Combinatorial Chemistry

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Synthesis of Diverse Lactam Carboxamides Leading to the Discovery of a New Transcription-Factor Inhibitor**

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The selective induction or disruption of protein–biopolymer interactions with small molecules is important for the chemical control of molecular pathways fundamental to cell and disease biology.[1,2] However, the structural requirements for compounds that selectively disrupt protein–protein and protein–DNA interactions are largely unknown.[3] The challenge of discovering selective modulators of protein–biopolymer interactions rests on the synthesis of compounds with sufficient complexity to interact with an appropriate interface site on a target protein. To address this challenge, we are developing new strategies for the efficient synthesis of complex libraries of small molecules for use in high-throughput screening experiments when the structural requirements for activity are not previously known.[4] Herein we describe how the synthesis and screening of a library of lactam carboxamides revealed a new inhibitor of HOXA13, a transcription factor that regulates mammalian development[5] and certain kinds of cancer.[6]

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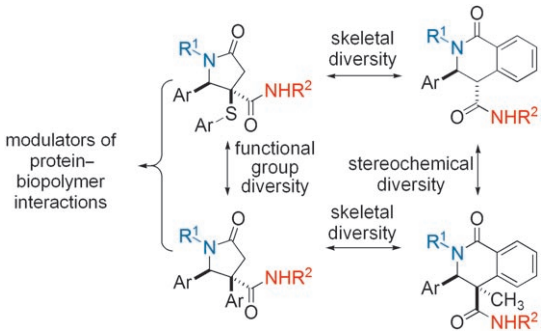
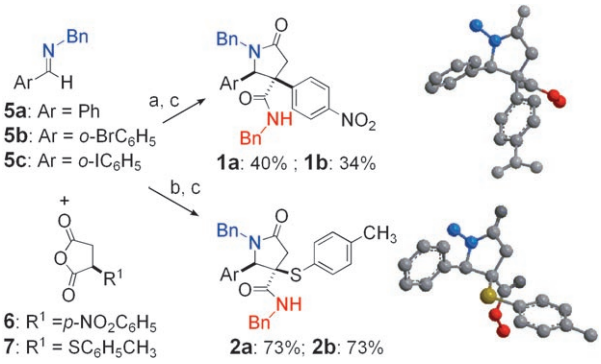
The formal cycloaddition between imines and anhydrides is an ideal starring point for the synthesis of small molecules with varied core structures that incorporate elements of functional group, skeletal, and stereochemical diversity (Scheme 1). Common imine **5** provides access to distinct



Scheme 1. Incorporation of elements of functional-group, skeletal, and stereochemical diversity.

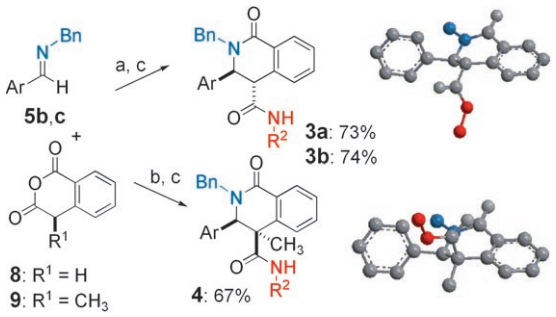
carboxy-γ-lactam and carboxy-2-quinolone structures, thus forming the basis for an efficient compound library synthesis. As a starting point, we have recently disclosed stereoselective methods for the synthesis of carboxy γ-lactams related to **1a** and **2a**, which provide distinct products based on the incorporation of either the aryl (**6**) or thioaryl (**7**) succinic anhydrides (Scheme 2).[7–8]

4-Carboxy-2-quiniolones in the *syn* (**3**) and *anti* (**4**) forms, which feature complementary skeletons when compared with



Scheme 2. Conditions: a) **5a** or **b** + **6**, toluene, 110°C; b) **5a** or **c** + **7**, toluene, 110°C, c) $BnNH_2$, HATU. Minimized (MM2) structures of **1a** and **2a**, *N*-CH_3 groups depicted for clarity. Yields based on isolation of final product after three steps. Yields of **1a** and **1b** include preparation of anhydride **6**. HATU = *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetra-methyluronium hexafluorophosphate.

carboxy-γ-lactams **1** and **2**, were also formed from imine **5** (Scheme 3). This reaction was first reported by Cushman et al.,[9] who has subsequently employed this transformation in a



