

Tetrahydroquinoline-Derived Macrocyclic Toolbox: The Discovery of Antiangiogenesis Agents in Zebrafish Assay

Shiva Krishna Reddy Guduru,[†] Srinivas Chamakuri,[†] Gayathri Chandrasekar,[‡] Satish Srinivas Kitambi,^{*,‡,§} and Prabhat Arya^{*,†}

[†]Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500 046, India

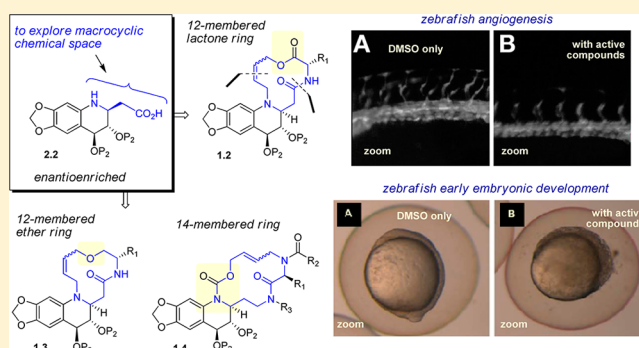
[‡]School of Life Sciences, Södertörns Högskola, Sweden

[§]Division of Molecular Neurobiology and Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Sweden

Supporting Information

ABSTRACT: A novel approach to incorporate the macrocyclic rings onto the privileged substructure, i.e., tetrahydroquinoline scaffold, is developed. The presence of an amino acid-derived moiety in the macrocyclic skeleton provides an opportunity to modulate the nature of the chiral side chain. Further, evaluation in a zebrafish screen identified three active small molecules (2.5b, 3.2d, and 4.2) as antiangiogenesis agents at 2.5 μ M.

KEYWORDS: Natural product-inspired compounds, macrocyclic diversity, zebrafish screen, antiangiogenesis agents

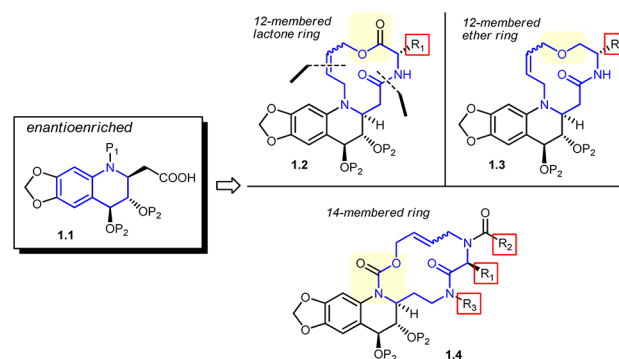


The growing quest to undertake biological targets that belong to the family of protein–protein^{1,2} and DNA/RNA–protein³ interactions in the signaling pathway arena^{4,5} is challenging the current thinking in going beyond the conventional chemical space to search for functional small molecules. Typically small molecules in the drug discovery research are rich in sp^2 character,^{6,7} whereas natural products⁸ that have an excellent track record in the domain of biomacromolecular interactions are relatively complex in nature and present a diverse array of chiral functional groups. In particular, macrocyclic natural products⁹ are proven to exhibit remarkable biological responses when it comes to modulating ppi through small molecules. There are several factors associated with macrocyclic natural product derivatives, and these include (i) an ability to map a large surface area, (ii) numerous binding interaction options, (iii) enhanced cell permeation properties when compared to their linear derivatives, and (iv) the dynamic preorganized structures to display various functional groups. Despite all these benefits that are associated with bioactive macrocyclic natural products, we have not seen a significant growth in building a chemical toolbox having diverse sets of different-types of macrocyclic shapes available to explore their biological value.^{9–15} Because of the inherited challenges associated with complex bioactive natural products (i.e., macrocyclic or nonmacrocyclic compounds), the interest in building a chemical tool box having small molecules that are obtained by inspirational approaches is also rising.^{16–22}

