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SHORT COMMUNICATION

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An Intramolecular Heck Approach To Obtain 17-Membered Macrocyclic Diversity and the Identification of an Antiangiogenesis Agent from a Zebrafish Assay

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We report a practical and modular approach to obtain two different types of 17-membered ring macrocyclic compounds through an intramolecular Heck reaction. These macrocyclic compounds are functionalized, that is, they contain two contiguous stereogenic hydroxy functional groups and an amino acid moiety in the macrocyclic ring skeleton. The macrocycles were then screened against a zebrafish assay to determine the antiangiogenesis activity of these small molecules. Macrocyclic compound **2.2a** was identified as a potent inhibitor at 2.5 μM, whereas its acyclic precursor and the other related macrocyclic compounds did not show any effect.

Introduction

As a result of the growing demand in accessing small molecules to search for modulators of protein–protein interactions[1,2] and selective dissectors of signaling pathways, we are seeing a rejuvenation in the interest in natural products. In addition, this is also leading to a growing desire to assemble small molecule toolboxes that are inspired from some of the features of bioactive natural products.[3–5] In this arena, in particular, macrocyclic natural products and the development of synthesis methods that lead to building the functionalized macrocyclic toolbox is gaining momentum.[6,7] In general, macrocyclic compounds are attractive because (1) they offer the ability to map a large surface area, (2) they have numerous binding interactions, (3) they have undergone preorganization, and (4) they show enhanced cell permeation properties. Some of these features are highly attractive in the search for compounds with high selectivity and affinity to protein targets and as modulators of protein–protein interactions and pathways.[4] Despite these valuable features and the proven record of several marketed macrocycle drugs derived from natural products, this structural class has been relatively poorly explored within drug discovery.[6,8–13] One of the major reasons is the lack of a wide variety of efficient synthesis methods to allow access to these compounds and their structurally related analogs in an efficient manner.

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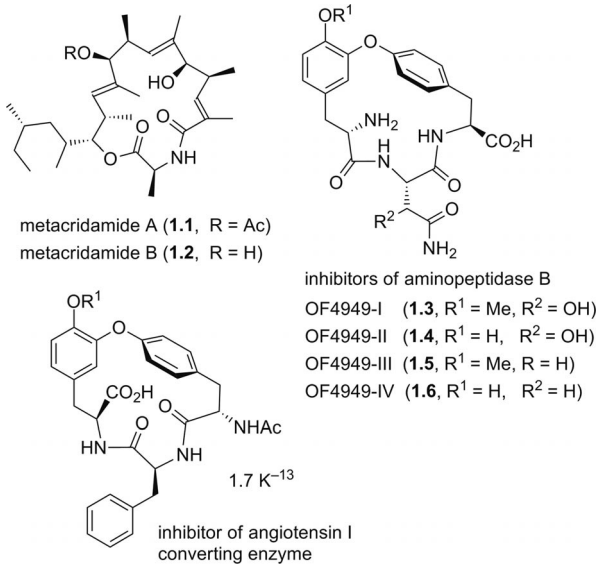
Results and Discussion

Synthesis

Herein, we report a practical and modular approach to obtain a diverse set of novel, natural product inspired, 17-membered macrocyclic architectures. Natural product inspired compounds are closer to natural products in terms of their 3D shapes and dense display of chiral functional groups, and they are highly likely to provide useful functional probes.[3,4] One of the major advantages with natural product inspired compounds is that it is easy to explore their stereochemical and skeletal diversity and the chemical space around theirs scaffolds; they are also easy to synthesize on a gram scale in a reasonable time period. These features are attractive when it comes to building a diverse chemical toolbox to explore biological potential in phenotypic assays. From the recent trend within the pharmaceutical sector, interest in phenotypic evaluation is also growing fast, and this approach is very different from the classical ways of target-based drug discovery.[14]

