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A Modular Approach to Build Macrocyclic Diversity in Aminoindoline Scaffolds Identifies Antiangiogenesis Agents from a Zebrafish Assay

Srinivas Chamakuri,^[a] Shiva Krishna Reddy Guduru,^[a] Sreedhar Pamu,^{[a],†}
Gayathri Chandrasekar,^[b] Satish Srinivas Kitambi,^{*,[b,c,d]} and Prabhat Arya^{*,[a]}

Keywords: Medicinal chemistry / Angiogenesis / Chemical probes / Molecular diversity / Nitrogen heterocycles / Macrocycles

A modular approach to explore the macrocyclic chemical space around an aminoindoline scaffold is developed. This is achieved by incorporating an amino acid moiety and subse-

quent "stitching technology". Through screening of a zebrafish assay, several antiangiogenesis agents are identified.

Introduction

With the goal of expanding the currently accepted chemical space to search for small molecule modulators of protein–protein^[1–3] and DNA/RNA–protein^[4] interactions and signaling pathways,^[5,6] the past few years have seen a growing interest in accessing small molecules^[7–9] that are more natural product like, and many of these small molecules are obtained through the inspiration of bioactive natural products.^[10–12] Traditionally, small molecules that are broadly available to the medicinal chemistry community for early biological evaluation are rich in sp² character, and in general, they lack the features that are commonly found in bioactive natural products.^[13,14] Several leading review articles have been written on this topic, and they challenge the organic synthesis community to access small molecules for biological evaluation in the broad areas of protein–protein interactions and other bio-macromolecular interactions.^[14,7,10,12] It is hoped that by going beyond the conventional chemical space and by enriching our small molecule collection to be more natural product like, we will identify functional small molecules for areas that are considered difficult.^[15,7] Moreover, this will allow us to expand the scope

of undertaking biological targets that are beyond our reach at present.^[15,7]

In particular, the interest in accessing functionalized large ring derivatives (i.e., macrocyclic compounds) is growing due to several reasons: (1) Medium-to-large ring structures present an opportunity to map a large surface area to interact with protein targets. (2) Preorganization in large rings allows several functional groups in specific orientations to be displayed. (3) In general, cyclic compounds possess better cell permeation properties.^[16] Despite all of these attractive features that large rings offer and despite the excellent track record of several macrocyclic natural products as drugs, the full potential of this area remains to be explored thoroughly.^[16–22] One of the challenges in this arena is the development of synthesis methods that are modular and practical in nature and that would allow different types of functionalized medium to large rings to be obtained.

A few years ago, we initiated a synthesis program that was aimed at developing methods to allow us access to functionalized, enantioenriched natural product inspired scaffolds.^[23–25] In one study,^[23] we developed a practical synthesis of an aminoindoline scaffold having three orthogonally protected functional groups. In this report, we demonstrate that this bicyclic scaffold can be converted into a tricyclic system through an in situ, stereocontrolled aza-Michael reaction. Our approach is general and can be transferred to the solid phase, which allowed us to obtain a proof of concept library for further biological evaluation.

Results and Discussion

As an extension of this work, with the objective to explore macrocyclic chemical space,^[26,27] we report herein a modular method that allowed us to incorporate different types of medium/large ring skeletons (see **1.2**, **1.3**, and **1.4**; Scheme 1) into enantioenriched aminoindoline scaffold **1.1**.

