



Pseudo-natural products and natural product-inspired methods in chemical biology and drug discovery

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Abstract

Through evolution, nature has provided natural products (NPs) as a rich source of diverse bioactive material. Many drug discovery programs have used nature as an inspiration for the design of NP-like compound classes. These concepts are guided by the prevalidated biological relevance of NPs while going beyond the limitations of nature to produce chemical matter that could have unexpected or novel bioactivities. Herein, we discuss, compare, and highlight recent examples of NP-inspired methods with a focus on the pseudo-NP concept.

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Introduction

Approaches that inspire new molecular discovery programs are at the core of chemical biology and medicinal chemistry. As the mode of action of small molecules compared to most genetic approaches can be rapid, tunable, conditional, and reversible [1], the development of selective probes is of high value for biological applications. A key challenge that arises is the identification of areas in chemical space, which are relevant for bioactivity [2], because the possible number of small molecules is at least 10^{60} , too many to ever be synthesized, nonetheless biologically evaluated [3].

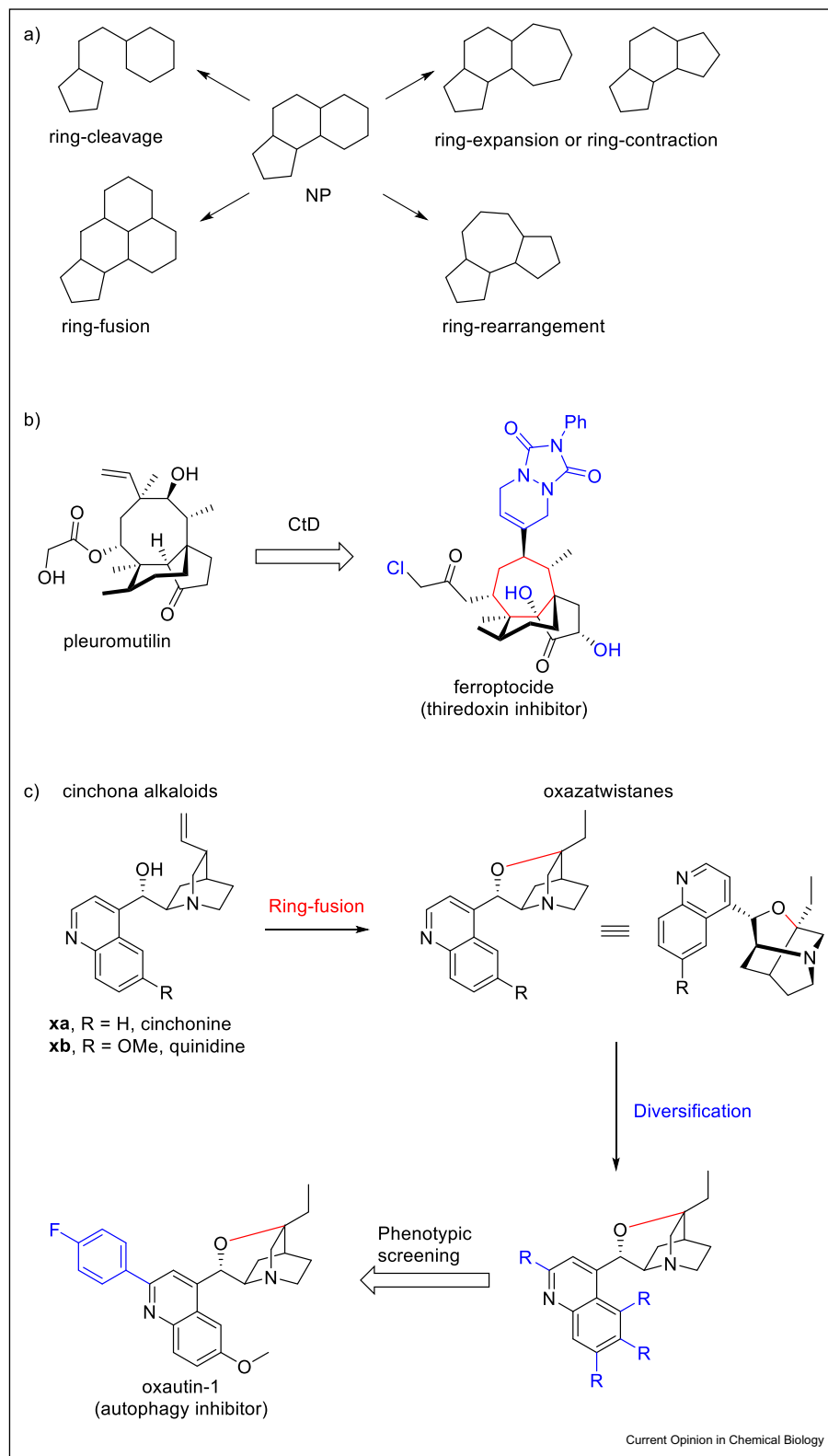
Natural products (NPs) contain privileged molecular scaffolds with inherent biological relevance, having themselves evolved to fulfill specific biological functions within the context of signaling pathways and protein interactions [4]. These frameworks are, therefore, ‘prevalidated’ representations of nature’s exploration of biologically relevant chemical space. While numerous NP-based drug discovery programs have successfully used NPs or their close derivatives as endpoints [5], other methods have thrived on using NPs as starting points to inspire new bioactive compound collections. In this review, recent developments in methods that are inspired by nature will be discussed with a focus on the recently disclosed pseudo-NPs concept.

