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Discovery of an Inhibitor of a Transcription Factor Using Small Molecule Microarrays and Diversity-Oriented Synthesis

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Transcription factors that become overactive in cancers are promising yet untested targets for cancer therapeutics.[1] These proteins mediate the excessive transcription of genes whose products are required for tumor growth and metastasis. Inhibiting the function of a transcription factor requires specific disruption of DNA−protein or protein−protein interactions. The discovery of small molecules that disrupt these interactions has thus far proven to be a significant challenge.[2] Diversity-oriented synthesis (DOS)[3] is a possible source of candidate antagonists of such interations.[4,5] To test this possibility, we devised a small molecule microarray-based screen aimed at identifying DOS-derived small molecules that directly bind and modulate the transcriptional activity of Hap3p, a subunit of the yeast Hap2/3/4/5p transcription factor complex involved in aerobic respiration and the nutrient-response signaling network.[6] The complex is particularly interesting because the regulation of mitochondrial function is relevant to numerous processes, including circadian rhythm, and several diseases, such as cancer and diabetes.[6]

Small molecule microarrays containing 12 396 compounds derived from three different DOS pathways[5] and prepared in a one bead−one stock solution format (Figure 1)[7] were printed onto chlorinated glass microscope slides as described previously.[8] The three microarrays were probed with purified Hap3p-GST fusion protein, and binding was detected using a Cy5-labeled antibody against the GST portion of the fusion protein. The screen revealed two reproducible positives **1** and **2** from a library of dihydropyrancarboxamides (Figure 1).[5a] Compound **2** also appeared as a positive when the library was screened with GST as a control, revealing that it, in contrast to **1**, binds to the GST portion of the Hap3p-GST fusion protein.[9]

Surface plasmon resonance studies revealed that **1**, or haptamide A, binds to Hap3p with a dissociation constant of 5.03 μM. To examine whether haptamide A modulates endogenous Hap3p function in cells, **1** was tested in a *GDH1-lacZ* reporter gene assay. *GDH1*, which encodes an NADP-dependent glutamate dehydrogenase, requires the Hap2/3/4/5p complex for optimal expression.[10] Haptamide A inhibited expression of the reporter in a dose-dependent manner and approached the level of an otherwise isogenic *hap3*Δ deletion strain at 50 μM (Figure 2). Treatment with negative control **2** did not affect expression of the reporter. After cells were treated with haptamide A for 60 min followed by a washout in fresh medium for 60 min (data not shown), expression levels approached the untreated sample, suggesting that **1** is a reversible inhibitor of Hap3p-mediated transcription.

To explore structure−activity relationships, 11 derivatives with systematic variations were synthesized as shown in Figure 1a. Dissociation constants and IC_{50} values for the reporter gene assay were determined for each compound (Table 1). In general, changes in potency were consistent with changes in binding affinity. As expected, the primary alcohol, the site of covalent attachment to

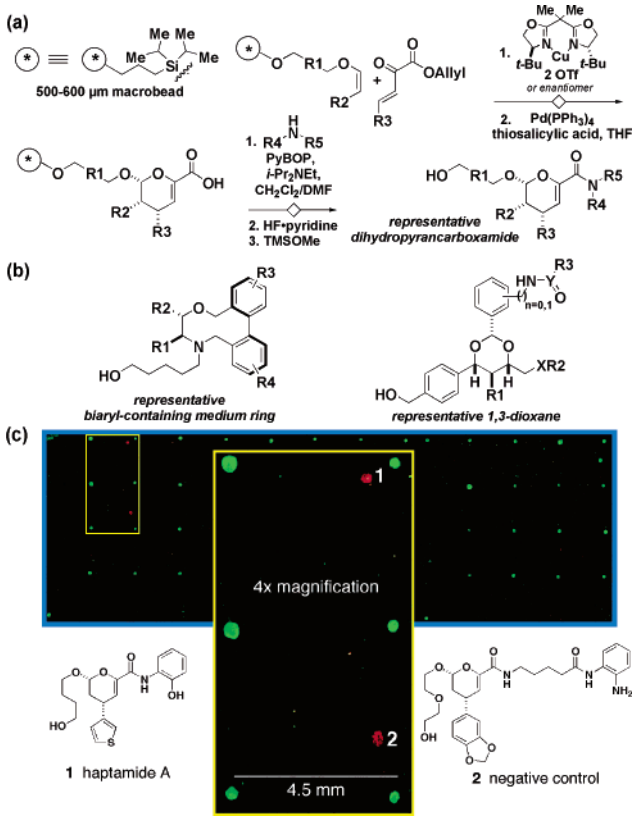

Figure 1. (a) DOS pathway leading to dihydropyrancarboxamides. (b) Representative structures for the biaryl-containing medium ring and 1,3-dioxane DOS libraries. (c) Small molecule microarray, containing 6504 printed dihydropyrancarboxamides (blue box), probed with Hap3p-GST followed by a Cy5-conjugated rabbit-anti-GST antibody. Rhodamine markers, printed in the upper right-hand corner of each 12 × 12 subarray, are false-colored green. The magnified portion of the array (yellow box) contains 288 printed small molecule features, including the two positives for Hap3p-GST binding (**1** and **2**) which are false-colored red.

the slide, is not required for binding or inhibition. Potency was reduced by replacing the thiophene moiety at C-4 with an isopropyl group (**5**) or removing the aryl amide (**4** and **12**). The enantiomer of haptamide A (**3**) was more potent with a submicromolar dissociation constant. Moving the *ortho*-hydroxyl group to the meta position (**6**) also resulted in improved potency and binding affinity. The effects of these two changes were additive as **13**, or haptamide B, was determined to have a K_D of 0.33 μM and an IC_{50} value of 23.8 μM.