[a] Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India
E-mail: prabhata@drils.org
Homepage: www.prabhatarya.org

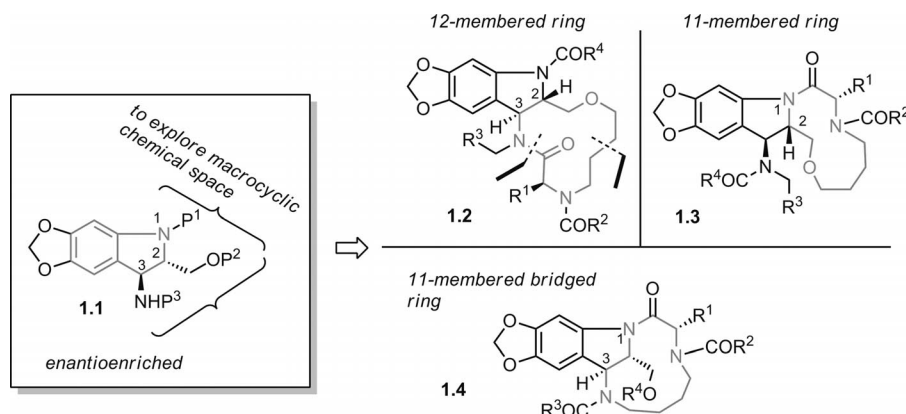
[b] School of Life Sciences, Södertörns Högskola, 14189 Huddinge, Sweden

[c] Department of Biosciences and Medical Nutrition, Karolinska Institutet, 17177 Stockholm, Sweden

[d] Division of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 17177 Stockholm, Sweden
E-mail: satish.kitambi@ki.se

[†] Current address: McGill University, Montreal

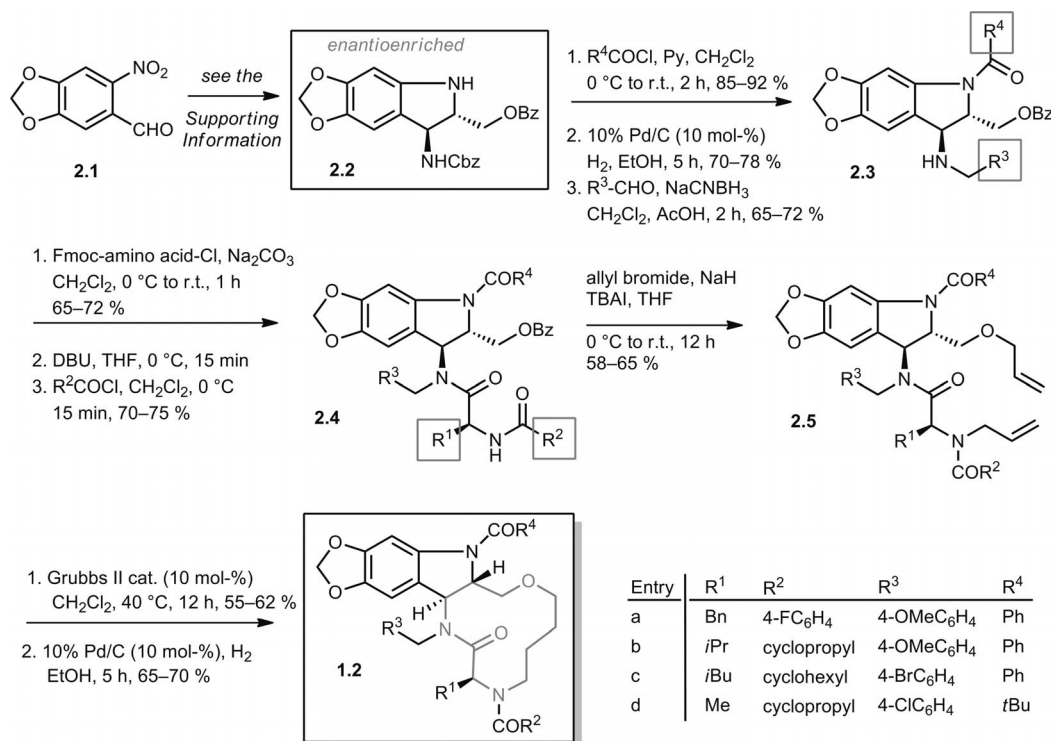
Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/ejoc.201300409>



Scheme 1. The proposed plan to obtain macrocyclic-derived compounds **1.2**, **1.3**, and **1.4** from enantioenriched aminoindoline scaffold **1.1**. P¹: NHTeoc; P²: OBz; P³: NHAlloc.