Ring distortion strategy – complexity-to-diversity (CtD)

An elegant approach to exploit nature’s pre-validated and rich pool of biologically relevant molecular scaffolds was introduced in 2013 as a ring distortion/modification strategy by Hergenrother and co-workers [6**]. Instead of regarding the complex structures of NPs as the final product in a synthesis or drug discovery effort, they are set as privileged starting points for the exploration of NP-derived chemical space through appropriate chemical modifications of orthogonal functional characteristics embedded in the chosen NPs. In contrast to the traditional optimization efforts, which intend to improve potency or drug-like properties of a NP, the ring distortion/modification approach aims for significant disruption of the overall topology of the parent NP by manipulation of core ring systems. This approach aims for a high degree of scaffold diversity and is, hence, referred to as the complexity-to-diversity (CtD) strategy.

CtD is inspired by the biosynthetic pathways leading to complex NPs where common intermediates are transformed by diverse arrays of various enzymes to generate compounds that are distinct from each other. Transferring this logic into the hands of organic chemists, enzymes become chemoselective reagents, which enable the strategic manipulation of a suitable NP through ring-cleavage, ring-rearrangement, ring-fusion and modification of ring-size (expansion or contraction), and/or its oxidation state (e.g., aromatization), as illustrated in [Figure 1a](#).

Figure 1



(a) General depiction of the ring distortion strategy. **(b)** Ring contraction (highlighted in red) and diversification (highlighted in blue) of pleuromutilin to give a thiredoxin inhibitor, ferroptocide. **(c)** Ring-fusion (highlighted in red) of cinchona alkaloids to provide oxazatwistanes (highlighted in red). Further diversification (highlighted in blue) and phenotypic screening provided the autophagy inhibitor oxautin-1.

A synthetic proof of concept was outlined by the diversification of several NPs, including gibberellic acid [6**], adrenosterone [6**], quinine [6**], abietic acid [7], yohimbine [8], sinomenine [9], and lycorine [10]. A recent example demonstrated the biological relevance of CtD by the diversification of pleuromutilin and subsequent biological evaluation (Figure 1b) [11*]. In total, 29 structurally diverse and highly complex derivatives of pleuromutilin were synthesized using CtD and screened in a phenotypic assay for anticancer activity. A ring-contraction product of pleuromutilin was further elaborated to ferroptocide and was found to rapidly induce ferroptotic death of cancer cells by inhibiting thioredoxin.