Toward this objective, a few years ago, we embarked a program in developing several novel approaches to allow

accessing different-types of functionalized large ring compounds. In one study, we were interested in utilizing an enantioenriched tetrahydroquinoline scaffold, (1.1, Scheme 1) that was reported by us earlier.^{23–27} The presence of a β -amino acid functionality and three contiguous chiral functional groups are the two attractive features of this scaffold. Our synthesis is practical and enantioselective in nature and allows us to access this scaffold in sufficient quantities in a short period that

Scheme 1. Incorporation of Different Macrocyclic Rings onto an Enantioenriched Tetrahydroquinoline Scaffold



Received: January 17, 2013

Accepted: May 24, 2013

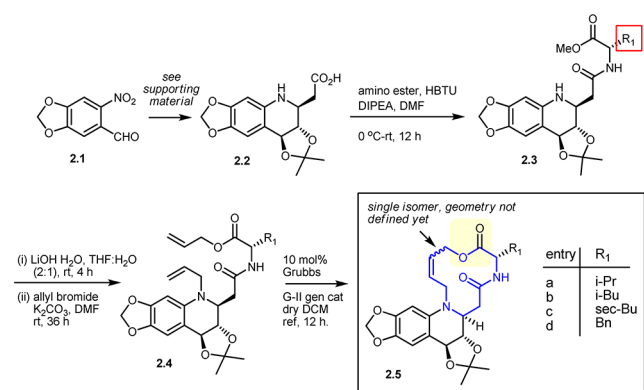
Published: May 24, 2013

utilized a stereoselective aza Michael reaction as the key step. To explore further the large ring-based chemical space around this scaffold, we report here our approach to build further the functionalized 12- and 14-membered rings onto this scaffold (see, 1.2–1.4).

There are two main objectives in our design strategy, first is to retain the functionalized privileged substructure, i.e., tetrahydroquinoline, and second is to map the macrocyclic chemical space with the additional functional groups. For example, target 1.2 has the additional 12-membered ring with an incorporation of an amino acid moiety in the skeleton. The ring-closing metathesis reaction was utilized as the stitching technology to obtain the macrocyclic rings. Using a similar approach, one can also obtain compound 1.3 with a 12-membered ring having the connectivity through the ether linkage. In addition to these two compounds, we also plan to incorporate 14-membered macrocyclic ring onto the tetrahydroquinoline scaffold having an amino acid moiety in the ring skeleton. Overall, our approach to building different types of large ring skeletons onto the tetrahydroquinoline scaffold provides an excellent opportunity to accessing a chemical tool box with a diverse set of functionalized large ring-based derivatives.

Our synthesis approach to incorporate a 12-membered ring onto an enantioenriched tetrahydroquinoline scaffold is shown in Scheme 2. As reported by us earlier,²⁸ we obtained an

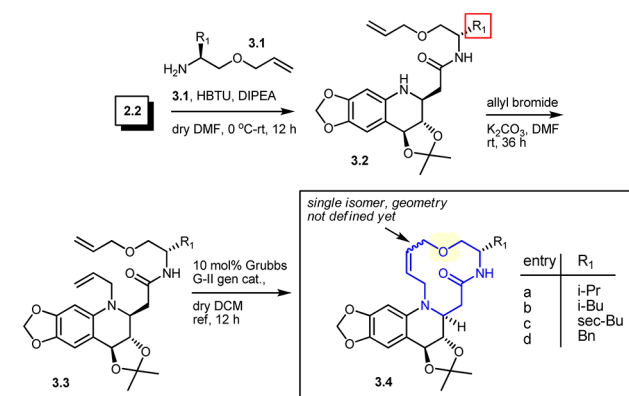
Scheme 2. Synthesis Route to Obtain a 12-Membered Macrocycle onto the Tetrahydroquinoline Ring



enantioenriched compound 2.2 from 2.1 in a number of well-established steps. One of the key advantages with this scaffold is that it can be accessed in several gms quantities in a short duration. The presence of the β -amino acid moiety in this scaffold is an attractive feature and is a subject of further modification. For example, the free carboxylic acid group (2.2) was coupled with several amino esters to obtain compound 2.3 (R₁ as the diversity site). This was easily converted to *bis*-allyl derivative 2.4 needed for building the 12-membered macrocyclic ring. Upon submission to the ring-closing metathesis stitching technology,^{29–31} we successfully obtained the cyclic product 2.5 in good yields as the single isomer (olefin geometry is not defined yet). Our approach is general in nature, and as a proof of concept studies, four macrocyclic compounds 2.5a–d were obtained. All the products in this scheme are thoroughly purified and characterized using HPLC-MS and NMR. The detailed procedure is provided in the Supporting Information.