There are several attractive features of aminoindoline scaffold **1.1** that make it an attractive starting point to incorporate various medium/large rings into the skeleton. These include the presence of (1) a *trans*-2,3-amino alcohol moiety that can further be utilized to build *trans*-fused 12-membered ring **1.2**, (2) a 1,2-amino alcohol that utilizes the indoline nitrogen atom to provide 11-membered ring **1.3**, and (3) 1,3-orthogonally protected diamino functional groups that can lead to bridged 11-membered ring **1.4**. In all three strategies, we utilized a common approach to incorporate the amino acid functionality, and ring-closing metathesis^[28–30] was used as stitching technology to construct the macrocyclic ring. For example, final target **1.2** presents several interesting features, including an enantioen-

riched aminoindoline substructure, a *trans*-fused 12-membered ring, and the presence of an amino acid functionality in the macrocyclic skeleton to allow variation in the nature of the chiral side chain. Similarly, target **1.3** provides the aminoindoline substructure and an additional 11-membered ring having an amino acid in the skeleton to allow variation in the chiral side chain. Finally, our third target, that is, compound **1.4**, contains an additional bridged 11-membered ring that utilizes both nitrogen atoms of an aminoindoline scaffold. Altogether, these three divergent strategies with a common objective provide an excellent opportunity to build a unique chemical toolbox having compounds that will represent the privileged substructure and additional different types of large-ring derivatives to explore



Scheme 2. The synthesis of **1.2** having a 12-membered macrocyclic ring on an aminoindoline scaffold. Py = pyridine, Bz = benzoyl, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, TBAI = tetrabutylammonium iodide.

their value in biology as modulators of protein–protein interactions and signaling pathways.

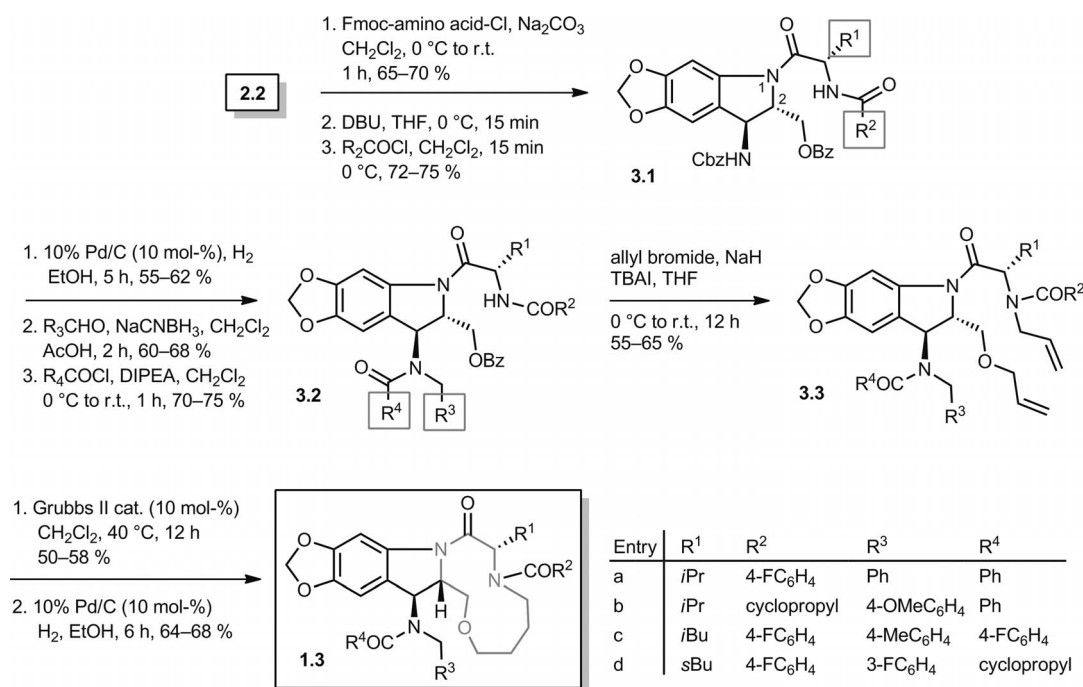
Our synthesis method to obtain 12-membered ring macrocyclic ring derivative **1.2** from an enantioenriched aminoindoline scaffold is outlined in Scheme 2. As we reported the synthesis of an aminoindoline scaffold earlier,^[23] a slightly revised procedure gave us easy access to **2.2** from **2.1** (note: the detailed synthesis procedure is provided in the Supporting Information).

