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Synthesis and Cellular Profiling of Diverse Organosilicon Small Molecules

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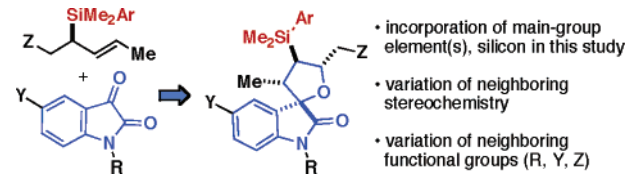
Small-molecule synthesis coupled with cellular profiling using multidimensional screening has been used to assess the role of stereochemical and skeletal variation on assay outcomes, albeit within a limited structure space.[1] Here, we provide an extension of this approach as a framework to assess the role of a main-group element on assay outcomes. We considered that incorporating, for example, boron, silicon, selenium, or germanium into small molecules could provide new structures where the unique properties of these elements may contribute to new biological activities.[2] The success of bortezomib (Velcade), a boron-containing proteasome inhibitor, highlights the use of a main-group element in a therapeutic context.[3] As a first step, a synthesis pathway was developed leading to organosilicon products[2,4,5] having silicon placed within a chiral environment (Scheme 1). This pathway allows the diversification of stereochemistry at nearby stereogenic centers and the variation of neighboring functional groups. In order to profile the biological activity of these organosilanes, multidimensional screening was performed, providing a first quantitative glimpse of the rich activities of silicon-containing small molecules within varying stereochemical and appendage contexts.

Our syntheses of test, silicon-containing small molecules relies on the stereoselective annulation pathways available for allylsilanes with π-electrophiles.[6] Crotylsilanes containing hydrogen-bond donors and acceptors (**2**−**4**) were derived from the common ester crotylsilane precursor **1** (Figure 1A).[7] Each of the (*R*)- and (*S*)-enantiomers of **2**−**4** was prepared to facilitate the synthesis of single enantiomers for biological evaluation. The anisyl crotylsilanes were selected based on their stability, enhanced nucleophilicity in the annulation reaction (relative to Ph), and ease of protodesilylation.[8]

Spiro-oxindoles represent an attractive framework for synthesis due to their precedent for biological activity.[9] Therefore, we developed a general method for Lewis acid-mediated annulations of isatins[10] (**5**) using macrobead-bound crotylsilanes **2**−**4**.[11] This method yields spirocyclic annulation products as single stereoisomers as judged by LCMS and ^{1}H NMR spectroscopy (Figure 1B). Use of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as a protic acid scavenger was essential to prevent Si−O deprotection or cleavage from the macrobead support. No protodesilylation or elimination of the silyl group was observed when HF/py (5% in THF) was used to cleave the Si−O linker, demonstrating the complementary reactivity of the Si−O bond and the Si−C bond under these conditions. Relative stereochemistry was confirmed by X-ray crystallographic analysis (**6l**).

In addition to incorporating functionality in the crotylsilane component, the modular placement of aryl iodide functional groups in the isatin component can be used in appending processes for further substitution and follow-up chemistry, for example, to facilitate target identification. The conversion of the aryl iodide to various amido functionalities was accomplished using the Buchwald amidation (for example, Figure 1C).[12]

Scheme 1



Ninety representative compounds resulting from the annulation pathway were next profiled using 41 diverse assays to evaluate the potential of silicon-containing small molecules to modulate cellular processes. These small-molecule screens use whole cells (mammalian cell lines), whole organisms (yeast, bacteria, plasmodium), and pure proteins that are relevant to cancer, developmental biology, infectious disease, and psychiatric disease.[13] To normalize the

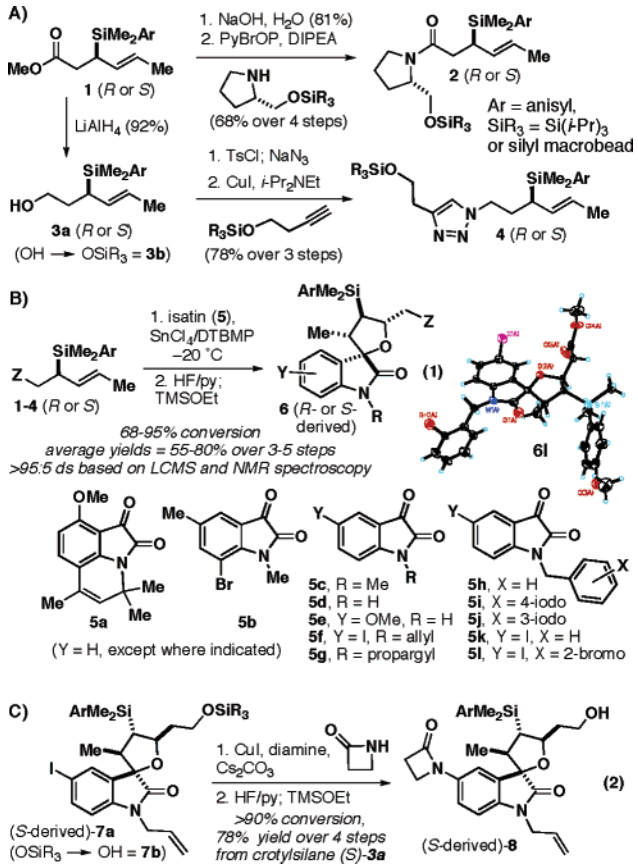


Figure 1. Annulations of functionalized crotylsilanes with isatins (**5**), where Ar = anisyl and Z = ester (**1**), amide (**2**), silyl ether (**3**), or triazole (**4**). To confirm the structure of the annulation product, on-bead analysis was performed using magic angle spinning (MAS) ^{1}H NMR spectroscopy; cleaved products were analyzed using LCMS and NMR spectroscopy (^{29}Si, ^{13}C, and ^{1}H). X-ray crystallography was performed with spirocycle **6l**, prepared from racemic crotylsilane **1** and isatin **5l**.