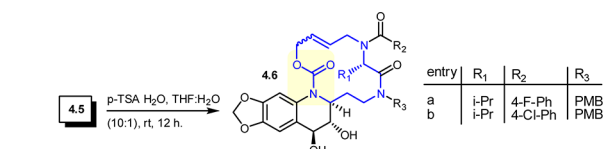
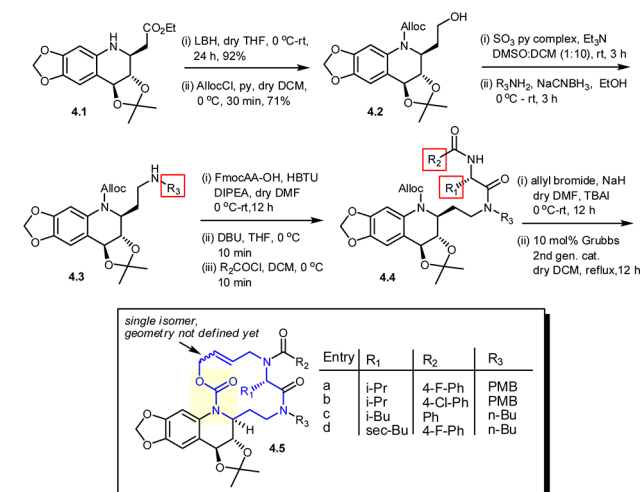
In another similar approach, we utilized the corresponding amino-alcohol derivatives 3.1 (see Scheme 3) to couple with

Scheme 3. Synthesis Route to Obtain a 12-Membered Macrocyclic Ring onto the Tetrahydroquinoline Ring That Utilizes Amino Alcohols



the free carboxylic acids, and this successfully led to the synthesis of 4 examples (3.4a–d). Once again, the products are obtained as a single isomer and the olefin geometry is not defined yet. Our plans to incorporate a 14-membered ring onto the tetrahydroquinoline scaffold are shown in Scheme 4.

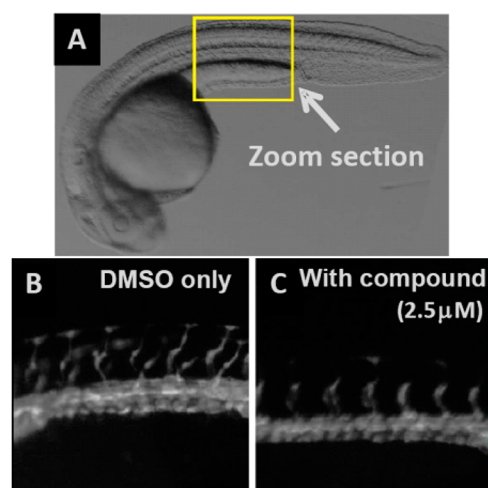
Scheme 4. Synthesis Route to Incorporate a 14-Membered Ring onto an Enantioenriched Tetrahydroquinoline Scaffold



Compound 4.1 as the starting material (see Supporting Information) was utilized to obtain 4.2 in two steps that followed the carboxyl ester reduction and aromatic amine protection as –NAlloc. Following the oxidation of a primary hydroxyl group, it was then reductively alkylated to obtain the secondary amine (with the first diversity as R₃) and then coupled with several amino acids and further amidation (second and third diversity sites, R₁ and R₂) to obtain 4.4. This was then subjected to *N*-allylation followed by ring closing metathesis stitching technology using second generation Grubbs catalyst giving the 14-membered ring-derived compounds 4.5. This reaction worked well and was also utilized to obtain four test macrocyclic compounds 4.5a–d. All the

products were purified over silica gel and thoroughly characterized by HPLC-MS and NMR (note: olefin geometry is not defined yet). As test studies, in two cases, the acetoneidone protection was removed under mild acidic conditions giving the macrocyclic compounds with two free hydroxyl groups (see 4.6).

