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MOLECULES

Recent developments in screening natural product combinatorial libraries

Screening a natural product based combinatorial library using FTICR mass spectroscopy

Fifteen new natural product-derived drugs were launched by the pharmaceutical industry between 2000 and 2003, highlighting the importance of natural products to drug discovery. An extension of synthetically derived combinatorial libraries is the use of natural product templates for the delivery of biologically relevant chemical libraries [1]. The carbonic anhydrase (CA) family of Zn(II) metalloenzymes catalyze the interconversion of CO_2 and HCO_3^-. This interconversion is a regulatory reaction that underpins many physiological processes associated with pH control, ion transport and fluid secretion [2]. Usually, an aromatic or heteroaromatic sulfonamide moiety ($ArSO_2NH_2$) is the primary recognition element for small molecules to bind the active site of CA [2].

Coordination of the ionized sulfonamide functional group with the active site Zn(II) of CA enables this protein–small molecule interaction [2]. The inhibition of CAs by aromatic sulfonamides has been exploited clinically for several decades for the treatment of a variety of conditions including glaucoma and gastric ulcers. More recently, a role for this class of compounds as anticancer agents has been identified, resulting from CA inhibition [3].

Recent work [4] has attempted to use a mixture-based screening methodology to reveal simultaneously both the presence and confirm the identity of any members from the synthesized natural product-based library based on the general template **(i)**, with affinity for bovine carbonic anhydrase II (bCAII). The methodology used bioaffinity characterization mass spectrometry (BACMS), originating from the seminal work of Smith and co-workers [5]. Workers here [4] found that the application of the BACMS screening technique identified a potent bCAII binder, found from random screening of a natural product-based synthetic library.

The mass spectral screening works as follows: electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) analysis of bCAII from a 10 mM NH_4OAc solution yielded the ESI-positive ion mass spectrum, acting as control free-protein spectra. Peaks corresponding to the +8 to +10 charge states of bCAII were observed, with the +9 charge state predominating. This charge state envelope (low charge states and few charge states) is typical for bCAII when in a compact, tightly folded structure [6]. A mixture of bCAII (30 μM) and the synthetic library component (30 μM of each compound, tested as singletons) in 10 mM NH_4Oac was incubated for 1 hour at room temperature and then analyzed by ESI-FTICR-MS under identical conditions to that for the free protein (above). The same charge state envelope as for free-protein bCAII was observed; however, if binding occurred between protein and ligand, each charge state consisted of a grouping of two peaks: a lower intensity peak that corresponded to unmodified bCAII and a more intense peak at a higher m/z value that corresponded to a bCAII–ligand complex.

In this way, identification of active compounds was possible. A solution phase competitive bCAII enzyme-binding assay validated the mass spectrometry screening results. From this mass spectral technique, the most potent compound identified was **(ii)**, which had a K_i of 77 nM when measured in the solution phase bCAII enzyme binding assay. This assay confirmed the original activity detected through mass spectral analysis.

This work is important because it applied bioaffinity characterization mass spectrometry (BACMS) to the analysis of a natural product-based combinatorial library in the presence of the protein target bCAII. ESI-FTICR-MS revealed and simultaneously identified a member from the natural product-based library mixture with affinity for bCAII. This mixture-based screening strategy permitted both a rapid and informative analysis, and enables an extension of this approach to bioactive-guided fractionation following high-throughput screening of natural product extracts.

Rational design of macrolides by virtual screening of combinatorial libraries generated through *in silico* manipulation of polyketide synthases

Many species such as bacteria and fungi produce secondary metabolites with diverse biological activities. Thus, these species represent a rich source of potentially valuable pharmacological agents [7]. Most drugs derived from secondary metabolites were discovered by random screening of biological samples, such as amphotericin and erythromycin [8]. Despite the benefits brought by high throughput screening, major limitations of these approaches still exist, such as: (1) focusing only on particular targets, which overlooks many useful kinds of biological activity, and (2) a low active:inactive molecule ratio among the compounds tested experimentally. Various strategies have been put forward for library design to increase the chance of finding active hits and leads. However, most of the existing target-based and ligand-based methods do not overcome the limitations described, and this is particularly true for libraries of natural compounds.