The biological relevance of the CtD approach was further verified by Laraia *et al.*, focusing on the diversification of oxazatwistanes (Figure 1c) [12*]. The synthesis commenced with an intramolecular etherification of cinchonine or quinidine to install the ring-fused non-natural oxazatricyclo[4.4.0.0]decane scaffold. Subsequent metal-catalyzed cross-coupling reactions and C–H functionalization were used to further modify the quinoline core to afford a total of 47 compounds that were subjected for biological evaluation in a range of phenotypic assays. The identification of several autophagy inhibitors, an activity not exhibited by cinchona alkaloids at concentrations up to 30 μM [13], demonstrated that significant changes in the topology of NPs might lead to novel NP-inspired molecular frameworks that are endowed with different bioactivity profiles than their natural predecessors.

Biology-oriented synthesis

Biology-oriented synthesis (BIOS) is a design principle to generate small molecules as tools for the study of complex biological systems [4,14,15**]. In nature, evolutionary conservation of molecular architectures leads only to a limited number of possible small-molecule binding sites [16]. Analogously, NPs have come from the function-driven evolution of biosynthetic pathways. Whereas the surrounding substitution patterns can be highly diverse, the core scaffolds are conserved. BIOS uses NPs as prevalidated starting points in the development of bioactive molecules. From a cheminformatics structural analysis of NPs [2], simplified core scaffolds have been identified that may transfer biologically relevant characteristics to the resulting synthetic molecule [17]. Diversification of substituent patterns can lead to specific selectivity profiles and can provide NP-inspired compound collections that are more elaborate and have enriched bioactivity relative to combinatorial libraries. Therefore, the size of the compound collection can be reduced while increasing bioactivity hit rates.

The application of BIOS in providing compounds for the development of optimized structures, for example, in

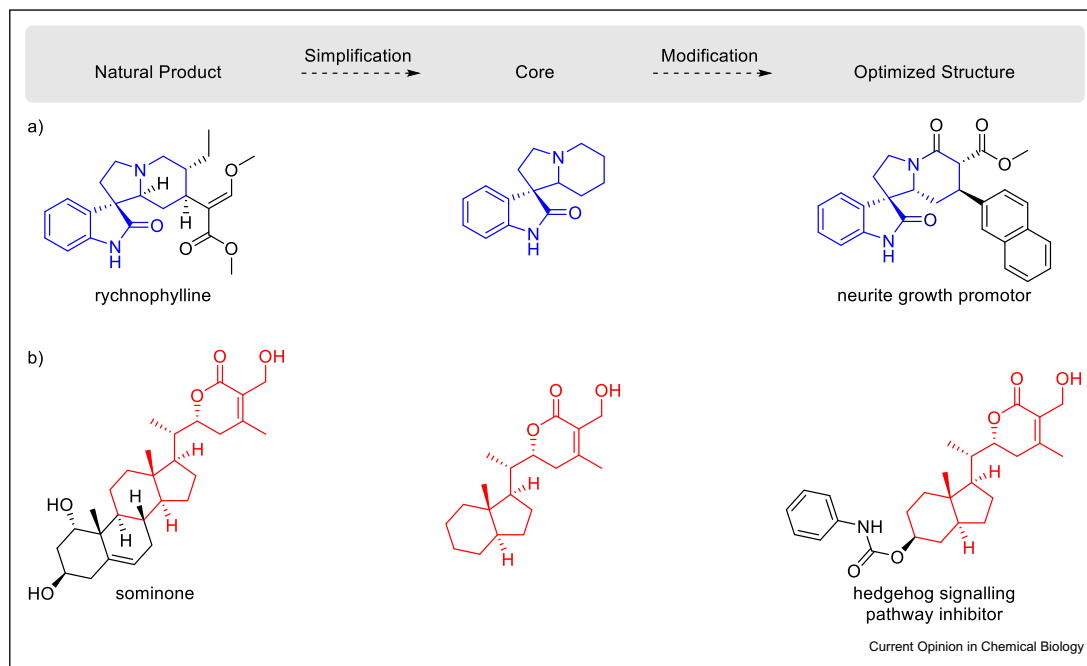
potency or pharmacodynamic properties, has been shown in various examples. Secoyohimane NPs such as rychnophylline (Figure 2a) are known for their neurite growth-promoting properties [18–20]. The simplification to the *spiro*-core scaffold composed of four connected rings allowed the synthesis of five derivatives, which are active in neurite growth assays [21]. Similarly, derivatives of the NP sominone (Figure 2b) with the dehydro- δ -lactone motif were identified as potent inhibitors of the Hedgehog signaling pathway [22]. Overall, the BIOS approach has structurally and, thereby, synthetically simplified NPs to provide small molecules that have new or improved bioactivities.