Having a chemical toolbox available to explore its biological value, we then decided to search for functional small molecules in three zebrafish screens,^{32,33} and these are (i) angiogenesis,^{34,35} (ii) an early embryonic development,³⁵ and (iii) neurogenesis.³⁶ These assays are well-established in our lab and utilize the procedure that is thoroughly documented in the literature.^{37,38} The detailed procedure is also provided in the Supporting Information. Of all the compounds tested from this toolbox (60 compounds in total), we identified three compounds (2.5b, 3.2d, and 4.2) that exhibited the inhibition of angiogenesis at 2.5 μM . These results are shown in Figure 1.



(note – the figures are shown with compound 2.5b)

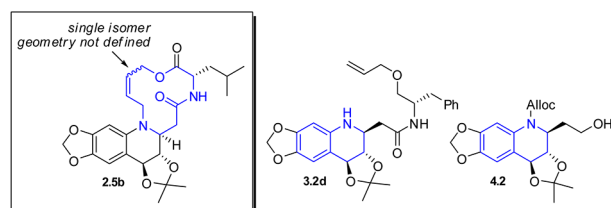
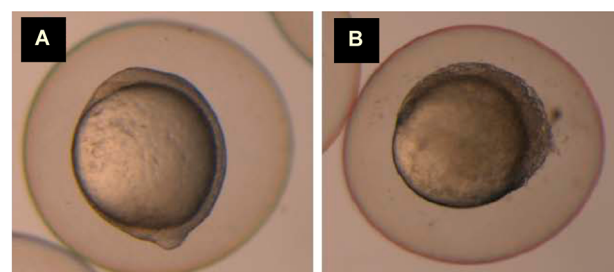


Figure 1. Zebrafish screen for angiogenesis: (A) zoom section of wild-type or vehicle treated embryo and (B,C) zoom sections after treatment with compound 2.5b. One macrocyclic derivative (2.5b) and two tetrahydroquinoline-based compounds (3.2d and 4.2) showed complete inhibition at 2.5 μM .

In another zebrafish screen to search for functional small molecules affecting an early embryonic development (see Figure 2), we identified three compounds (2.5c, S8d, and 4.2) that inhibited at 2.5 μM .

In our zebrafish studies, we exposed a total of 30 embryos per compound producing defects in angiogenesis and early embryo developmental stage. We then quantified the number of embryos exhibiting severe defects in each treatment. Embryos at 2.5 μM completely exhibited severe phenotype, and this percentage dropped drastically when the concentration was slightly lowered (note: the detailed information is provided



(note: the figures are shown with compound 2.5c)

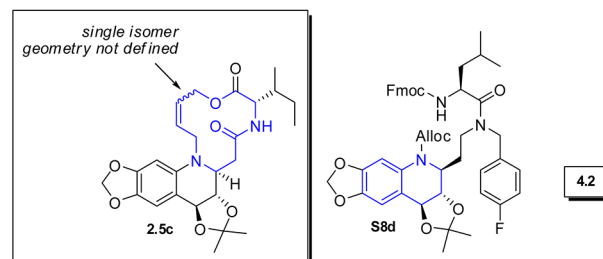


Figure 2. Zebrafish screen for an early embryo development: (A) DMSO exposed embryos at 10 hpf of development and (B) small molecule 2.5c exposed embryos causing a delay in epiboly. One macrocyclic derivative (2.5c) and two tetrahydroquinoline-based compounds (S8d and 4.2) exhibited the complete inhibition of an early embryo development at 2.5 μM .

in the Supporting Information). This may indicate that the minimum concentration required to produce any significant effect on these biological processes is above 2.5 μM . The severe effect was seen as the complete inhibition of angiogenesis and epiboly, and the partial inhibition was characterized by the inhibition of angiogenesis of more than 50% of vessels.

It is interesting to note that the functional macrocyclic compounds (2.5b and 2.5c) in both assays are structurally related. It would be excellent to find the exact mechanism of action of these compounds and to determine if there is any common mode of action in these two phenotypic experiments.