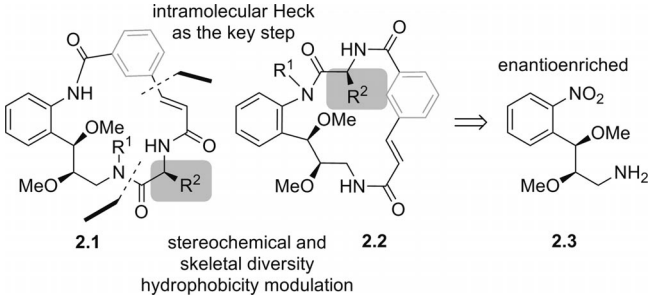
In our study, we decided to develop a modular method to obtain a diverse set of 17-membered functionalized macrocyclic compounds because there are several examples of bioactive natural products that have functionalized 17-

membered rings. Some of the representative examples with the assigned biological functions are shown in Scheme 1 and include metacridamide A&B (**1.1** and **1.2**),[15] OF4949 I–IV (**1.3**–**1.6**),[16–19] and K13 (**1.7**).[17,20,21]



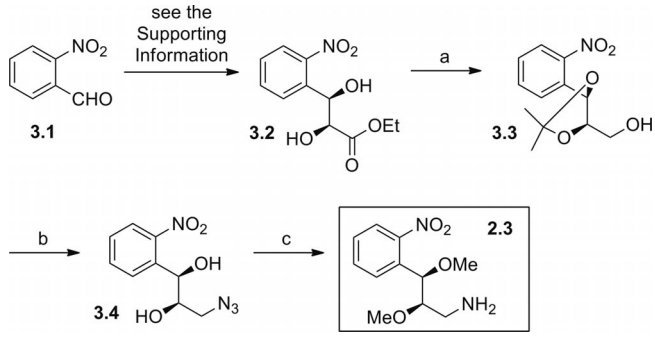
Scheme 1. A few examples of bioactive natural products having a 17-membered ring.

Shown in Scheme 2 are our proposed macrocyclic targets **2.1** and **2.2** that could be derived from monocyclic, enantioenriched starting material **2.3**. Both enantiomers of **2.3** needed for these macrocyclic targets could be easily accessed through a Sharpless dihydroxylation approach[22–24] in several-gram quantities in short duration. Macrocyclic targets **2.1** and **2.2** are attractive because of the presence of a functionalized 17-membered ring skeleton. The possibility of using both enantiomers further allows us to explore stereochemical diversity on a similar macrocyclic ring. The incorporation of an amino acid moiety as part of the macrocyclic ring provides an opportunity to introduce various nonpolar and polar groups as a chiral side chain. In both cases, the key reaction in our approach to obtain the 17-membered ring is an intramolecular Heck reaction.[25–27] Further, in our present design targets, each macrocyclic ring has two diversity points that could easily be explored to generate structurally related analogs as library members.



Scheme 2. Our key synthetic plan to access 17-membered macrocycles **2.1** and **2.2** from enantioenriched amine **2.3**.

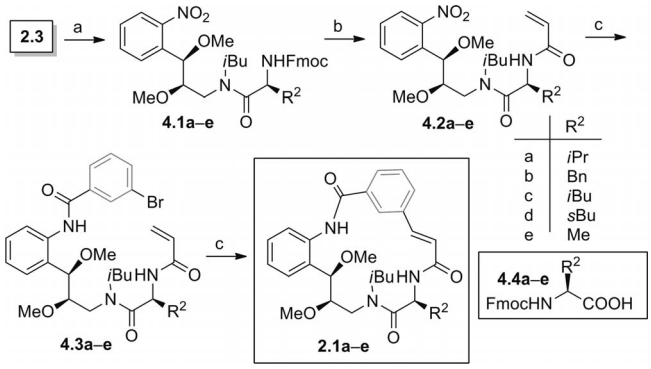
As shown in Scheme 3, 2-nitrobenzaldehyde (**3.1**) was converted into α,β-unsaturated carboxy ester through a Horner–Wadsworth–Emmons reaction and then subjected to Sharpless asymmetric dihydroxylation to give enantiopure dihydroxy derivative **3.2**. Following acetonide protection of the diol, the carboxy ester was then reduced with lithium borohydride to give primary alcohol **3.3**. Diolazide **3.4** was then obtained from **3.3** in three steps as follows: (1) Mesylation of the primary alcohol with methane sulfonyl chloride (MsCl). (2) Treatment with sodium azide. (3) Deprotection of the acetonide. It was further subjected to dimethylation with methyl iodide and reduction of the azide by Staudinger reaction to obtain primary amine **2.3**.