Recent work [9] has attempted to generate a general approach to hit finding based on *in silico* chemoinformatics applied to the rational design of secondary metabolites, coupled to their corresponding biosynthetic pathways. In essence, the approach attempts to predict and redesign the structures of bacterial secondary metabolites based on information known about their bio-

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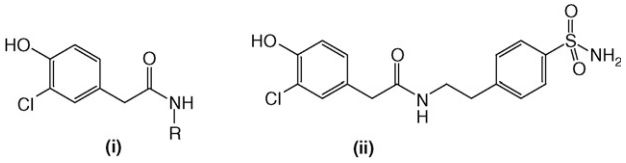
synthetic pathways. Biosynthesis of macrolides, governed by modular polyketide synthases (PKS), obeys certain rules that can be simulated *in silico*. PKS mode of action theoretically enables a huge number of macrolides to be produced upon combinatorial manipulation. Because engineering of all possible PKS variants is not possible, 'Biogenerator' software was developed that simulated manipulation of PKS to generate virtual libraries of macrolides. These libraries were screened with computer-aided prediction of biological activities, as exemplified by analysis of erythromycin and macrolactin libraries. This approach enabled rational selection of macrolides with desired biological activities and provided instructions regarding the composition of the PKS gene clusters necessary for the microbial production of such molecules. Erythromycin and macrolactin were chosen as examples by these workers [9] because compounds such as these are assembled biosynthetically by the modular polyketide synthase (PKS) enzymes.

The macrolides biosynthesized by bacteria and fungi display a wide range of activities, including antibacterial and antifungal activities [10]. As the presence of specific biological activity has a key role in the discovery of new leads, computer-aided prediction of biological activity can be used as a first filter for selecting the most interesting compounds in virtual combinatorial libraries of macrolides produced by the 'Biogenerator' software. When validating the approach with generation and *in silico* analysis of erythromycin analogues in a virtual library, for example, an *in silico* screen on a library of 285 erythromycin analogues with experimentally confirmed biological activities from the MDDR database (http://www.mdl.com) was undertaken. The results of this analysis shows that the *in silico* analysis failed to predict biological activities in only 18 of 471 cases, giving an accuracy of prediction of ~96%.

This work is of interest because it has designed and implemented *in silico* a new approach to generating and screening virtual macrolide libraries that can lead to discovery of new drug-like activities for these molecules. From this analysis and software developed, molecular biologists might be able to design and construct artificial PKS gene clusters for production of the most interesting molecules in bacteria. Validation of this approach performed on large numbers of macrolides known from the literature has demonstrated the reasonable accuracy of computer prediction. Further work is warranted to validate and develop this software approach with this and other biologically active systems.



1 Nicolaou, K.C. *et al.* (1998) Solid and solution phase synthesis and biological evaluation of combinatorial sarcodictyin libraries. *J. Am. Chem. Soc.* 120, 10814–10826
2 Scozzafava, A. and Supuran, C.T. (2001) Carbonic anhydrase inhibitors. *Curr. Med. Chem. Immun. Endocr. Metab. Agents* 1, 61–69
3 Scozzafava, A. *et al.* (2003) Anticancer and antiviral sulfonamides. *Curr. Med. Chem.* 10, 925–953
4 Poulsen, S.-A. *et al.* (2006) Screening a natural product-based combinatorial library using FTICR mass spectrometry. *Bioorg. Med. Chem.* 14, 510–515
5 Cheng, X. *et al.* (1995) Using electrospray ionization FTICR mass spectrometry to study competitive binding of inhibitors to carbonic anhydrase. *J. Am. Chem. Soc.* 17, 8859–8860
6 Smith, R.D. *et al.* (1997) New mass spectroscopic methods for the study of noncovalent associations of biopolymers. *Chem. Soc. Rev.* 26, 191–202
7 Koehn, F.E. and Carter, G.T. (2005) The evolving role of natural products in drug discovery. *Nat. Rev. Drug. Discov.* 4, 206–220
8 Bindseil, K.U. *et al.* (2001) Pure compound libraries; a new perspective for natural product based drug discovery. *Drug Discov. Today* 6, 840–847
9 Zotchev, S.B. *et al.* (2006) Rational design of macrolides by virtual screening of combinatorial libraries generated through *in silico* manipulation of polyketide synthases. *J. Med. Chem.* 49, 2077–2087
10 Omura, S., ed. (2002) *Macrolide Antibiotics: Chemistry, Biology, and Practice*, (2nd edn) Academic Press

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