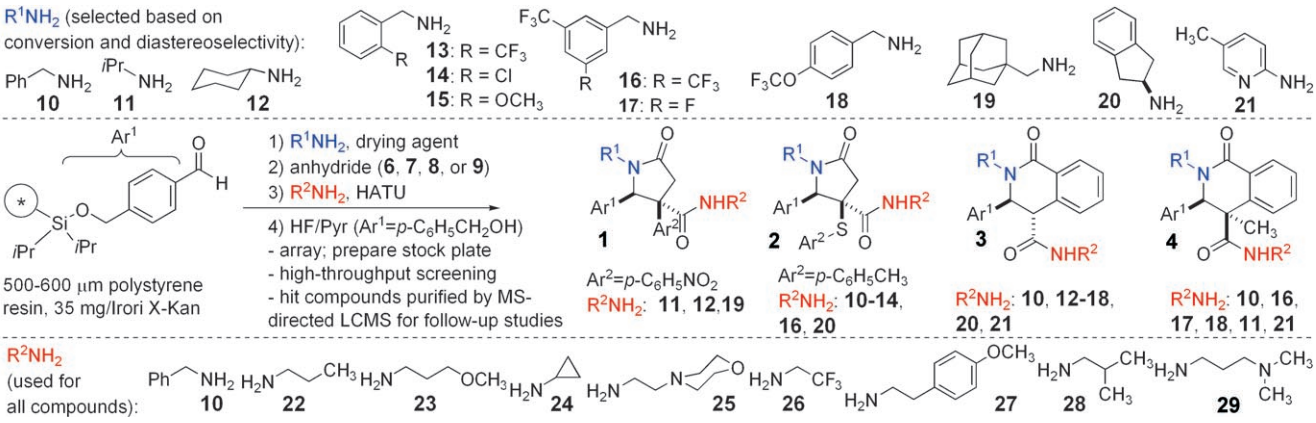
Scheme 3. Conditions: a) **5a** or **b** + **8**, CH_2Cl_2, 23 °C; b) **5b** or **c** + **9**, benzene, 23 °C, c) (p-$CH_3OC_6H_5$)$CH_2CH_2NH_2$, HATU. Minimized (MM2) structures of des-iodo-**3b** and **5**, N-CH_3 groups depicted for clarity.

variety of syntheses.[10] Although the initial reaction of anhydride **8** with imines **5a** and **5b** provides the *cis* cycloadducts, complete epimerization to the *trans* isomer is observed during conversion to amide **3a** and **3b**. Anhydride **9** also forms the *cis*-isoquinolone, but in this case epimerization during amide formation is prevented by the presence of the quaternary stereogenic center. Although these compounds only differ by a single methyl substituent, the different stereochemical array (*syn* versus *anti*) has a profound impact on the orientation of the two substituents.

The pathways described for the synthesis of lactams **1**–**4** were developed to prepare a structurally diverse library of 400 discrete compounds on a solid phase by using split-pool techniques.[11] As a wide array of imines are available for the stereoselective cycloaddition reactions with anhydrides **6**–**9**, diverse amine reagents were selected for each anhydride based on optimal conversion and diastereoselectivity (Scheme 4). Arylsuccinic anhydride **6** reacted with high (>90:10) diastereoselectivity to afford the γ-lactam products. Anhydrides **7** and **8** exhibited greater reactivity and improved substrate tolerance to react with a wider variety of imines with excellent (>95:5) diastereoselectivity. The cycloaddition reaction of 4-methylhomophthalic anhydride (**9**) was included to increase stereochemical diversity, in which the incorporation of the 4-methyl substituent prevents isomerization and provides the *cis*-fused product with good diastereoselectivity (80:20–93:7). Primary-amine (R^2NH_2) building blocks with lower than average molecular weights were chosen for the final amide-formation reaction owing to their high reactivity and incorporation of additional hydrogen-bonding capabilities.

This diverse library is useful for the study of biological process by using phenotypic readouts in whole cells and multicellular organisms when the structural requirements for activity are not known or when a pathway with multiple potential targets is examined. In addition, the structural requirements for compounds that selectively disrupt protein–protein and protein–DNA interactions, which can be studied biochemically, are still emerging. This collection of compounds has been included in over 150 high-throughput screens, of which the majority involved phenotypic readouts or the detection of protein–biopolymer interactions.[12] Although we sought to explore a broad range of biological processes that will contribute to global analyses and activity profiling,[13] this study describes our efforts to investigate the small-molecule control of gene expression by inhibiting transcription-factor–DNA binding.

