Natural product pharmaceuticals

the third generation

Compounds derived from natural products have made a big impact on the pharmaceutical industry. Of the 1,010 NCEs approved between January 1981 and June 2006, 43 were unaltered natural products (NPs) and a further 232 (23%) were second generation NP derivatives[1]. The second generation compounds were primarily semi-synthetic NP analogues created to improve properties such as solubility and pharmacokinetics. Famous examples include the cancer drugs Taxol (paclitaxel) and its derivative Taxotere (docetaxel) and the antibiotics erythromycin and its derivatives Biaxin (clarythromycin) and Zithromax (azithromycin). This article discusses the exciting emergence of the third generation

By Dr Melanie McCullagh

The third generation of NPs relies upon bioengineering: using genetically altered producer organisms to generate libraries of analogue compounds with modifications that are difficult to make by synthetic organic chemistry. The resulting compounds retain the high potency typical of natural products, but have improved selectivity and toxicity profiles, and are more suitable for some new medical uses. As well as improved versions of approved pharmaceutical NPs, this approach of biosynthetic medicinal chemistry opens access to a huge range of NPs with potentially novel mechanisms of action which otherwise might not be on the top priority of development as pharmaceuticals.

Why natural product drug discovery?

NPs can have very high affinity to the target that they are naturally evolved to bind, very distinct from low-affinity synthetic fragments. Although NPs can be large in molecule size and complex in structure with multiple chiral centres and do not comply with the Lipinski's rule-of-five[2,3], these molecules tend to have well balanced conformational pre-organisation and flexibility. They bind to protein targets with little loss of entropy and in the meantime are bioavailable because of their flexibility of adopting different conformations in aqueous and lipophilic environments.

Furthermore, NPs seem to be able to address a different range of targets from combinatorial chemistry-derived small molecules. Calcineurin, for example, is the target of the major immunosuppressant drugs Sandimmune/Neoral (cyclosporine), Prograf/Protopic (tacrolimus, FK506) and Elidel (pimecrolimus). Even though cyclosporine was approved in 1983, there are still no approved small molecule inhibitors of calcineurin on the market. Targeting of mTOR is similarly dominated by rapamycin and its analogues.

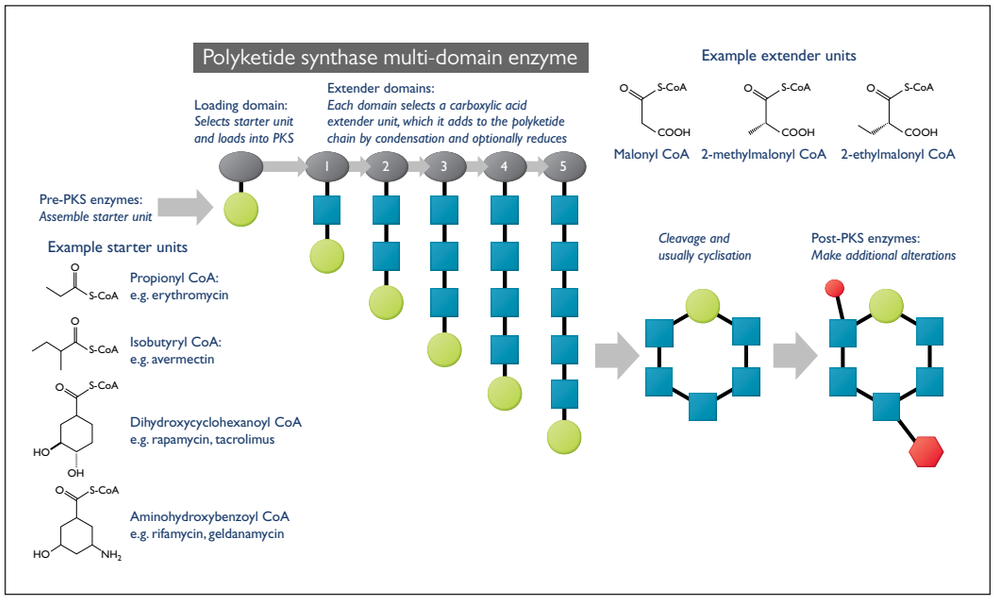

Figure 1
Schematic representation of the action of the polyketide synthase multi-enzyme and the associated pre- and post-PKS enzymes. Swapping, deleting or otherwise changing the enzymes at any stage in polyketide production results in a predictable change in the resulting polyketide molecule

The large size and complex structure of NPs may make them especially suitable for traditionally difficult targets, such as protein-protein interactions.

