JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Perspective

pubs.acs.org/JACS

Biology-Oriented Synthesis: Harnessing the Power of Evolution

Hilde van Hattum[†] and Herbert Waldmann*[,†,‡]

[†]Max Planck Institute of Molecular Physiology, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany

[‡]Department of Chemistry and Chemical Biology, Technical University of Dortmund, Otto-Hahn-Strasse 6, 44227 Dortmund, Germany

ABSTRACT: For scientists to gain a better understanding of nature, biological research is greatly aided by small-molecule modulators that perturb protein activity without fundamentally altering the underlying biological systems. The number of possible interfering molecules, however, is so vast that, due to limitations in existing matter and time required for synthesis, they cannot be covered comprehensively. Because proteins and their cognate natural product ligands and substrates co-evolved, these naturally occurring ligands can serve as structural starting points to explore the biologically relevant chemical space. To this end, known natural products are structurally classified on the basis of their core scaffolds and hierarchically arranged in the "natural product tree", which can be annotated for bioactivity and intuitively navigated with currently available software. Biologically relevant scaffolds inspire the synthesis of compound libraries enriched in biological activity. This Perspective describes the development of "biology-oriented synthesis" as a guiding principle to harness the power of evolution in the quest for novel bioactive small molecules for chemical biology research and drug discovery.

INTRODUCTION

Protein−ligand binding events mediate a plethora of biological processes and are essential for the development, growth, and sustainability of living systems. Naturally occurring small-molecule protein ligands are metabolites, which are intermediates and products of biosynthesis and metabolism. Both natural products (NPs) and non-natural but biologically relevant small molecules bind proteins and can then either be subject to modification or influence protein activity. One leading objective in chemical biology is the identification of bioactive small molecules for selective modulation of individual components of biomolecular complexes, typically proteins, for the study of complex biological systems. If the biology of interest is disease-related, such protein ligands may inspire drug discovery. However, the number of possible biologically relevant drug-like small molecules exceeds a staggering 10^{60}, and due to time and matter limitations,[1,2] it is unfeasible to comprehensively cover by means of organic synthesis the chemical space they define. Likewise, the chemical space covered by small molecules synthesized in the course of evolution, which is matched by their corresponding protein binding sites, cannot have been fully explored in evolution due to limited matter and time.[2] Therefore, it is crucial to develop an intuitively accessible and logical reasoning to identify and explore by synthesis the biologically relevant fraction of chemical space without missing important compound classes. In this endeavor, NPs are a major source of inspiration, as they co-evolved with proteins and can hence be regarded as biologically validated.

Nature is very economical in the design and synthesis of proteins and metabolites and repeatedly employs and exploits only a minute fraction of chemical space, which was selected in evolution, obviating the need for extensive coverage. For example, the human genome is estimated to encode ca. 25 000 proteins, which is far less than the 10^{390} possible proteins of 300 amino acids.[1] This restriction can, to a large extent, be explained by selection-driven protein production under physical chemical pressure.[3] Computational and experimental insight indicate that protein formation is mostly based on thermodynamic stability rather than function,[4,5] and consequently stable single-domain proteins embody only ca. 900 fold types.[6] In addition, concave ligand-accommodating shapes within these folds can be represented by a mere 1300 binding pockets, a surprisingly small number.[4] However, since a given fold type can be formed from differing peptide sequences, amino acid diversity ensures structural variability in these conserved pockets. Evolutionary pressure thus fine-tunes interactions of binding sites with their cognate ligands. By analogy to conservation of protein fold structure, the number of core scaffolds in NPs is also limited, and NPs are differentiated by diversity of functional groups attached to the common scaffolds. In a sense, both structural conservation (protein fold and NP scaffold structure) and diversity (amino acid side chain and NP scaffold substituents) are characteristic for both worlds (Figure 1).

Moreover, proteins synthesize multiple NPs. Individual NPs can bind a variety of proteins during biosynthesis and often have diverse biological activities. These insights suggest that structural parameters that enable binding to evolutionarily conserved protein binding sites may be encoded in NP structure at the scaffold level and fine-tuned by substituent decoration. As a consequence, arguments derived from the evolution of protein and NP structure can be applied to guide the identification of novel synthetic modulators of protein function and activity. Systematic analysis of NP scaffolds and substituents should allow distillation of the features that are important for the design and synthesis of NP-inspired compound collections with simplified structure but still endowed with the basic biological relevance of the guiding NPs.