Whole-genome transcription profiling[11] was first used to study the global effects of haptamide B in cells growing in glucose. Deletion of *HAP3* or treatment with 50 μM **13** led to reduced expression of genes regulated by Hap2/3/4/5p (*GDH1*, *GDH3*,

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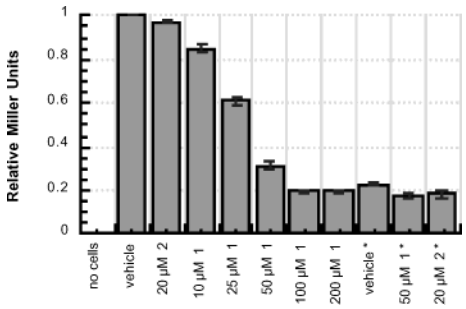
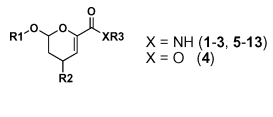
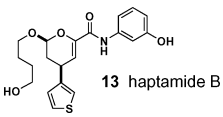


Figure 2. Dose-response of **1** in a reporter gene assay. BY4741 cells or BY4741 *hap3*Δ cells (*) expressing a *GDH1-LacZ* reporter were treated with **1** or **2**. After 60 min of treatment at 30 °C, liquid β-galactosidase assays were performed in quadruplicate. Data are expressed in fold Miller units relative to wild-type cells treated with vehicle (0.5% DMF).

Table 1. Structure−Activity Relationship Data for Derivatives of **1**





13 haptamide B

					(μM)	
compound	R1	R2	catalyst	R3[a]	IC_{50}	K_D [b]
1	HO−$(CH_2)_4$	thiophene	S	*o*-PhOH	42.1	5.03
3	HO−$(CH_2)_4$	thiophene	R	*o*-PhOH	30.6	0.66
4	HO−$(CH_2)_4$	thiophene	S	H	136	
5	HO−$(CH_2)_4$	*i*-Pr	S	*o*-PhOH	82.9	42.9
6	HO−$(CH_2)_4$	thiophene	S	*m*-PhOH	35.3	1.66
7	HO−$(CH_2)_4$	thiophene	S	*o*-$PhNH_2$	66.6	10.7
8	HO−$(CH_2)_4$	thiophene	S	*o*-PhSH	47.6	10.1
9	HO−$(CH_2)_4$	thiophene	S	Ph	49.6	17.2
10	BdtO−$(CH_2)_4$	thiophene	S	*o*-PhOH	48.9	3.59
11	TrtO−$(CH_2)_4$	thiophene	S	*o*-PhOH	53.7	
12	HO−$(CH_2)_4$	thiophene	S	*t*-cycOH	>200	
13	HO−$(CH_2)_4$	thiophene	R	*m*-PhOH	23.8	0.33

[a] *t*-cycOH, *trans*-2-hydroxycyclohexyl. [b] K_D's were not determined for compounds **4** (nonspecific binding), **11** (insoluble), and **12** (no binding).

YHB1, *COX4*).[6] The Hap2/3/4/5p meta complex also regulates several genes under nonfermentative conditions.[6a,12] Treating these genes as reporters, we additionally studied the effects of gene deletion or haptamide B on transcription in cells shifted from growth medium containing 2% (w/v) glucose to medium containing 2% (w/v) lactate. With the exception of *JEN1*,[12d] the induction of genes encoding the major enzymes of lactate metabolism[6a,12] (*CYB2*, *DLD1*, and *CYC1*) was reduced, relative to wild-type shifted cells, by deletion of *HAP3* or treatment with 50 μM **13**. In addition, we observed that the induction of several genes involved in regulating hexose transport (*HXT4*, *HXT6*, *HXT7*, *MTH1*, and *SUC2*) was also reduced in both the genetic "knockout" and the chemical genetic "knockdown". As a whole, transcriptional profiling analysis provided further evidence that haptamide B selectively inhibits Hap2/3/4/5p-mediated transcription in cells.

Although the mechanism for inhibition by haptamide B is unknown, this study demonstrates that an in vitro high-throughput screen for DOS-derived small molecules targeted to a subunit of a transcription factor can be used to discover inhibitors of transcription in cells. Future studies are aimed at determining whether haptamide B disrupts a protein−protein interaction among subunits or a DNA−protein interaction and if it inhibits the orthologous human NFY complex.[6c]

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Supporting Information Available: Experimental procedures, characterization, and SAR data (PDF). Complete transcription profiling data are available at http://www.schreiber.chem.harvard.edu. This material is available free of charge via the Internet at http://pubs.acs.org.

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