size difference. As an organic molecule, **4** is resistant to proteolytic degradation and thus probably has a longer lifetime. In addition, structural studies suggest that while many natural activation domains are unstructured in solution, helix formation accompanies binding for at least some activator−target interactions;[14] in contrast, **4** likely populates conformations more closely related to the final bound state due to structural constraints imposed by the ring. Both factors should also contribute advantageously to the function of **4** in cells. Future cell-based functional comparisons with ATF14, a strong activator in cells,[8] and related natural activation domains should thus provide insight into factors contributing to transcriptional activator function. Finally, when the sequences of minimal modules such as ATF14 are reiterated to make longer activation domains, the levels of transcription elicited increase synergistically.[6] Thus, considering **4** as a minimal functional module, oligomers of this isoxazolidine should exhibit increased potency and in the future offer a mechanism for creating artificial activators tuned for a particular level of gene transcription.

In summary, we have described the first small molecule transcriptional activation domain. This molecule, an amphipathic isoxazolidine, is approximately as potent as the natural activation peptide ATF14. With this discovery, the creation of wholly artificial transcriptional activators can now be envisioned. These will be valuable tools for studying the mechanistic details of transcriptional regulation and may in the long run assist in the development of transcription-based therapeutic agents.

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Supporting Information Available: Details of the synthesis of isoxazolidines **2**−**6** and in vitro transcription experiments. This material is available free of charge at http://pubs.acs.org.

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