Received: June 11, 2014
Published: July 29, 2014

   dx.doi.org/10.1021/ja505861d | J. Am. Chem. Soc. 2014, 136, 11853−11859

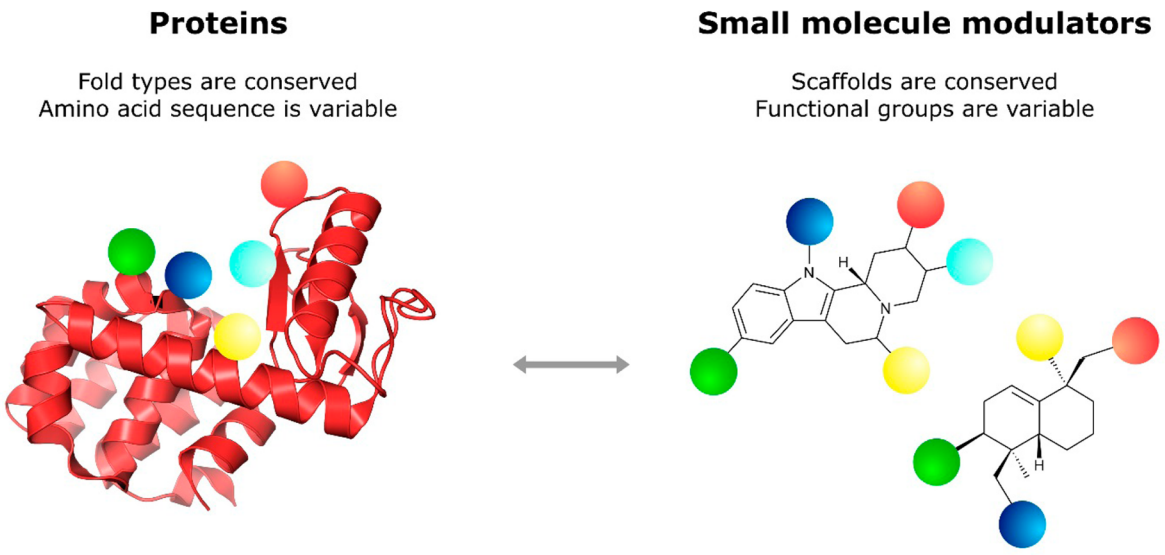

Figure 1. Natural products and proteins both show conserved features in their scaffold structure but display additional diversity in substituent decoration and amino acid sequence.