Pseudo-natural products

While several approaches have been developed to produce novel bioactive compound collections, they may have limitations. The CtD approach gives rapid access to diverse biological space through chemical derivatization of NPs. However, the use of NPs as a starting point may present a chemical limitation because the availability of sufficient quantities of many NPs is restricted. Nevertheless, complex polycyclic scaffolds that are NP-like can be directly synthesized and distorted [23]. In BIOS, NPs inspire the synthesis of structurally simplified but biologically relevant compound collections. NPs occupy a large yet limited amount of chemical space [24]. However, NP-like chemical space is significantly larger than existing NP scaffolds and therefore presents a chemical limitation of BIOS. From a biological viewpoint, these compounds may retain similar bioactivities to the structurally related parent NP, thereby limiting the exploration of biological space [17].

Methods that allow for rapid access to biologically pertinent chemical space while retaining biological relevance to NPs may provide useful compound collections. This matching led to the development of the pseudo-NP approach [25**,26**] in which bioactive compound classes are obtained through combining strategies that take inspiration from nature, such as BIOS, with principles that efficiently cover a vast amount of chemical space, such as fragment-based compound design. In a cheminformatics analysis, more than 180,000 NPs were analyzed and simplified to approximately 2,000 NP-based fragment classes in which the properties of the NPs were retained [27]. It was hypothesized that the *de novo* combination and arrangement of the NP fragments could give unprecedented combinations or fusion patterns that may provide novel scaffolds that are significantly different from NPs and other NP-based collections (Figure 3a). These compounds could retain biological and chemical relevance to NPs but extend beyond the biologically relevant chemical space explored by nature. The new compound classes resemble NPs but are not accessible through existing biosynthetic pathways. Therefore, they

Figure 2



The BIOS approach with (a) rychnophylline and (b) sominone, which provided compounds that promote neurite growth and inhibit the Hedgehog signaling pathway, respectively.

were termed ‘pseudo-natural products’ (pseudo-NPs). The term pseudo-NPs has been previously used by Suga et al. and Oshima et al. to describe cyclic peptides [28,29] and products from intercepted biosynthetic pathways [30,31], respectively.