The free amine was acylated (i.e., R^4 as the first diversity point), and following removal of the benzyloxycarbonyl (Cbz) group (10% Pd over C), it was then subjected to reductive alkylation to obtain secondary amine **2.3** in good yield (i.e., R^3 as the second diversity point). The secondary amine was then coupled with different 9-fluorenylmethoxycarbonyl (Fmoc) amino acids that allowed us to introduce the diversity site as R^1 (i.e., the side chain of an amino acid). The Fmoc group was removed, and the free amine was then acylated with acid chlorides to provide **2.4** with the fourth diversity point (R^2). Upon treatment under basic conditions with allyl bromide, bisallylated product **2.5** was produced in respectable yield. Under reactions conditions that may have trace amounts of moisture, hydrolysis of the benzoyl ester was also observed, which was then followed by *O*-allylation. The next crucial step was to explore the ring-closing metathesis based stitching technology on highly functionalized substrate **2.5**. This reaction worked well, and as we proposed, the 12-membered macrocyclic ring was easily obtained. The cyclic product was then subjected to hydrogenation to obtain the saturated version as product **1.2**, which was characterized through various analytical techniques (HPLC–MS and NMR spectroscopy). Our approach is general in nature, and this was tested by

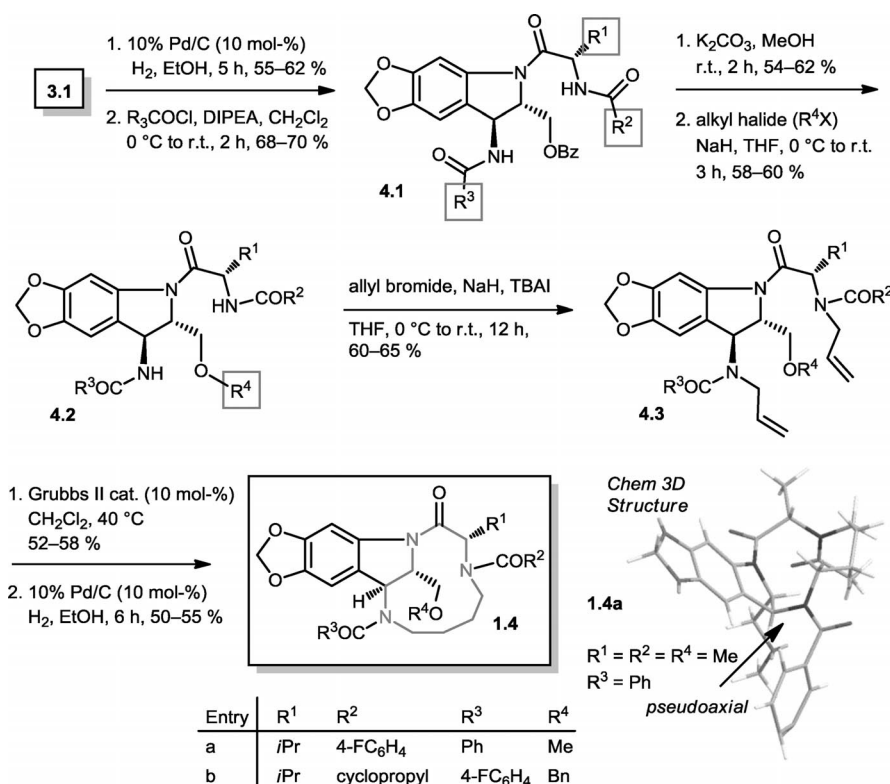
completing the synthesis of macrocyclic products **1.2**. All products were thoroughly purified and characterized (see the Supporting Information).