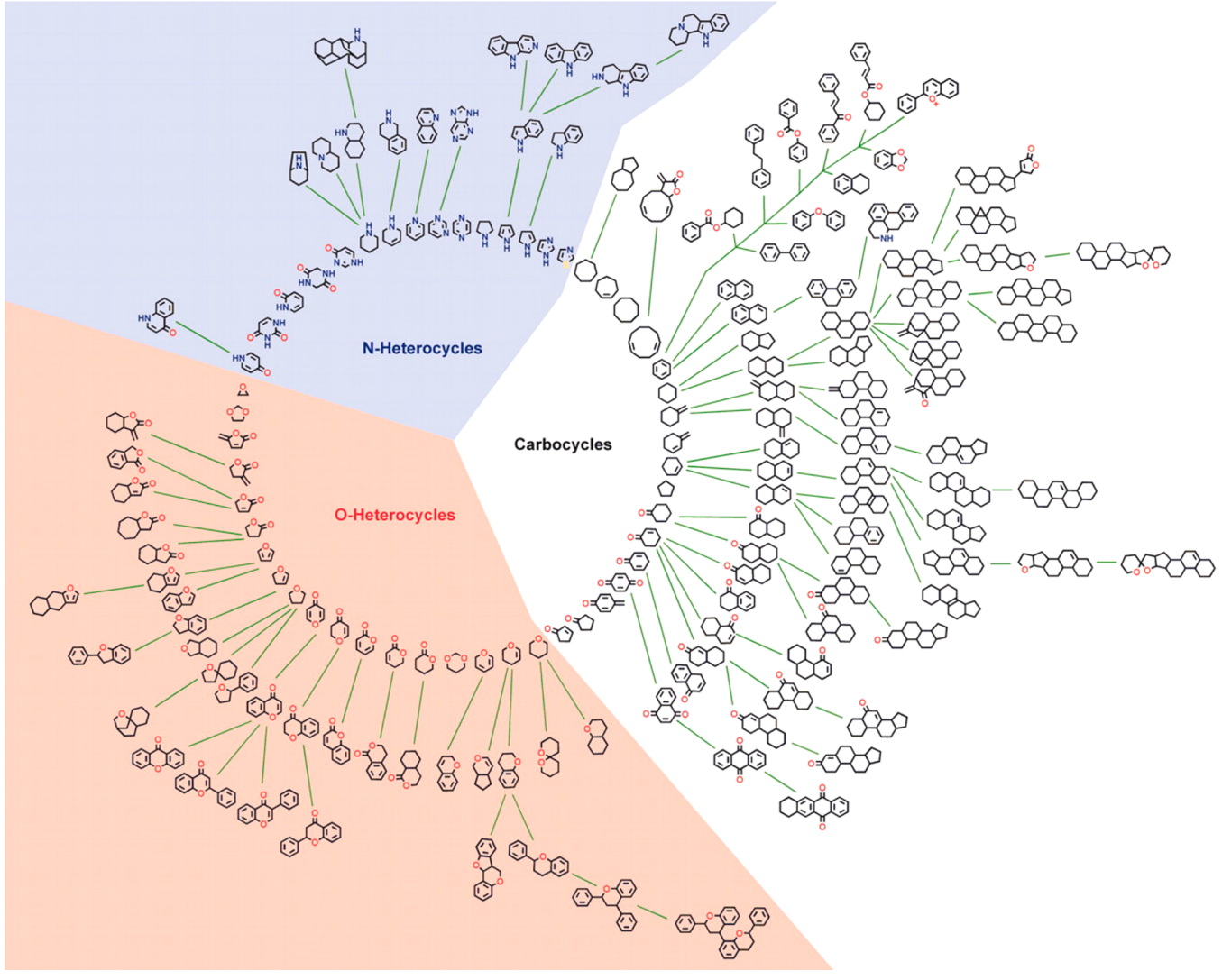

Figure 2. Schematic representation of the Structural Classification of Natural Products (SCNOP) scaffold tree. Natural products were reduced to single ring structures according to a set of chemical rules for the identification of biologically relevant scaffolds. Reproduced with permission from ref 9.

dx.doi.org/10.1021/ja505861d | J. Am. Chem. Soc. 2014, 136, 11853−11859

This fundamental reasoning led us to propose and develop "biology-oriented synthesis" (BIOS) as an intellectual framework for the discovery of novel bioactive small molecules.[2,7,8] In BIOS the analysis of evolutionarily selected NP structures is employed to define prevalidated, educated-guess starting points for the synthesis and further development of biologically relevant small molecules for chemical biology and medicinal chemistry research.

In this Perspective we discuss the logic and philosophy of BIOS, the development of the necessary bioinformatic and cheminformatic tools to navigate through biologically relevant chemical space, and the application of this intellectual framework for the synthesis of small-molecule modulators of protein function.

STRUCTURAL CLASSIFICATION OF NATURAL PRODUCTS

Development of the BIOS logic initially required cheminformatic analysis of NPs and their scaffold structures. To this end, all NPs encoded in the Dictionary of Natural Products (DNP) database were reduced to their scaffolds, which were subsequently subjected to tree analysis to arrive at a Structural Classification of Natural Products (SCONP; Figure 2).[9]

During cheminformatic building of structural trees, like the NP tree, not all branches may be reducible to a single ring structure, because there may be "gaps" of structures that are not encoded in the underlying database. For NPs this means that they either do not exist or have not been identified. Therefore, the initial NP-focused logic for tree generation was modified and extended to non-NPs. Accordingly, "virtual scaffolds" were introduced to fill the gaps and enable tree completion and comparison by overlay. The new structural classification also embraced medicinal chemistry experience, which to some extent involves subjective reasoning. However, 13 argued rules ensured the applicability of the identified scaffolds.[10] Additional tree building based on bioactivity as the guiding criterion for structural simplification ensures that neither chemical accessibility nor natural occurrence but rather biological relevance is of major importance while building the trees and filling the gaps. Moving from complex structures to simpler scaffolds along the branches of trees, like the NP tree (termed "brachiation" after the anthropological description of gibbons moving in botanical trees), then assures that the bioactivity encoded in the scaffolds is retained, although possibly at lower potency. The software called Scaffold Hunter was developed to interface SCONP analysis in a user-friendly manner and to enable intuitive and interactive analysis by chemists and biologists without expert knowledge in cheminformatics and knowledge of descriptors like SMILES strings, InChI keys, or graphs that allow for efficient computing (software freely available at http://scaffoldhunter.sourceforge.net/). More information and an example for the application of the Scaffold Hunter in the discovery of new modulators of pyruvate kinase can be found in ref 11. This program may motivate the synthesis of NP-inspired compound libraries based on evolutionarily prevalidated structures that are, therefore, enriched in biological activity. The Scaffold Hunter software also allows prospective assignment of bioactivity for novel compound classes by means of overlay and comparison of differently annotated trees.

POSSIBILITIES AND IMPLICATIONS OF BIOLOGY-ORIENTED SYNTHESIS

BIOS and SCONP lay a foundation for the synthesis of various NP-inspired compound collections, which may involve challenging synthetic routes exceeding the demands of standard compound library synthesis, for example syntheses including the stereoselective establishment of multiple stereocenters. However, in our experience and also based on the work of others, these challenges can be successfully met by contemporary synthesis methods.[2,12,13] Moreover, despite these higher demands on synthesis, like their guiding NPs, the collection members are biologically relevant. Since NPs show significantly higher hit rates compared to the compounds from traditional synthetic and combinatorial libraries, enhanced hit rates are expected for BIOS collections as well.[14] In our experience, this expectation is in agreement with reality. The hit rate of BIOS compounds in our biochemical and cell-based screens is typically 0.5−1.5%, and thus libraries of only 200−500 compounds usually suffice for identification of an initial set of modulators. In a sense, higher investment into more demanding chemistry pays off as smaller library sizes are sufficient and extensive automation is avoided, which is particularly relevant to academic settings.