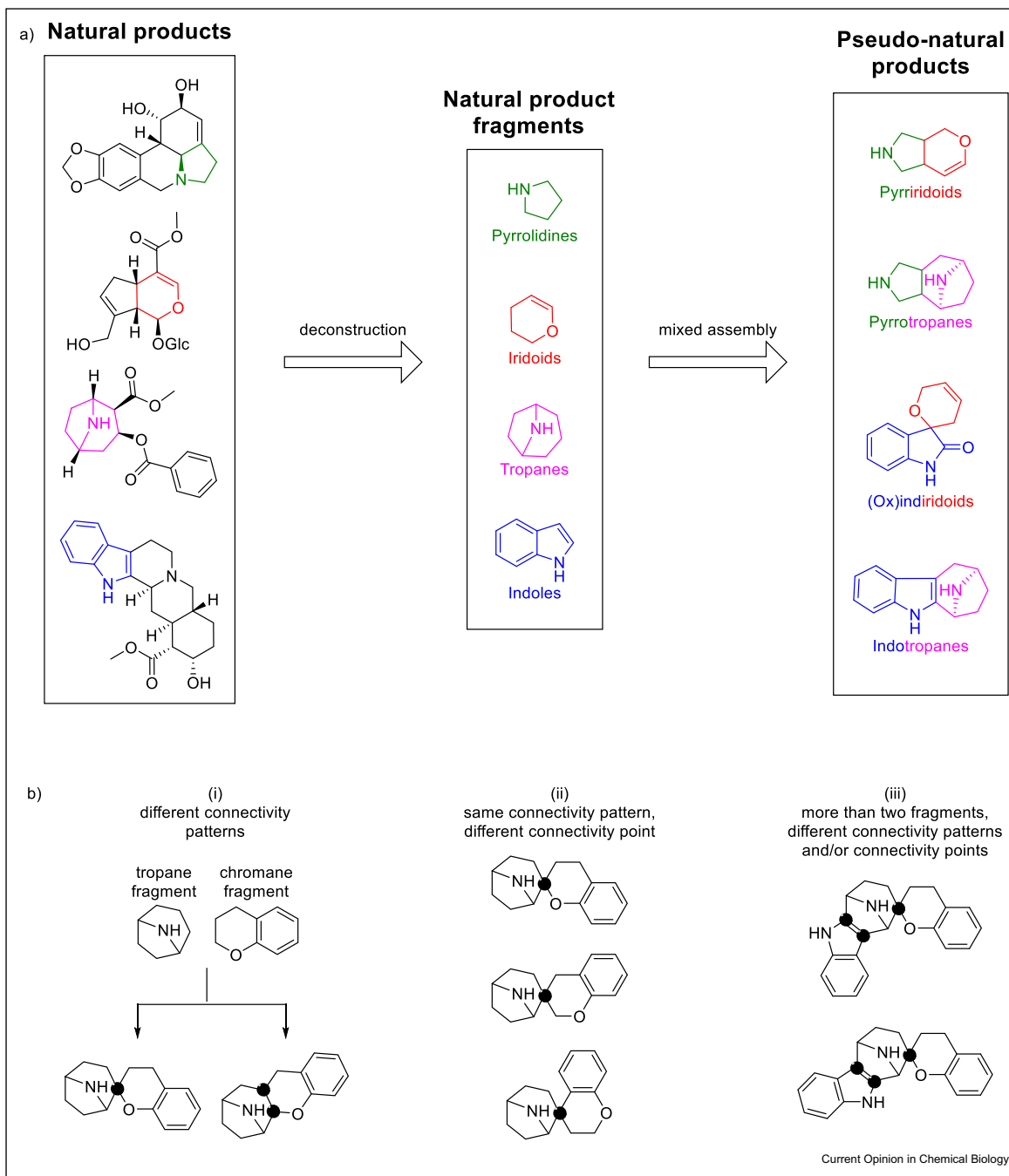
The general design criteria of pseudo-NP compound classes should combine NP-fragments with diverse bioactivities, providing structures that have chirality and complementary heteroatom content, such as oxygen and nitrogen. Furthermore, the merging of biosynthetically unrelated fragments may be advantageous for inducing new bioactivities. Further principles can provide expansive libraries based on different connectivity patterns of the same NP-fragment combinations. For example, the chromane and tropane fragment could be combined in different ways to provide spiro-fused or edge-fused compounds (Figure 3bi). Other compounds could retain the same connectivity pattern but have different connection points (Figure 3bii). Finally, more than two fragments could be connected in various patterns (Figure 3biii).

The pseudo-NP design principle may lead to new scaffolds that could have unexpected or unprecedented biological targets. To biologically evaluate these compounds, unbiased assays can be used to monitor entire biological systems, such as signaling cascades and phenotypic changes, instead of individual targets [32].

Beyond individual phenotypes, multiparametric high-content image screening technologies, such as the ‘cell painting’ assay, can be used to assist hypothesis generation of the biological target or pathway that is being affected [33]. In addition, such phenotypic profiling enables evaluation of library performance diversity and activity distributions across sub-library classes, hence allowing informed library designs toward high performance diversity or hit expansion into promising structure classes [34].