With an objective to incorporate different macrocyclic rings onto the tetrahydroquinoline scaffold, we successfully developed several approaches. The presence of the privileged substructure and the additional macrocyclic rings (for example, functionalized 12- and 14-membered rings) are two unique features in our design strategy. Further, the incorporation of an amino acid in the large ring skeleton allows an opportunity to modulate the nature of the side chain (for example, chiral polar to nonpolar groups through utilizing natural and un-natural amino acids). Finally, when tested this tool box in a zebrafish screen, we identified three functional small molecules active as antiangiogenesis agents at 2.5 μM and three as inhibitors of an early embryonic development. To understand the precise mode of action of these compounds that are active in phenotypic screens, much work would be needed. This will be reported as these findings become available.

■ ASSOCIATED CONTENT

📄 Supporting Information

Detailed synthesis procedure along with the analytical data for all new compounds and the zebrafish screen. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*(P.A.) E-mail: prabhata@drils.org.

Author Contributions

The manuscript was written through contributions of all authors.

Funding

This work was supported by DST (SR/S1/OC-30/2010) and DBT (102/IFD/SAN/PR-2863) grants to P.A.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

S.K.R.G. and S.C. thank CSIR funding agency for the award of the Ph.D. fellowships. We also thank the ILS analytical team for providing an excellent HPLC-MS and NMR support. S.S.K. thanks Södertörns Högskola for the financial support to perform zebrafish assays.

REFERENCES

(1) Arkin, M. R.; Wells, J. A. Small-molecule inhibitors of protein–protein interactions: progressing towards the dream. *Nat. Rev. Drug Discovery* **2004**, *3*, 301–317.

(2) Wells, J. A.; McClendon, C. L. Reaching for high-hanging fruit in drug discovery at protein–protein interfaces. *Nature* **2007**, *450*, 1001–1009.

(3) Boger, D. L.; Desharnais, J.; Capps, K. Solution-phase combinatorial libraries: modulating cellular signaling by targeting protein–protein or protein–DNA interactions. *Angew. Chem., Int. Ed.* **2003**, *42*, 4138–4176.

(4) Scott, J. D.; Pawson, T. Cell signaling in space and time: where proteins come together and when they're apart. *Science* **2009**, *326*, 1220–1224.

(5) Pawson, T.; Linding, R. Network medicine. *FEBS Lett.* **2008**, *582*, 1266–1270.

(6) Schreiber, S. L. Organic synthesis toward small-molecule probes and drugs. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6699–6702.

(7) Dandapani, S.; Marcaurelle, L. A. Grand challenge commentary: Accessing new chemical space for 'undruggable' targets. *Nat. Chem. Biol.* **2010**, *6*, 861–863.

(8) Newman, D. J.; Hill, R. T. New drugs from marine microbes: the tide is turning. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 539–544.

(9) Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. The exploration of macrocycles for drug discovery: an underexploited structural class. *Nat. Rev. Drug Discovery* **2008**, *7*, 608–624.

(10) Marcaurelle, L. A.; Comer, E.; Dandapani, S.; Duvall, J. R.; Gerard, B.; Kesavan, S.; Lee, M. D. T.; Liu, H.; Lowe, J. T.; Marie, J. C.; Mulrooney, C. A.; Pandya, B. A.; Rowley, A.; Ryba, T. D.; Suh, B. C.; Wei, J.; Young, D. W.; Akella, L. B.; Ross, N. T.; Zhang, Y. L.; Fass, D. M.; Reis, S. A.; Zhao, W. N.; Haggarty, S. J.; Palmer, M.; Foley, M. A. An aldol-based build/couple/pair strategy for the synthesis of medium- and large-sized rings: discovery of macrocyclic histone deacetylase inhibitors. *J. Am. Chem. Soc.* **2010**, *132*, 16962–16976.

(11) Moretti, J. D.; Wang, X.; Curran, D. P. Minimal fluorine tagging strategy that enables the synthesis of the complete stereoisomer library of SCH725674 macrolactones. *J. Am. Chem. Soc.* **2012**, *134*, 7963–7970.