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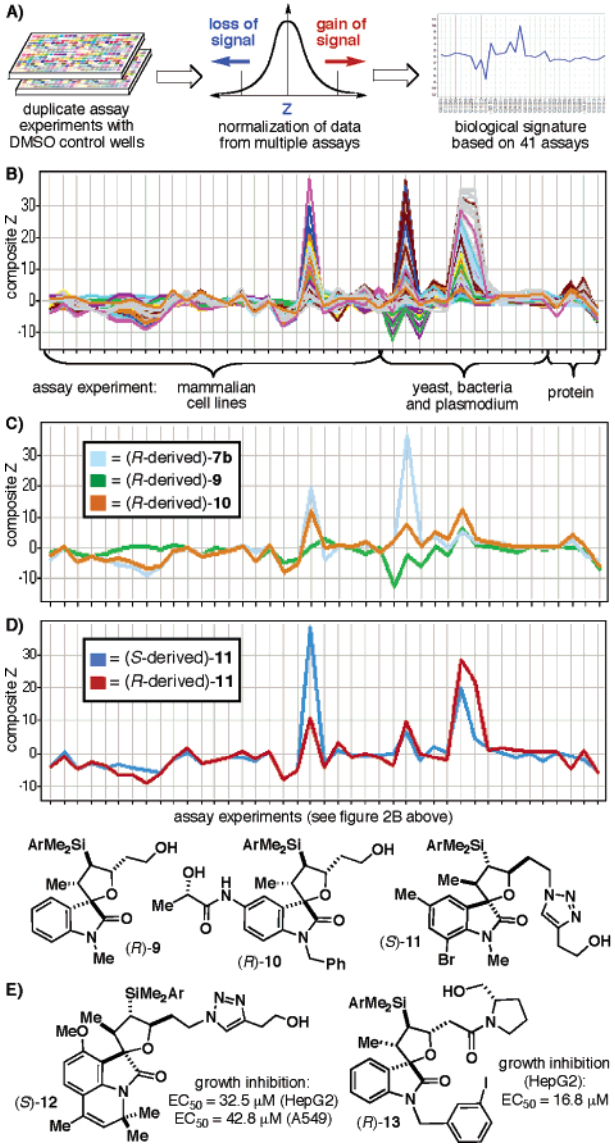

Figure 2. (A) Overview of compound signatures generated from screening data. (B) Global view of 90 compound signatures based on composite *Z*-scores derived from 41 biological assays, grouped based on assay type (biological object). Color coding reflects the substitution of the isatin reagent. (C) Signatures comparing the effect of varying appendage contexts, in this case, iodo spirocycle (*R*)-**7b**, spirocycle (*R*)-**9**, and amido spirocycle (*R*)-**10**. (D) Signatures comparing the effect of absolute stereochemistry for triazole spirocycles (*S*)-**11** and (*R*)-**11**. (E) Examples of EC_{50} values for spirocycles (*S*)-**12** and (*R*)-**13** in growth inhibition assays with A549 cells (assay #6) and HepG2 cells (assay #7). (*R*)- and (*S*)-Descriptors refer to the stereochemistry of the chiral allylic silane from which the compounds are derived. Ar = anisyl.

primary screening data from multiple assays, each compound was independently assigned a composite *Z*-score (+ or −) corresponding to the confidence of biological activity (gain or loss in signal relative to DMSO control wells) and the reproducibility of duplicate experiments.[1,14]

The composite *Z*-scores enable the generation of a high-feature, biological signature for each silicon-containing compound (Figure 2A,B).[14] These signatures reflect both the activity and selectivity of these compounds across 41 assays and provide the means to perform comparative analyses by a variety of mathematical methods. Most importantly, they demonstrate a rich breadth of activities of silicon-containing small molecules. Not surprisingly, key roles for functional group and stereochemical diversity (cf. differential performance of enantiomeric pairs of products) can also be ascertained (Figure 2C,D), where activity trends are observed within each assay. Secondary assays were performed to demonstrate dose-dependent activity and to obtain EC_{50} values for representative compounds. Examples are shown for the growth inhibition of lung adenocarcinoma (A549) cells and hepatocellular carcinoma (HepG2) cells (Figure 2E).[15]

We are currently working to understand the role of silicon in the observed biological activity.[16] This work provides a framework to assess the roles of silicon in assay outcomes, including comparative analyses of compounds having different substituents attached to silicon and of compounds having silicon replaced with carbon and other main-group elements.

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Supporting Information Available: General experimental details, spectral characterization data, X-ray structure, data analysis methods, summary of assay type, and dose−response curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Screening was performed at the Broad Institute Chemical Biology Screening Platform with duplicate experiments and mock treatment (DMSO) control wells to determine the statistical significance of the measurements. Refer to Figure S-1 and Table S-1 in the Supporting Information for a summary of assay type and related disease biology.
(14) (a) Clemons, P. A. Unpublished results. Also see http://www.chembank.harvard.edu. (b) Data visualization was performed using *DecisionSite 8.2*; Spotfire, Inc.: Somerville, MA.
(15) Refer to Figures S-3 and S-4 in the Supporting Information.
(16) Preliminary studies indicate that conversion of the arylsilane affords compounds with reduced activity for the assays described here.

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