BIOS, furthermore, may help to overcome the two most important problems with NPs in drug discovery and may foster a renaissance of NP-inspired strategies. First, reduction of structural complexity at retained bioactivity (but not necessarily potency) and subsequent development of NP-inspired libraries ensure reliable and efficient synthetic tractability, which often is not the case for the guiding NPs. Second, compound supply in amounts sufficient for full biological and pharmacological development will not be an issue. Therefore, at the heart of BIOS is the continuous development of efficient and highly practical synthetic methods, amenable to compound library synthesis, while giving access to complex structures incorporating multiple stereocenters with high levels of selectivity.

Structural simplification of the original NPs to scaffolds in order to arrive at synthetically tractable NP-inspired compound collections may result in compounds with lower affinities than their guiding NPs, e.g., in the micromolar range, and lower target specificity. However, BIOS only sets the starting point for full-fledged compound development to a biologically relevant core structure for which the usual rules of medicinal chemistry and chemical biology fully apply. The initial reduction of NP complexity and decoration during scaffold isolation needs to be balanced by rational increase of complexity through synthesis to enhance affinity and selectivity. Moreover, binding of multiple proteins by a given ligand need not be a uniformly negative feature. Biological systems are robust and often wired redundantly. Tuning, for instance, signal propagation through such systems below a therapeutically relevant level may require simultaneous interference at multiple signaling nodes. Kinase inhibitors, which often need to target multiple proteins simultaneously for therapeutic effect, are relevant examples.[15−17] While the principles for logical design of "selective non-selectivity" are mostly unclear, NPs evolved to modulate dynamic, robust, and redundantly wired biological systems, and this property must be encoded in their structure. Thus, NP-inspired collections offer the ambitious opportunity to identify compounds endowed with this property as well. Such chemical modulators of biological systems could be invaluable tools for systems biology research.

dx.doi.org/10.1021/ja505861d | J. Am. Chem. Soc. 2014, 136, 11853−11859

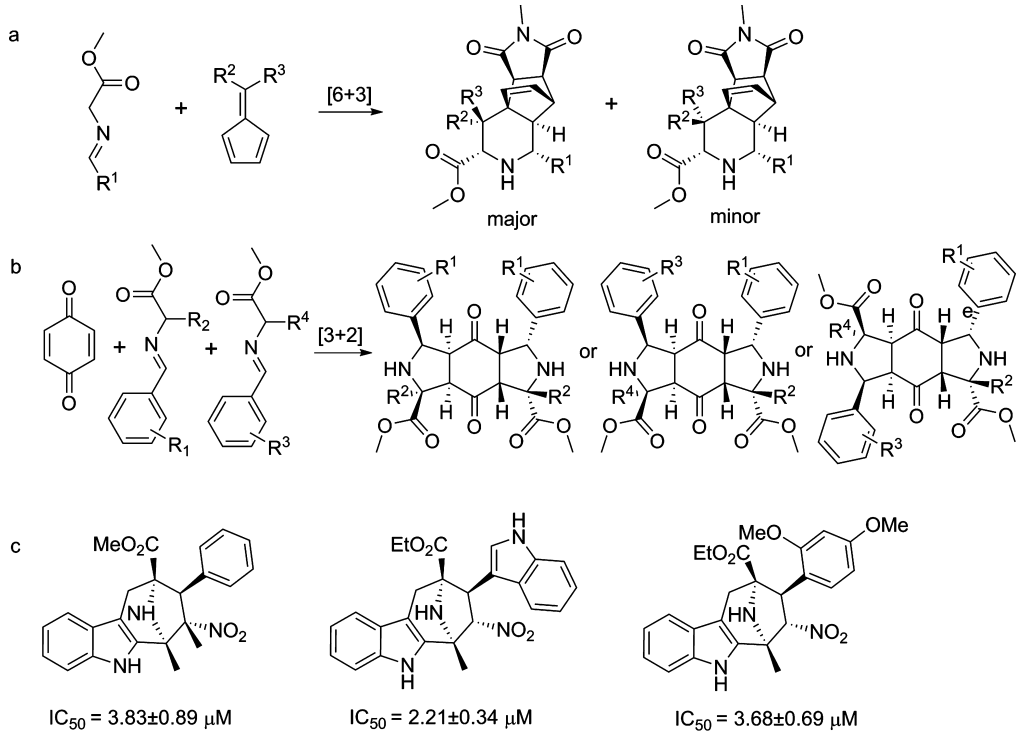

Figure 3. (a) [6+3] Cycloaddition of azomethine ylides with fulvenes.[19] (b) Double enantioselective [3+2] cycloaddition of azomethine ylides to *p*-benzoquinone.[20] (c) Hedgehog inhibitors obtained from a Cu^{I}-catalyzed [3+2] cycloaddition of 1,3-fused cyclic azomethine ylides and nitroalkenes.[21]