(12) Ajay, A.; Sharma, S.; Gupta, M. P.; Bajpai, V.; Kumar, B.; Kaushik, M. P.; Konwar, R.; Ampapathi, R. S.; Tripathi, R. P. Diversity oriented synthesis of pyran based polyfunctional stereogenic macrocycles and their conformational studies. *Org. Lett.* **2012**, *14*, 4306–4309.

(13) Dockendorff, C.; Nagiec, M. M.; Weiwer, M.; Buhrlage, S.; Ting, A.; Nag, P. P.; Germain, A.; Kim, H. J.; Youngsaye, W.; Scherer, C.; Bennion, M.; Xue, L.; Stanton, B. Z.; Lewis, T. A.; Macpherson, L.; Palmer, M.; Foley, M. A.; Perez, J. R.; Schreiber, S. L. Macrocyclic

hedgehog pathway inhibitors: optimization of cellular activity and mode of action studies. *ACS Med. Chem. Lett.* **2012**, *3*, 808–813.

(14) Stanton, B. Z.; Peng, L. F.; Maloof, N.; Nakai, K.; Wang, X.; Duffner, J. L.; Taveras, K. M.; Hyman, J. M.; Lee, S. W.; Koehler, A. N.; Chen, J. K.; Fox, J. L.; Mandinova, A.; Schreiber, S. L. A small molecule that binds hedgehog and blocks its signaling in human cells. *Nat. Chem. Biol.* **2009**, *5*, 154–156.

(15) Peng, L. F.; Stanton, B. Z.; Maloof, N.; Wang, X.; Schreiber, S. L. Syntheses of aminoalcohol-derived macrocycles leading to a small-molecule binder to and inhibitor of sonic hedgehog. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6319–6325.

(16) Arya, P.; Joseph, R.; Gan, Z. H.; Rakic, B. Exploring new chemical space by stereocontrolled diversity-oriented synthesis. *Chem. Biol.* **2005**, *12*, 163–180.

(17) Reayi, A.; Arya, P. Natural product-like chemical space: search for chemical dissectors of macromolecular interactions. *Curr. Opin. Chem. Biol.* **2005**, *9*, 240–247.

(18) Dasari, B.; Jogula, S.; Borhade, R.; Balasubramanian, S.; Chandrasekar, G.; Satish Srinivas Kitambi, S. S.; Arya, P. Macrocyclic glycohybrid toolbox identifies novel antiangiogenesis agents from zebrafish Assay. *Org. Lett.* **2013**, *15*, 432–435.

(19) Aeluri, M.; Pramanik, C.; Lakshindra Chetia, L.; Mallurwar, N. K.; Balasubramanian, S.; Chandrasekar, G.; Kitambi, S. S.; Arya, P. 14-Membered macrocyclic ring-derived toolbox: the identification of small molecule inhibitors of angiogenesis and early embryo development in zebrafish assay. *Org. Lett.* **2013**, *15*, 436–439.

(20) Aeluri, M.; Gaddam, J.; Trinath, D. V. K. S.; Chandrasekar, G.; Kitambi, S. S.; Arya, P. An intramolecular heck approach to obtain 17-membered macrocyclic diversity and the identification of an antiangiogenesis agent from a zebrafish assay. *Eur. J. Org. Chem.* **2013**, DOI: 10.1002/ejoc.201300408.

(21) Chamauri, S.; Guduru, S. K. R.; Pamu, S.; Chandrasekar, G.; Kitambi, S. S.; Arya, P. A modular approach to build macrocyclic diversity in aminoindoline scaffolds identifies antiangiogenesis agents from a zebrafish assay. *Eur. J. Org. Chem.* **2013**, DOI: 10.1002/ejoc.201300409.

(22) Jogula, S.; Dasari, B.; Khatravath, M.; Chandrasekar, G.; Kitambi, S. S.; Arya, P. Building a macrocyclic toolbox from C-linked carbohydrates identifies novel antiangiogenesis agents from zebrafish assay. *Eur. J. Org. Chem.* **2013**, accepted for publication.

