Bioorganic & Medicinal Chemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Review

Biased and unbiased strategies to identify biologically active small molecules

Valentina Abet [a], Angelica Mariani [a], Fiona R. Truscott [a], Sébastien Britton [b], Raphaël Rodriguez [a,*]

[a] Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette, France
[b] Institut de Pharmacologie et de Biologie Structurale, CNRS and Université de Toulouse-Université Paul Sabatier, Equipe labellisée Ligue contre le Cancer, F-31077 Toulouse, France

ARTICLE INFO

Article history:
Received 21 February 2014
Revised 3 April 2014
Accepted 10 April 2014
Available online xxxx

Keywords:
Drug discovery
Dynamic combinatorial chemistry
In situ click chemistry
Fragment-based drug discovery
Diversity-oriented synthesis

ABSTRACT

Small molecules are central players in chemical biology studies. They promote the perturbation of cellular processes underlying diseases and enable the identification of biological targets that can be validated for therapeutic intervention. Small molecules have been shown to accurately tune a single function of pluripotent proteins in a reversible manner with exceptional temporal resolution. The identification of molecular probes and drugs remains a worthy challenge that can be addressed by the use of biased and unbiased strategies. Hypothesis-driven methodologies employs a known biological target to synthesize complementary hits while discovery-driven strategies offer the additional means of identifying previously unanticipated biological targets. This review article provides a general overview of recent synthetic frameworks that gave rise to an impressive arsenal of biologically active small molecules with unprecedented cellular mechanisms.

Contents

1. Introduction

Synthetic small molecules and natural products are key players in molecular medicine programs. Drug substances can perturb cellular processes underlying diseases, providing the means to reveal biological targets suitable for therapeutic intervention. Small molecules have been shown to accurately tune a single function of pluripotent proteins in a reversible and dose-dependent manner with temporal resolution that cannot be achieved with RNA silencing strategies. As such, forward chemical genetic approaches offer the additional means of identifying associated chemical hits suitable for drug development.[1]

The discovery of potent and selective agents remains a worthy challenge that can be addressed by the establishment of novel synthetic and screening methodologies. Combinatorial chemistry, a process designed to produce large libraries of closely related structural analogues, has emerged as a result of technological advancement associated to solid-phase synthesis.[2] The use of a solid support can increase reaction yields, facilitate purifications

* Corresponding author. Tel.: +33 169823031.
E-mail address: raphael.rodriguez@cnrs.fr (R. Rodriguez).

http://dx.doi.org/10.1016/j.bmc.2014.04.019
0968-0896/

and enable split-pool synthesis to mix and match reagents in a strategic manner, thereby generating a high number of compounds readily available for biological evaluation. Based on this, combinatorial chemistry has long been considered a powerful process in drug discovery programs.

Compounds from combinatorial libraries, however, remain structurally closely related with a common central core harboring appendages with a high degree of variability. While combinatorial chemistry can provide a useful starting point, the fairly limited chemical space covered by combinatorial libraries may not be sufficient to discover new biologically active structures.[3] Over the past two decades, several approaches have been introduced with the aim of covering biologically-relevant chemical space. This includes dynamic combinatorial chemistry (DCC),[4] in situ click chemistry,[5] fragment-based drug discovery (FBDD)[6] and diversity-oriented synthesis (DOS).[7] While these strategies rely on conceptually distinct principles, recent examples from the literature demonstrate that these methods display complementary features and can be strategically used to fulfill different purposes.

This article describes recent strategies implemented to accelerate the process of drug discovery and the production of small molecule probes to study biology. The development of such diverse approaches reflects the inherent difficulties chemical biologists and medicinal chemists are facing to identify new biologically active compounds. No method has proven to be a 'one size fits all' route. This review outlines some of the advantages and pitfalls of each methodology, illustrating relevant examples that helped identify compounds with unprecedented properties.