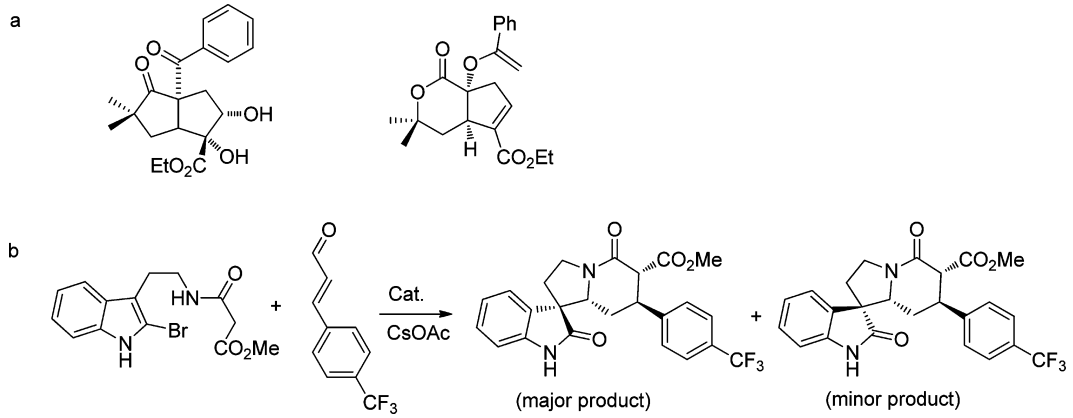

Figure 4. (a) Iridoid-inspired neurite outgrowth modulators.[22] (b) Neurite outgrowth enhancers based on the secoyohimbane scaffold.[23]

In agreement with the modulation of ligand and protein structure promiscuity by evolutionary pressure, it is to be expected that NPs—and by analogy also NP-inspired compounds—may have multiple cellular targets, which in turn leads to the expectation that a given NP-inspired compound collection of sufficient diversity may actually contain modulators of different proteins or biological programs. In-house examples indicate that this notion may hold its promise.

Additionally, the property of NPs to target multiple, often still unidentified proteins, and to modulate complex biological systems, frequently with very high potency and selectivity, may offer the opportunity to identify novel target proteins and to create unprecedented possibilities for drug discovery. Thus, a very demanding but equally promising application of NPs and NP-inspired compound collections in chemical biology and medicinal chemistry is their use in cell-based phenotypic screens, which then need to be coupled to powerful target identification technology.[18]

APPLICATIONS OF BIOLOGY-ORIENTED SYNTHESIS

Among the various examples of BIOS developed to date, in particular one-pot sequences and cascade and domino reactions deserve to be mentioned because of their high practicality and efficiency.

The SCONP was used, for example, for the identification of specific biologically active heterocycles, which were subsequently synthesized using an enantioselectively catalyzed [6+3] cycloaddition of azomethine ylides with fulvenes. This reaction provided piperidine derivatives with four stereocenters with high regio- and enantioselectivity. The method could be extended to a one-pot transformation, including a subsequent

dx.doi.org/10.1021/ja505861d | J. Am. Chem. Soc. 2014, 136, 11853−11859

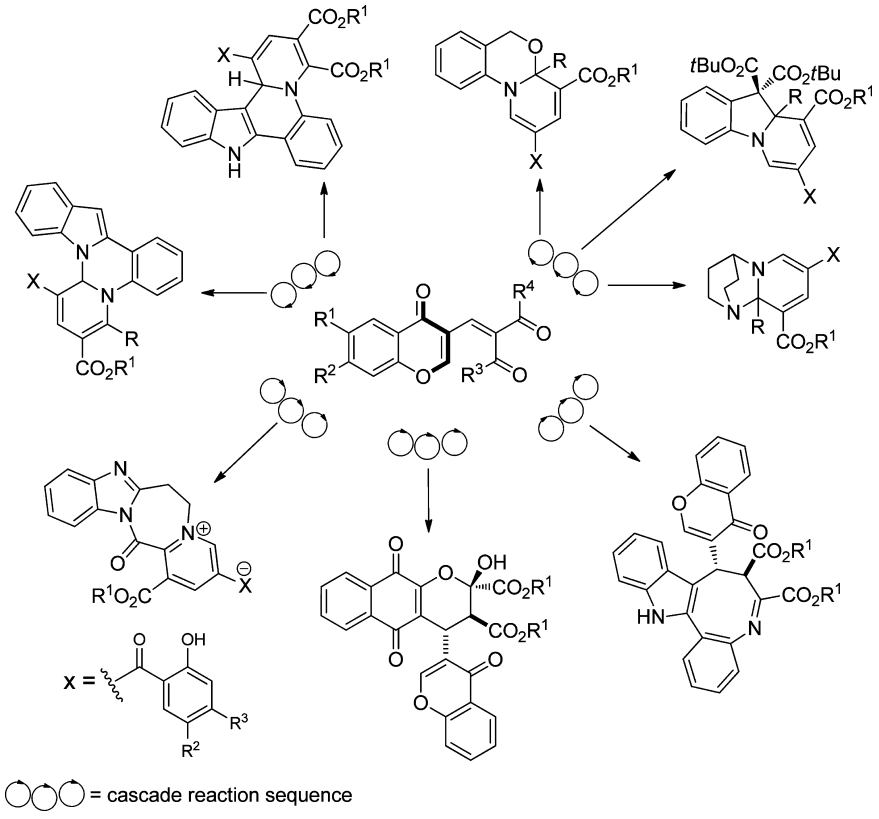

Figure 5. An example of branching cascades.[24]