A high-throughput screen by using fluorescence polarization[14] revealed lactam carboxamides **30** and **31** that inhibit the interaction of the HOXA13 DNA binding domain to its target DNA sequence (Figure 1).[15] HOXA13 is a member of the conserved Hox transcription-factor family whose function is required for the normal development of the limb and genitourinary tissues.[16] Recognizing that tumor formation closely mimics the developmental processes controlled by Hox proteins and that Hox proteins are often aberrantly expressed in cancerous tissues, strategies to disrupt Hox protein–DNA interactions could prove useful in the treatment of the disease state.[17] The known DNA intercalator 9-hydroxyelipticine[18] and other planar compounds suspected of interacting unselectively with the DNA oligonucleotide were also identified. The concentration-dependent activity of **31** was confirmed and an IC_{50} value of 6.5 μM was determined.[19] No disruption of the control Trp-repressor–DNA



Scheme 4. Synthesis of 400 diverse lactams from the reactions of solid-phase imines with anhydrides **6**–**9** followed by amide formation.

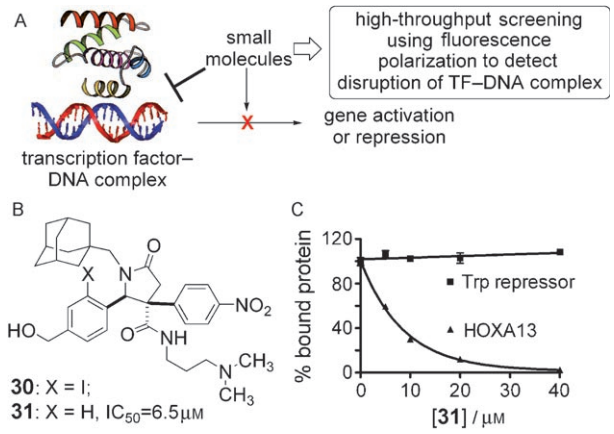


Figure 1. A) High-throughput screen for small molecules that disrupt a transcription factor–DNA complex. B) Primary-screening hits. C) Concentration-dependence of the activity of **31** against HOXA13–DNA and Trp repressor–DNA complexes.

complex was observed, indicating that **31** specifically interferes with HOXA13 DNA binding and does not unselectively bind to DNA.

The activity of **31** in cells was confirmed by using a reporter gene assay in which the expression of luciferase is under the control of HOXA13. Addition of compound **31** had little effect on the expression of luciferase (Figure 2, columns 1 and 2). In the presence of HOXA13, luciferase activity is suppressed by roughly 80%. Adding compound **31** produced a concentration-dependent increase in luciferase activity, indicating that repression by HOXA13 was effectively inhibited in cells.

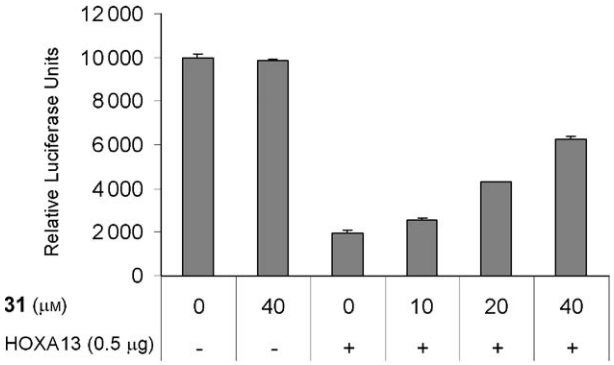


Figure 2. Inhibition of HOXA13 in NG108-15 cells. Addition of **31** reverses the ability of HOXA13 to repress expression of luciferase.

Our discovery of a new transcription-factor inhibitor from this library of lactam carboxamides affirms the idea that structurally diverse compounds are useful starting points in the search for chemical modulators of protein–biopolymer interactions. Given the difficulty in selectively targeting transcription factors,[3,20–22] the activity of **31** is a significant advance in the chemical control of transcription. We are currently establishing the structure-activity relationships of this compound in preparation for future studies in vivo. A full account of the synthesis, computational analysis of diversity, and biological profiling will be disclosed shortly.

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