The first example of the pseudo-NP concept provided an unprecedented combination of a chromane and a tetrahydropyrimidone (THPM) fragment through a bridged fusion connection (Figure 4a) [25**]. The ‘chromopynone’ library was synthesized in a telescoped manner via a variant of the Biginelli reaction followed by a single-flask, multistep reaction cascade. Biological evaluation of the chromopynone collection showed inhibition in glucose uptake assays. (–)-(R,R)-Chromopynone-1 was the most potent compound in the library, selectively inhibited glucose transporters GLUT-1 and GLUT-3, and inhibited cancer cell growth. It is interesting to note that the bioactivity of the pseudo-NP was neither shared by 2,801 compounds that contain the chromane fragment or substructure nor 1,270 compounds containing the THPM fragment or substructure in glucose uptake assays. Therefore, the combination of chromane and THPM fragments provided novel

Figure 3



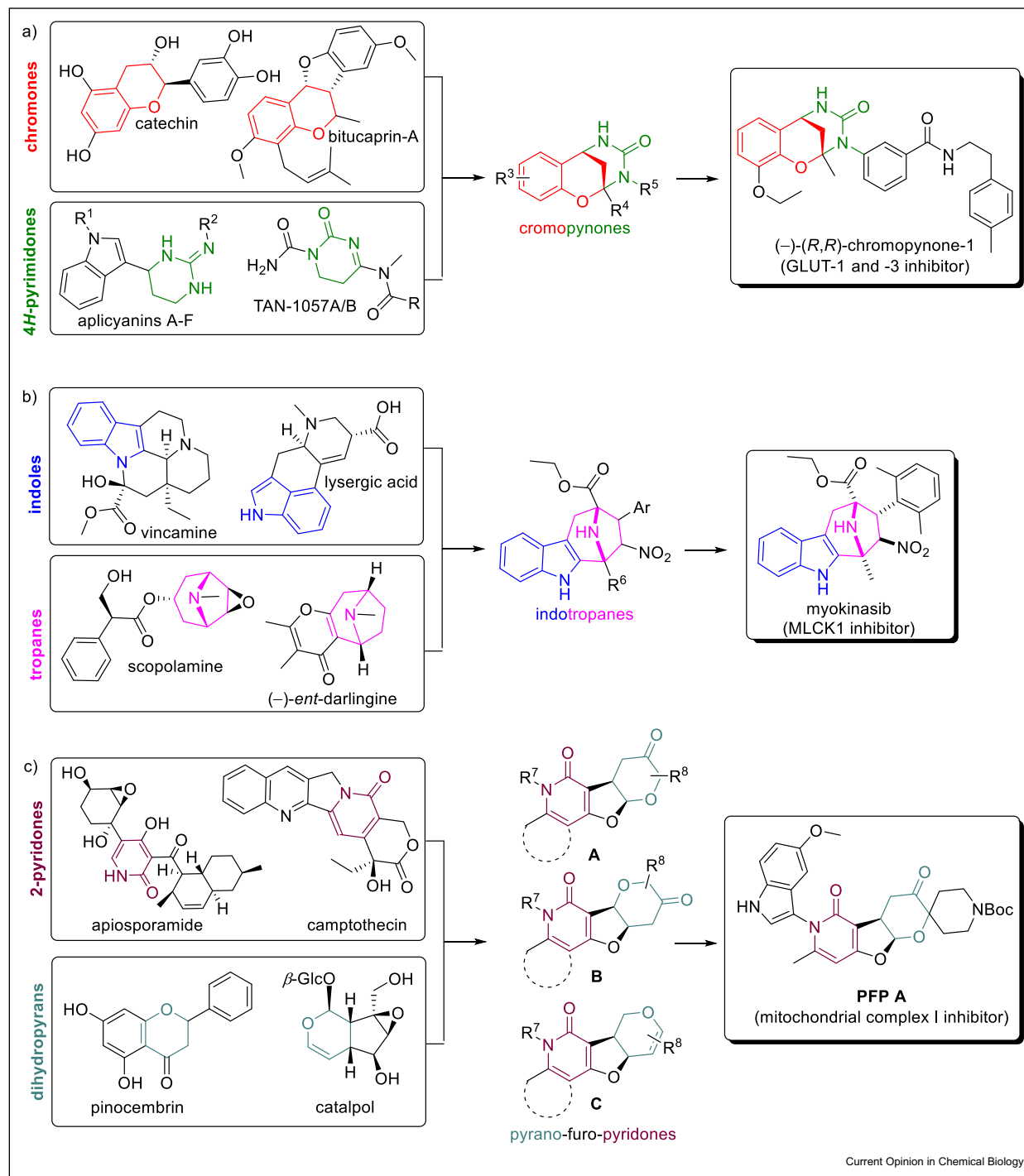
(a) Design of pseudo-NPs through deconstruction of NPs and mixed reassembly. **(b)** Variation of (i) connectivity patterns, (ii) connection points, and (iii) the combination of more than two NP-fragments in pseudo-NP design.