Scheme 3. Reagents and conditions: (a) 1. 2,2-Dimethoxypropane, *p*-toluenesulfonic acid (PTSA), CH_2Cl_2, r.t., 12 h; 2. $LiBH_4$, dry THF, 0 °C to r.t., 90–95% for two steps. (b) 1. MsCl, Et_3N, dry CH_2Cl_2, 0 °C to r.t., 1 h; 2. NaN_3, DMF, 80 °C, 12 h; 3. PTSA, H_2O, THF, reflux, 5 h. (c) 1. MeI, NaH, DMF, –78 °C to r.t., 0.5 h; 2. PPh_3, H_2O, THF, r.t., 8 h; 50–55% for six steps (from **3.3** to **2.3**).

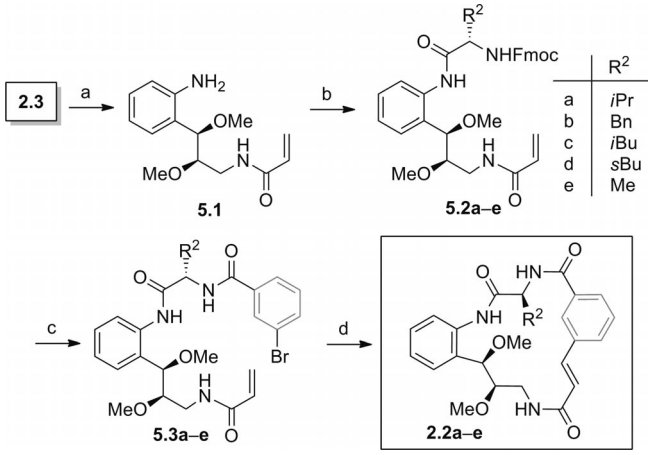
Scheme 4 outlines our plan to obtain macrocyclic compounds **2.1a**–**e** from primary amine **2.3**. First, **2.3** was subjected to reductive alkylation conditions to obtain the secondary amine. This was then coupled with *N*-Fmoc-protected (Fmoc = 9-fluorenylmethoxycarbonyl) amino acids **4.4a**–**e** by using *O*-benzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) to obtain **4.1a**–**e**. *N*-Fmoc removal with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) followed by acryloylation gave **4.2a**–**e**. The nitro compounds were reduced to aromatic primary amines under Zn/AcOH conditions, and the amines were then coupled with 3-bromobenzoyl chloride to give **4.3a**–**e**. Finally, macrocycles **2.1a**–**e** were obtained from an intramolecular Heck reaction in the presence of $Pd(OAc)_2$, P(*o*-tol)$_3$ (tol = tolyl), and *N*,*N*-diisopropylethylamine (DIPEA) in acetonitrile in good yields. The *trans* geometry of the double bond was confirmed by the coupling constant in the individual ^{1}H NMR spectra (see the Supporting Information).

Our plan to obtain macrocyclic compounds **2.2a**–**e** from key intermediate **2.3** is shown in Scheme 5. Primary amine **2.3** was converted into the amide with acryloyl chloride, and the aromatic nitro group was then reduced to give amine **5.1**. This was coupled with *N*-Fmoc-protected amino acids **4.4a**–**e** by using the 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) coupling reagent in acetonitrile to give coupled products **5.2a**–**e**. The *N*-Fmoc group was removed with DBU, and the free amine was coupled with 3-bromobenzoyl chloride to give **5.3a**–**e**. Finally, these compounds were then subjected to an intra-



Scheme 4. Synthesis of macrocycles **2.1a**–**e**. Reagents and conditions: (a) 1. Isobutyraldehyde, EtOH, $NaCNBH_3$, AcOH; 2. **4.4a**–**e**, HBTU, DIPEA, dry DMF, 6 h, 80–90%. (b) 1. DBU, THF, r.t., 5 min; 2. acryloyl chloride, CH_2Cl_2, r.t., 5 min. (c) 1. Zn/AcOH, EtOH, r.t., 10 min; 2. 3-bromobenzoyl chloride, r.t., 5 min, 60–70% for four steps. (d) $Pd(OAc)_2$, P(*o*-tol)$_3$, DIPEA, CH_3CN, reflux, 20 h, 55–60% (based on recovered starting material).

molecular Heck reaction in the presence of $Pd(OAc)_2$, P(*o*-tol)$_3$, and DIPEA in acetonitrile to obtain macrocycles **2.2a**–**e** in good yields. Once again, the *trans* olefin was obtained from the Heck reaction, and this was assigned on the basis of the coupling constant in the individual ^1H NMR spectra (see the Supporting Information).