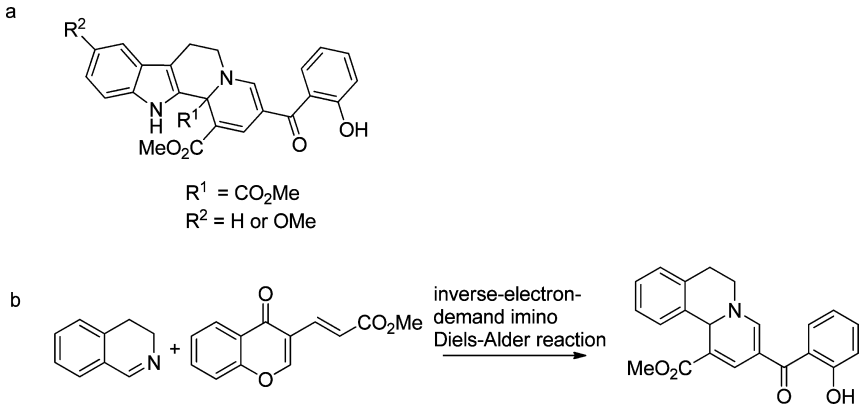

Figure 6. (a) Indoloquinolizines, formed via a 12-step cascade reaction sequence in one pot.[25] (b) An enantioselective inverse-electron-demand imino-Diels–Alder reaction yielding centrocountin analogues.[26]

[4+2] cycloaddition to yield complex piperidines with eight stereocenters (Figure 3a).[19] Also, sequential enantioselective cycloadditions employing azomethine ylides were applied to *p*-benzoquinone to yield NP-inspired compounds with high structural and chemical diversity (Figure 3b).[20] Moreover, the Cu^{I}-catalyzed [3+2] cycloaddition of 1,3-fused cyclic azomethine ylides and nitroalkenes yielded a collection of a novel class of inhibitors for the hedgehog-signaling pathway (Figure 3c).[21]

The BIOS strategy was also applied in the search for neurite outgrowth modulators. Enantioselective synthesis of an iridoid-inspired compound collection yielded modulators of neurite outgrowth from primary hippocampal neurons and motor neurons derived from mouse embryonic stem cells (Figure 4a).[22] In addition, compounds with a secoyohimbane scaffold were accessed with high enantioselectivity. They influenced the complexity of neuronal network formation and promoted neurite outgrowth (Figure 4b).[23] These examples show that NP structure simplification may lead to tractable synthesis targets and that the corresponding products may retain the kind of biological activity characteristic for the guiding NPs.

The examples mentioned above concern singular cascade reactions for the formation of novel NP-inspired compound libraries. Another strategy is to use branching cascades, in which a more diverse set of compounds is generated, which can subsequently be narrowed to more focused compounds for different target proteins (Figure 5).[24] This approach was also applied for the discovery of phosphatase inhibitor classes, in which brachiation from the pentacyclic yohimbane scaffold to the simpler scaffolds of substituted indoles via indolo-

dx.doi.org/10.1021/ja505861d | J. Am. Chem. Soc. 2014, 136, 11853–11859

quinolizidines resulted in four novel selective phosphatase inhibitor classes for the MptpB, Cdc25A, PTP1B, and VE-PTP tyrosine phosphatases.[8]

Furthermore, inspired by NPs with relevance to cancer and mitosis, new synthetic routes were developed to transform readily available substrates into complex indoloquinolizines via a 12-step cascade reaction sequence in one pot. The sequence included two opposing kinds of organocatalysis and nine different reactions to finally form scaffolds that resemble the tetracyclic core structure of numerous polycyclic indole alkaloids (Figure 6a).[25]

Biological investigation of a focused compound library led to the discovery of modulators of centrosome integrity, termed centrocountins. These compounds impair mitosis by binding to the centrosome-associated proteins nucleophosmin (NPM1) and Crm1, which results in the formation of supernumerary and fragmented centrosomes, multipolar mitotic spindles, chromosome congression defects, multipolar cell division, and acentrosomal spindle poles. Further development of the synthetic strategy made the centrocountin structure available by means of a newly developed enantioselective inverse-electron-demand imino Diels–Alder reaction. This asymmetric reaction involved electron-deficient dienes and electron-rich imines and yielded a centrocountin analogue with a 4-fold higher potency than the guiding centrocountin-1 (Figure 6b).[26]

■ CONCLUSIONS AND OUTLOOK

The use of small-molecule modulators of biological systems offers unique opportunities to investigate the function of individual biomacromolecules and their complexes. The effects of small molecules often are fast, temporary, conditional, and tunable in perturbing protein activity and do not fundamentally alter the biological systems to be analyzed. In addition, small molecules are often selective, although the degree of selectivity may vary (e.g., flavonoids can be relatively nonselective). The ready availability of potent small-molecule modulators will foster biological research and novel therapeutic opportunities. Biology-oriented synthesis represents a strategy to quickly and efficiently navigate biologically relevant space to guide and inspire subsequent syntheses.