Our next plan was to develop a synthesis method to access various macrocyclic compounds from **1.3** (Scheme 3). To achieve this objective, we again utilized compound **2.2** as the starting material. The direct coupling with various amino acid chlorides (i.e., to obtain the first diversity point as R^1) was achieved. The Fmoc group was removed, and the amine was acylated to add the second diversity point as R^2 . Because we planned to utilize the aminoindoline side chain oxygen atom to obtain our macrocyclic ring, the indoline amine was converted into a tertiary amide following removal of the Cbz group to give us two additional diversity sites (R^3 and R^4). As described earlier, allylation of **3.2** was performed, and as in the previous case, under the reaction conditions **3.2** was also debenzoylated, which led to *O*-allylation at the primary hydroxy group. The bisallylated product was then subjected to ring-closing metathesis by using Grubbs chemistry. Once again, this reaction worked well and the 11-membered cyclic ring was easily obtained. To validate the generality of our approach, we tried this method in four cases and all of them successfully produced the macrocyclic products. We subjected all four macrocycles to double bond removal under hydrogenation conditions, and their corresponding saturated derivatives were easily obtained as **1.3**. All the products were thoroughly purified and characterized by HPLC–MS and NMR spectroscopy.

Our final approach to develop a method to access 11-membered bridged macrocyclic derivatives (see, **1.4**) is shown in Scheme 4. As in the previous case, we obtained **4.1** from **3.1** in easy steps. At this stage, the amino group



Scheme 3. Synthesis of **1.3** with an 11-membered ring. DIPEA = *N,N*-diisopropylethylamine.



Scheme 4. The incorporation of the 11-membered bridged macrocycle.

on scaffold **3.1** was deprotected and further acylated to obtain **4.1**. Following the conversion of the side chain hydroxy group from OBz to OMe or OBn, the substrate was subjected to the bisallylation reaction conditions. The bisallylated product was then subjected to crucial stitching technology by using ring-closing metathesis. To our pleasant surprise, this reaction worked well, and final product **1.4** obtained after double bond removal, and it was thoroughly purified and characterized (see the Supporting Information). Once again, to explore the validity of this method, we examined two other substrates, and our approach to build the 11-membered bridged macrocyclic ring worked in both cases. Careful analysis of the 3D model of **1.4a** indicated that the benzylic nitrogen atom occupies a pseudoaxial position and this brings the CH₂OMe group out of the plane due to a relative 1,2-*trans* orientation. This may be a crucial factor in bringing the two olefinic groups in proximity to facilitate formation of the 11-membered bridged macrocycle. To the best of our knowledge, this strategy to obtain different macrocyclic rings attached to the aminoindoline scaffold is unique and has not been reported. It also provides an excellent opportunity to build a novel, natural product inspired chemical toolbox to explore its value in a search of functional small molecules.