Scheme 5. Synthesis of macrocycles **2.2a**–**e**. Reagents and conditions: (a) 1. Acryloyl chloride, CH_2Cl_2, r.t., 5 min; 2. Zn/AcOH, EtOH, r.t., 10 min, 85% for two steps. (b) **4.4a**–**e**, EDC·HCl, CH_3CN, 1 h, 80–90%. (c) 1. DBU, THF, r.t., 5 min, 2. 3-bromobenzoyl chloride, r.t., 5 min, 90–95% for two steps. (d) $Pd(OAc)_2$, P(*o*-tol)$_3$, DIPEA, CH_3CN, reflux, 20 h, 55–60% (based on recovered starting material).

Zebrafish Assay Related to Angiogenesis and Early Embryonic Development

The next plan was to subject our macrocyclic collection and several intermediates (34 compounds in total) to various zebrafish assays to evaluate the biological effect of these small molecules. The structural information of all compounds tested during this screen is provided in the Supporting Information. These screens were related to a search for compounds affecting epiboly during early embryonic development,[28–33] angiogenesis,[34–37] and neurogenesis[38] in zebrafish embryo assays.[39–41] All three assays are well documented in the literature,[33,36,42–44] and a detailed experimental procedure is provided in the Supporting Information. Figure 1 shows the identification of macrocycle **2.2a** as an antiangiogenesis agent (i.e., complete inhibition at 2.5 μM). It is interesting to note that the acyclic precursor of **2.2a** (see Figure 2, **5.3a**) as well as the other related macrocyclic compounds did not show any effect on angiogenesis or early embryonic development.

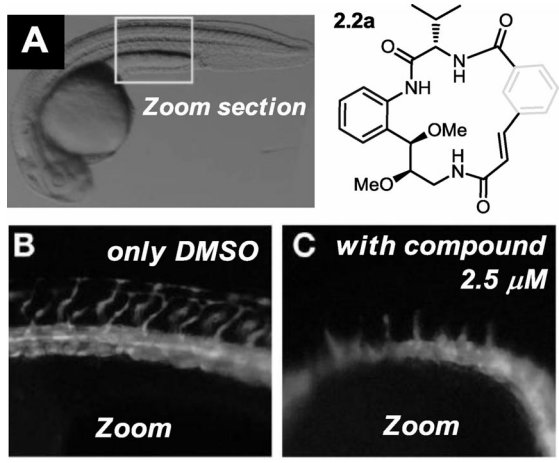


Figure 1. (a) Wild-type zebrafish embryo at 30 hpf of development; region zoomed in panels b and c is shown by the box. (b) Zoom section of wild-type or vehicle-treated embryo and (c) zoom section after treatment with a small molecule. Macrocycle **2.2a** showed complete inhibition at 2.5 μM.

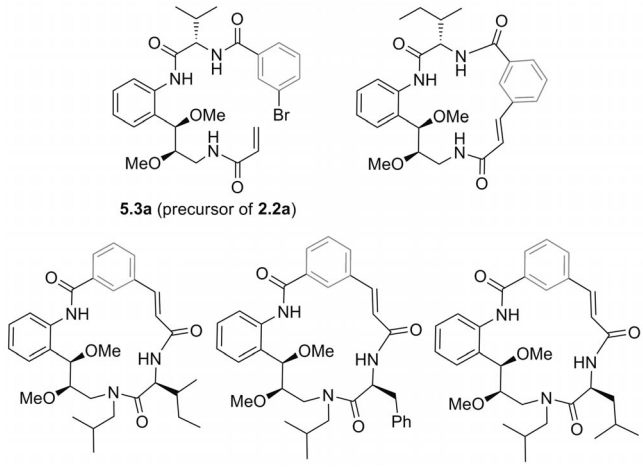


Figure 2. Compound **5.3a**, which is the acyclic precursor of **2.2a**, and the other related macrocycles that did not show any response to angiogenesis and early embryo development.

Conclusions

To summarize, we developed a modular method that allowed us to build a toolbox having two different types of 17-membered macrocyclic compounds. An intramolecular

Heck reaction was the key step in our approach. When tested to search for functional small molecules as antiangiogenesis agents in a zebrafish assay, we discovered that **2.2a** is a potent inhibitor at 2.5 μM. Further biological studies are needed to understand the mode of action of this active compound, and the results of these studies will be reported when they become available.

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

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