In recent years, various novel approaches were initiated for further development of the field. Thus, Aubé et al. synthesized compound collections from four families of biologically active alkaloids. Sophisticated synthesis routes yielded a diverse set of NP-inspired compounds which were comparable in structural complexity and sp^3 content to their guiding NPs.[27] In an alternative approach, Hergenrother et al. started from readily accessible NPs and developed divergent synthesis strategies to form related but different complex products in quantities sufficient for biological research. For instance, the tetracyclic diterpene hormone gibberellic acid was used as a guiding NP to enable several divergent transformations across the ring systems, validating this NP as a potent scaffold from which large numbers of complex molecules could be synthesized.[28] By analogy, the steroid hormone adrenosterone was subjected to chemical exploration.[27] These examples prove the potential of NPs as starting points for population of biologically relevant chemical space.

Burke et al. developed an iterative approach to the synthesis of polyene NPs based on the repeated use of bifunctional vinyl boronate reagents.[29] Twelve MIDA-protected boronate building blocks were synthesized and employed to form the polyene motifs that occur in more than 75% of the known polyene NPs. The synthesis strategy includes iterative cross coupling of a pinacol boronate to a bifunctional halo MIDA boronate and removal of the MIDA protecting group to initiate a new round of coupling. The modular character of the approach and the reagents may enable the rapid and efficient synthesis of hitherto unaccessible polyene NP libraries.

Another approach to explore biologically relevant chemical space is the use of NP fragments. Over et al. fragmented, filtered, and clustered more than 180 000 NP structures, resulting in 2000 clusters of NP-derived fragments.[30] These natural scaffolds are rich in sp^3-configured centers, which are crucial for spatial binding in protein pockets. NP fragments were employed to identify inhibitors of several phosphatases and stabilizers of an inactive conformation of p38α MAP kinase, which define a truly novel kinase inhibitor chemotype. Synthetically combining these NP fragments into novel compounds may lead to compound libraries with interesting biological properties. Structural analysis of protein binding pockets should indicate the corresponding space for binding and interacting residues.

Moreover, the field of NP-inspired synthesis could be advanced drawing from novel biological methods to obtain new NPs. A particularly relevant example comes from synthetic biology research in which baker's yeast is used as an NP generator.[31] Novel compounds were discovered by combinatorial genetics mimicking "co-evolution" of NP-synthesizing target proteins. This strategy allows to identify a variety of novel compounds that are quickly produced.

NP-inspired compound libraries have proven their worth in the discovery of novel, biologically active structures. The approaches described above could foster more efficient use of NPs as prevalidated starting points for exploring biologically relevant chemical space. These efforts may greatly forward chemical biology research for a better understanding of biological processes and programs and ultimately may inspire novel drug discovery applications.

■ AUTHOR INFORMATION

Corresponding Author

herbert.waldmann@mpi-dortmund.mpg.de

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to our co-workers who have contributed to the work described in this Perspective. This research was supported by the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013; ERC Grant 268309 to H.W.) and the Max Planck Society. H.v.H. thanks the Humboldt Foundation for a fellowship.

■ REFERENCES

(1) Dobson, C. M. *Nature* **2004**, *432*, 824.
(2) Wetzel, S.; Bon, R. S.; Kumar, K.; Waldmann, H. *Angew. Chem., Int. Ed.* **2011**, *50*, 10800.
(3) Skolnick, J.; Gao, M. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 9344.
(4) Gao, M.; Skolnick, J. *PLoS Comput. Biol.* **2013**, *9*, e1003302.
(5) Patel, S. C.; Bradley, L. H.; Jinadasa, S. P.; Hecht, M. H. *Protein Sci.* **2009**, *18*, 1388.
(6) Zhang, Y.; Hubner, I. A.; Arakaki, A. K.; Shakhnovich, E.; Skolnick, J. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 2605.
(7) Bon, R. S.; Waldmann, H. *Acc. Chem. Res.* **2010**, *43*, 1103.