(23) Couve-Bonnaire, S.; Chou, D. T. H.; Gan, Z.; Arya, P. A solid-phase, library synthesis of natural-product-like derivatives from an enantiomerically pure tetrahydroquinoline scaffold. *J. Comb. Chem.* **2004**, *6*, 73–77.

(24) Prakesch, M.; Sharma, U.; Sharma, M.; Khadem, S.; Leek, D. M.; Arya, P. Part 1. Modular approach to obtaining diverse tetrahydroquinoline-derived polycyclic skeletons for use in high-throughput generation of natural-product-like chemical probes. *J. Comb. Chem.* **2006**, *8*, 715–734.

(25) Sharma, U.; Srivastava, S.; Prakesch, M.; Sharma, M.; Leek, D. M.; Arya, P. Part 2: Building diverse natural-product-like architectures from a tetrahydroaminoquinoline scaffold. Modular solution- and solid-phase approaches for use in high-throughput generation of chemical probes. *J. Comb. Chem.* **2006**, *8*, 735–761.

(26) Prakesch, M.; Srivastava, S.; Leek, D. M.; Arya, P. Part 3. A novel stereocontrolled, in situ, solution- and solid-phase, aza michael approach for high-throughput generation of tetrahydroaminoquinoline-derived natural-product-like architectures. *J. Comb. Chem.* **2006**, *8*, 762–773.

(27) Arya, P.; Couve-Bonnaire, S.; Durieux, P.; Laforce, D.; Kumar, R.; Leek, D. M. Solution- and solid-phase synthesis of natural product-like tetrahydroquinoline-based polycyclics having a medium size ring. *J. Comb. Chem.* **2004**, *6*, 735–745.

(28) Arya, P.; Durieux, P.; Chen, Z.-X.; Joseph, R.; Leek, D. M. Stereoselective diversity-oriented solution and solid-phase synthesis of tetrahydroquinoline-based polycyclic derivatives. *J. Comb. Chem.* **2004**, *6*, 54–64.

- (29) Grubbs, R. H. Olefin-metathesis catalysts for the preparation of molecules and materials (Nobel Lecture). *Angew. Chem., Int. Ed.* **2006**, *45*, 3760–3765.
- (30) Grubbs, R. H. Olefin metathesis. *Tetrahedron* **2004**, *60*, 7117–7140.
- (31) Grubbs, R. H.; Miller, S. J.; Fu, G. C. Ring-closing metathesis and related processes in organic synthesis. *Acc. Chem. Res.* **1995**, *28*, 446–452.
- (32) Peterson, R. T.; Fishman, M. C. Designing zebrafish chemical screens. *Methods Cell Biol.* **2011**, *105*, 525–541.
- (33) Kitambi, S. S.; McCulloch, K. J.; Peterson, R. T.; Malicki, J. J. Small molecule screen for compounds that affect vascular development in the zebrafish retina. *Mech. Dev.* **2009**, *126*, 464–477.
- (34) Serbedzija, G. N.; Flynn, E.; Willett, C. E. Zebrafish angiogenesis: a new model for drug screening. *Angiogenesis* **1999**, *3*, 353–9.
- (35) Vogt, A.; Cholewinski, A.; Shen, X.; Nelson, S. G.; Lazo, J. S.; Tsang, M.; Hukriede, N. A. Automated image-based phenotypic analysis in zebrafish embryos. *Dev. Dyn.* **2009**, *238*, 656–663.
- (36) Kitambi, S. S.; Malicki, J. J. Spatiotemporal features of neurogenesis in the retina of medaka, *Oryzias latipes*. *Dev. Dyn.* **2008**, *237*, 3870–81.
- (37) Kitambi, S. S.; Nilsson, E. S.; Sekyrova, P.; Ibarra, C.; Tekeoh, G. N.; Andang, M.; Ernfors, P.; Uhlen, P. Small molecule screening platform for assessment of cardiovascular toxicity on adult zebrafish heart. *BMC Physiol.* **2012**, *12*, 3.
- (38) Kimmel, C. B.; Ballard, W. W.; Kimmel, S. R.; Ullmann, B.; Schilling, T. F. Stages of embryonic development of the zebrafish. *Dev. Dyn.* **1995**, *203*, 253–310.