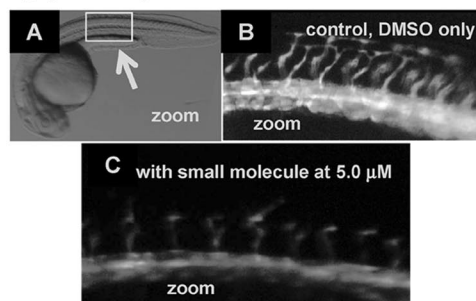
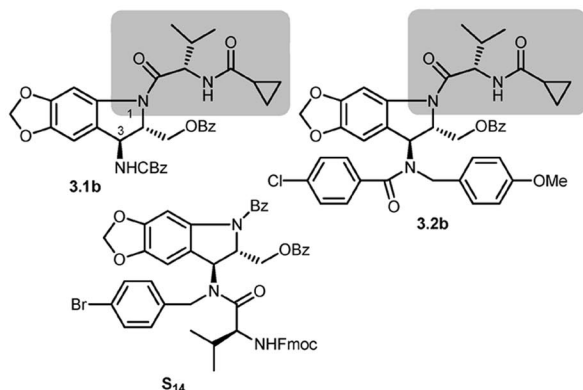
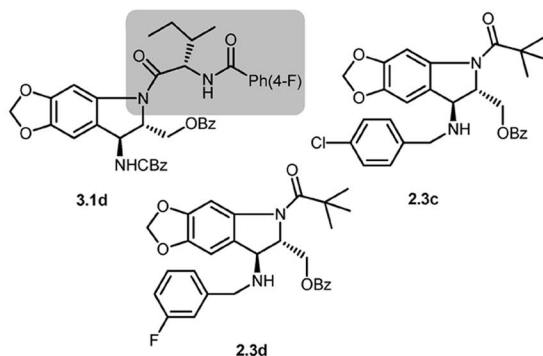
All the small molecules obtained from this project (in total 39 compounds) were then subjected to an embryo zebrafish screen in three different assays: angiogenesis,^[31–33] early embryonic development,^[34] and neurogenesis.^[33,35] A detailed list of all compounds tested in this study is shown in the Supporting Information. These assays are well docu-

mented in the literature^[33,35] and are routinely utilized in our laboratories. A major advantage with the use of the zebrafish technology is that it is close to the *in vivo* model, and it is a cheap way to evaluate compounds in a high-throughput manner. Details of the screening procedure are provided in the Supporting Information. The zebrafish screen identified **3.1b**, **3.2b**, and **S₁₄** (Figure 1) as antiangiogenesis agents with partial inhibition at 2.5 μm and complete inhibition at 5.0 μm. Compounds **3.1d**, **2.3c**, and **2.3d** were found to exhibit complete inhibition at 2.5 μm. In another screen of early embryonic development, we identified **S₂₆** functioning at 3.0 μm.

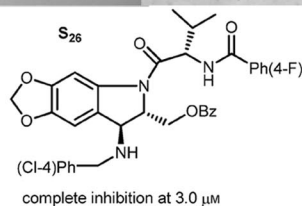
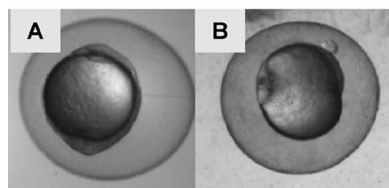
Conclusions

With the goal of building a diverse set of small molecules having a privileged substructure and different types of medium to large ring derivatives, we developed a diversity-based method. The first study utilized the primary hydroxy on the side chain and the benzylic amine to build a 12-membered macrocyclic ring. Using a similar strategy and through the use of the primary hydroxy group and the indoline amino group, an 11-membered ring was synthesized. Finally, the use of both nitrogen atoms (i.e., primary and secondary amines) led to the successful synthesis of an 11-membered bridged macrocycle. Upon evaluation of our small molecules in a zebrafish assay, we identified three novel compounds as antiangiogenesis agents. These findings are at an early stage and further work is required to

Angiogenesis assay

(note: the figures are shown with only compound **3.1b**)Partial inhibition at 2.5 μM and complete inhibition at 5.0 μM Complete inhibition at 2.5 μM 

Early development assay

complete inhibition at 3.0 μM

gain deeper insight into the mode of action of these functional small molecules.

Supporting Information (see footnote on the first page of this article): General methods, experimental procedures, spectral data, and zebrafish screening.

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Figure 1. Zebrafish angiogenesis assay: (a) embryonic zebrafish, (b) control with DMSO, (c) antiangiogenesis effect of **3.1b** at 5.0 μM ; embryo development assay: (a) normal development, (b) the effect of small molecule **S₂₆** at 3.0 μM .

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