(8) Noren-Muller, A.; Reis-Correa, I., Jr.; Prinz, H.; Rosenbaum, C.; Saxena, K.; Schwalbe, H. J.; Vestweber, D.; Cagna, G.; Schunk, S.; Schwarz, O.; Schiewe, H.; Waldmann, H. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 10606.
(9) Koch, M. A.; Schuffenhauer, A.; Scheck, M.; Wetzel, S.; Casaulta, M.; Odermatt, A.; Ertl, P.; Waldmann, H. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17272.
(10) Schuffenhauer, A.; Ertl, P.; Roggo, S.; Wetzel, S.; Koch, M. A.; Waldmann, H. *J. Chem. Inf. Model.* **2006**, *47*, 47.
(11) Wetzel, S.; Klein, K.; Renner, S.; Rauh, D.; Oprea, T. I.; Mutzel, P.; Waldmann, H. *Nat. Chem. Biol.* **2009**, *5*, 581.
(12) Kumar, K.; Waldmann, H. *Angew. Chem., Int. Ed.* **2009**, *48*, 3224.
(13) Nandy, J. P.; Prakesch, M.; Khadem, S.; Reddy, P. T.; Sharma, U.; Arya, P. *Chem. Rev.* **2009**, *109*, 1999.
(14) Sukuru, S. C.; Jenkins, J. L.; Beckwith, R. E.; Scheiber, J.; Bender, A.; Mikhailov, D.; Davies, J. W.; Glick, M. *J. Biomol. Screen.* **2009**, *14*, 690.
(15) Davis, M. I.; Hunt, J. P.; Herrgard, S.; Ciceri, P.; Wodicka, L. M.; Pallares, G.; Hocker, M.; Treiber, D. K.; Zarrinkar, P. P. *Nat. Biotechnol.* **2011**, *29*, 1046.
(16) Fabian, M. A.; Biggs, W. H., III; Treiber, D. K.; Atteridge, C. E.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lelias, J. M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A. M.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J. *Nat. Biotechnol.* **2005**, *23*, 329.
(17) Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. *Nat. Biotechnol.* **2008**, *26*, 127.
(18) Ziegler, S.; Pries, V.; Hedberg, C.; Waldmann, H. *Angew. Chem., Int. Ed.* **2013**, *52*, 2744.
(19) Potowski, M.; Bauer, J. O.; Strohmann, C.; Antonchick, A. P.; Waldmann, H. *Angew. Chem., Int. Ed.* **2012**, *51*, 9512.
(20) Potowski, M.; Schürmann, M.; Preut, H.; Antonchick, A. P.; Waldmann, H. *Nat. Chem. Biol.* **2012**, *8*, 428.
(21) Narayan, R.; Bauer, J. O.; Strohmann, C.; Antonchick, A. P.; Waldmann, H. *Angew. Chem., Int. Ed.* **2013**, *52*, 12892.
(22) Dakas, P. Y.; Parga, J. A.; Hoing, S.; Scholer, H. R.; Sterneckert, J.; Kumar, K.; Waldmann, H. *Angew. Chem., Int. Ed.* **2013**, *52*, 9576.
(23) Antonchick, A. P.; Lopez-Tosco, S.; Parga, J.; Sievers, S.; Schurmann, M.; Preut, H.; Hoing, S.; Scholer, H. R.; Sterneckert, J.; Rauh, D.; Waldmann, H. *Chem. Biol.* **2013**, *20*, 500.
(24) Liu, W.; Khedkar, V.; Baskar, B.; Schurmann, M.; Kumar, K. *Angew. Chem., Int. Ed.* **2011**, *50*, 6900.
(25) Dückert, H.; Pries, V.; Khedkar, V.; Menninger, S.; Bruss, H.; Bird, A. W.; Maliga, Z.; Brockmeyer, A.; Janning, P.; Hyman, A.; Grimme, S.; Schürmann, M.; Preut, H.; Hübel, K.; Ziegler, S.; Kumar, K.; Waldmann, H. *Nat. Chem. Biol.* **2012**, *8*, 179.
(26) Eschenbrenner-Lux, V.; Kuchler, P.; Ziegler, S.; Kumar, K.; Waldmann, H. *Angew. Chem., Int. Ed.* **2014**, *53*, 2134.
(27) McLeod, M. C.; Singh, G.; Plampin Iii, J. N.; Rane, D.; Wang, J. L.; Day, V. W.; Aubé, J. *Nat. Chem.* **2014**, *6*, 133.
(28) Morrison, K. C.; Hergenrother, P. J. *Nat. Prod Rep.* **2014**, *31*, 6.
(29) Woerly, E. M.; Roy, J.; Burke, M. D. *Nat. Chem.* **2014**, *6*, 484.
(30) Over, B.; Wetzel, S.; Grütter, C.; Nakai, Y.; Renner, S.; Rauh, D.; Waldmann, H. *Nat. Chem.* **2013**, *5*, 21.
(31) Klein, J.; Heal, J. R.; Hamilton, W. D.; Boussemghoune, T.; Tange, T. O.; Delegrange, F.; Jaeschke, G.; Hatsch, A.; Heim, J. *ACS Synth. Biol.* **2014**, *3*, 314.