bioactivity that is unrelated to the two independent NP fragments.

Indotropanes were reported as a class of pseudo-NPs that combine the biosynthetically unrelated fragments indole and tropane through a bipodal connection (Figure 4b). Cu-catalyzed 3 + 2 cycloaddition reactions were

used to provide a stereogenically complex library of indotropanes [35]. After biological evaluation, myokinasib was found to induce extensive cell rounding and to impair cell division, causing multinucleation [36*]. It was hypothesized that this phenotype may be due to failed cytokinesis. From a kinase panel and several validation experiments, the phenotype could be attributed

Figure 4



Design and implementation of the pseudo-NP method to provide **(a)** chromopynone, **(b)** indotropane, and **(c)** pyrano-furo-pyridone compound classes. Unique bioactivities were found within the compound classes of GLUT-1/-3, MLCK1, and mitochondrial complex I inhibition, respectively.

to the inhibition of MLCK1. Myokinasib is the first selective, isoform-specific inhibitor of MLCK1. Relative to typical ATP-competitive kinase inhibitors, which are usually flat and aromatic, myokinasib is stereogenically

complex and has a three-dimensional shape. The unprecedented combination of the indole and tropane led to a pseudo-NP that has unprecedented bioactivity and is a novel chemotype for kinase inhibition.

2-Pyridone and dihydropyran NP fragments comprise several unique structures and biological activities. Using either a Pd-catalyzed Tsuji-Trost cascade, a Pd-catalyzed Tsuji-Trost oxa-Michael cascade or a quinine-mediated Michael transacetalization cascade, these rarely related NP fragments could be fused in three distinct bipodal attachment point isomers to provide a compound library of pyrano-furo-pyridones (PFPs) (Figure 4c) [37*]. Morphological profiling by means of a cell painting assay enabled the prioritization of substructure class **A** and guided the discovery and identification of **PFP A** as a structurally novel inhibitor of mitochondrial complex I. Further analysis of the cell painting data indicated general trends in structure–phenotype relation, which could be subsequently confirmed in a structure–activity relationship study by a cell-based fluorogenic assay measuring the production of mitochondrial superoxide (MitoSOX Red assay).

Conclusions and outlook

Nature continues to be a source of inspiration for the design of novel bioactive compound collections. The CtD approach synthetically mimics enzymatic processes by chemically functionalizing and distorting NPs to structurally diverse compound collections. In BIOS, NPs inspire the synthesis of structurally simplified compound collections that are enriched in bioactivity. Nevertheless, the CtD and BIOS approaches may be limited due to their focus on specific NP scaffolds as starting points.

The pseudo-NPs principle aims to combine the biological relevance of NP-guided methods with the rapid chemical exploration of fragment-based ligand discovery through the deconstruction of NPs to fragments and their mixed reassembly to provide scaffolds that are unprecedented in nature. The three classes of pseudo-NPs discussed here provide support that pseudo-NPs may display novel and unexpected bioactivities that differ from the parent NPs. We expect that the design principle of pseudo-NPs will provide compound collections that will have significant value toward the exploration of biologically relevant chemical space, eventually disclosing novel molecular architectures eliciting potentially unprecedented modes of actions, which can be developed further to chemical probes or serve as privileged starting points in chemical biology and drug discovery projects.

Conflict of interest statement

Nothing declared.

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