2. Target guided synthesis (TGS)

Target guided synthesis (TGS) represents a subset of combinatorial approaches designed to produce biologically active small molecules. These nature-inspired strategies rely on adaptive libraries in which the biological target itself is able to select the best small molecule binder, thus avoiding the difficult task of drug design, cost of individual synthesis, characterization and screening of each library component. In contrast to traditional combinatorial chemistry, TGS methods are inherently biased towards a pre-defined biological target that is used to select for small molecule binders. Therefore, the impact of such an approach highly depends on the choice of the biological target and whether its targeting leads to a phenotype that can be exploited for therapeutic benefits. TGS may be illustrated by two types of processes: the 'thermodynamic' approach, named *dynamic combinatorial chemistry* (DCC) and the 'kinetic' approach, among which *in situ click chemistry* has received considerable attention.[8]

2.1. Dynamic combinatorial chemistry (DCC)

Dynamic combinatorial chemistry (DCC), independently conceptualized in the mid-90s by Lehn and Sanders,[9] can be described as a chemical process taking advantage of the reversible nature of chemical bonds to drive the composition of a mixture of building blocks at steady-state to a different composition upon introduction of a bias to the mix (i.e. protein or nucleic acid targets).

To do so, a dynamic combinatorial library (DCL) can be obtained by mixing building blocks capable of undergoing reversible bond formation, thereby producing adducts in variable proportion. The distribution of each adduct relies on the initial composition of the mixture and intrinsic reactivity of each building block. The constituents present at any moment are just a subset of all those that are potentially accessible, hence defining a virtual library.[10] By subjecting the mixture to an external pressure, it becomes possible to drive the equilibrium and influence the product distribution. In particular, when the *stimuli* is an external template able to engage supramolecular interactions with specific members of the library, the change in product distribution can lead to the amplification, and thus to the identification of the best binder. This concept has been mainly exploited in two different settings: (i) 'substrate casting', where a biomolecule acts as a host for the assembly of the fittest ligand and (ii) 'receptor molding', where a small molecule acts as a guest for the optimal assembly of a synthetic receptor.[9b]

Huc and Lehn reported the first example of this concept in 1997, using carbonic anhydrase (CAII) as target.[9b] The library generated by DCC purposely included products with structural features close to known inhibitors; the positive outcome validated the method and outlined the basis of subsequent research in the field.

The design of a dynamic combinatorial systems depends on a set of basic principles, that can be divided in few key steps: (i) selection of building blocks, (ii) choice of the reversible chemistry for the generation of dynamic diversity and (iii) the external template that can 'trap' and amplify the best binder at the expense of the other members of the library.

To efficiently produce a DCL, building blocks must fit several requirements. Firstly, they should contain functional groups that can be engaged in reversible covalent or non-covalent interactions. Secondly, to avoid the bias imposed by the competition, it is important that all members of the library display a comparable reactivity. Finally, library members should be designed to interact with the target in the most diverse geometrical and functional ways. It is noteworthy that dynamic combinatorial selections must be carried out at physiological conditions, which limits the choice of reversible reactions at use. A desirable feature to implement in the system is a 'switch off' mechanism to freeze the exchange process and analyze the composition of the mixture after selection. This includes pH, temperature, solvent composition or the use of quenching reagents. As a result, organic reactions most frequently associated with DCC involves condensation reactions (e.g. imine and hydrazone exchange) and disulfide chemistry.

DCC relies on the generation of dynamic libraries in the presence of a template; if one of the products better interacts with the template, it will be subtracted from the equilibrating pool causing a redistribution of the mixture according to Le Chatelier's principle (Fig. 1, pathway A). The preparation of such libraries, known as 'adaptive libraries', is restricted by several factors such as the need to use mild equilibrating reaction conditions and stoichiometric amounts of template to achieve high amplification turn over. Alternatively, 'pre-equilibrated libraries' (Fig. 1, pathway B) can be generated and frozen in the absence of the template, then screened with standard assays. In this case, no amplification takes place. Here, identification of the active components may be achieved through dynamic deconvolution protocols, where sub-libraries are generated in the absence of one of the building blocks

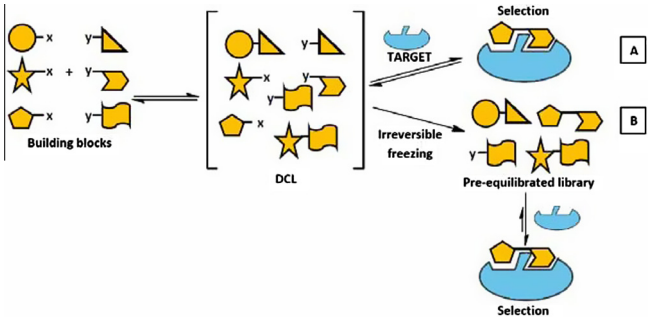

Figure 1. Dynamic combinatorial chemistry with (A) adaptative libraries and (B) pre-equilibrated libraries.