Polyketides

Polyketides are a particularly rich NP source of new pharmaceuticals, with nearly 50 approved products out of the 5-10,000 known polyketides[4]. In addition to anti-bacterials, the polyketides include anti-hyperlipidaemics, anti-cancers, immunosuppressants, anti-fungals and anti-helminthics. There are six recent blockbuster polyketide-based pharmaceuticals including three HMG-CoA reductase inhibitors [Mevacor (lovastatin), Pravachol (pravastatin) and Zocor (simvastatin)], two antibiotics [Biaxin (clarythromycin) and Zithromax (azithromycin)], and the immunosuppressant Prograf/Protopic (sirolimus). Peak sales of these six compounds alone totalled $15 billion.

Although polyketides are structurally diverse, they share common biosynthetic pathways (**Figure 1**). They are assembled in the producer organism by the PKS through stepwise condensation of carboxylic acids (CoAs). First, pre-PKS enzymes assemble a CoA 'starter unit', which is then selected by a 'loading module' of the PKS and loaded on to the PKS via a high-energy thioester linkage. Multiple extension modules in the PKS each contain a set of enzymes which collectively contribute to the addition of a 2-carbon unit to the growing chain of a polyketide. After the completion of the chain extension, the linear polyketide is usually cyclised and cleaved from the PKS resulting in a cyclic intermediate polyketide that may be further glycosylated or otherwise altered by post-PKS enzymes to form the final compound[5].

Bioengineering

Engineering or altering the biosynthetic pathways of NPs to generate novel analogues has been feasible for some time, and its use as a viable drug discovery tool has been further facilitated by advances in genome-sequencing technology. The reduction of time and effort required for genome sequencing has made it possible to gather much genetic information before bioengineering starts. Dr Jesus Cortez was a member of Professor Leadlay's research group in Cambridge University, which reported sequencing of the *Saccharopolyspora erythraea*[6] erythromycin gene cluster in 1990. He says that it took "a year of hard work from several scientists" to complete even a PKS gene cluster sequencing. Today, it takes a couple of days for the entire genome of a polyketide producer to be sequenced.

Bioengineering harnesses the natural flexibility of the PKS, using it to expand structural diversity. For example, the loading module of the PKS can be swapped with that of another organism to allow it to incorporate a wider range of starter acids. Some loading modules, such as that from avermectin, will even accept a range of synthetic starter acids if they are fed to the producer organism. ATs within the PKS can be swapped with those of another PKS to incorporate a different CoA. Post-PKS enzymes can also be added, subtracted or altered to introduce further structural diversity.

Combination of all bioengineering changes can result in a library of diverse analogues of a parent polyketide compound. A six-module type-I PKS could produce any of 100,000 different structures, taking into account the possibility of incorporation of different extender units, different stereochemistry and different oxidative states, even before post-PKS modification. This combinatorial approach makes drug discovery by bioengineering very complementary with conventional medicinal chemistry methods.

Kosan

One of the first companies to work on polyketide bioengineering was Kosan, a US biotechnology company which was acquired by Bristol-Myers Squibb for $190 million in June 2008. At its IPO in 2000, Kosan's portfolio was an excellent demonstration of how bioengineering one polyketide lead can result in multiple drug discovery programmes in completely different indications from the parent compound. It included three projects based on erythromycin (ketolide antibacterials, motilin agonists and mucus hypersecretion suppressants) and two based on the immunosuppressant FK520, one to create a more metabolically stable version and the other to engineer out calcineurin binding for use in neurodegenerative disorders. Kosan also had programmes in cancer, including epothilone and geldanamycin analogues, although the initial leads were semi-synthetic.

Biotica

Biotica is a private UK biotechnology company with a pipeline of therapeutic compounds based on bioengineering type I polyketides. The company's focus is on optimising polyketides in indications in which there is clinical validation for the mechanism of action. For example, it is creating new analogues of Astellas's Prograf (tacrolimus, FK506) for inhaled use in asthma, an indication for which tacrolimus has not been developed. Biotica is reducing the systemic exposure of the molecule, eg by minimising oral bioavailability, to alleviate the side effects associated with its systemic use.

Biotica's lead programme is on analogues of Wyeth's immunosuppressant Rapamune (sirolimus, rapamycin) (**Figure 2**). Biotica engineered a strain of the *Streptomyces hygroscopicus* producer organism to lack *rapK*, a pre-PKS gene involved in biosynthesis of the starter unit, and used it to produce a library of rapamycin analogues by feeding the new strain with synthetic starter acids. Compounds in this series were found to be more stable against metabolism by CYP3A and were more readily able to cross the blood brain barrier than rapamycin. One such compound, BC210, was shown to substantially reduce tumour growth in an orthotopic xenograft mouse model of glioblastoma multiforme, a tumour of particularly low median survival and high mortality.

The company has also used bioengineering to resolve issues that semi-synthetic chemistry had not previously been able to address in other programmes. Among the lead optimisation projects Biotica has been working on is an HSP90 inhibitor programme based on macbecin[7]. HSP90 is a chaperone protein that is involved in maintenance of various cancer-associated proteins including p53, HER2/neu, and a range of kinases. However, its activity is somewhat dependent on reduction of a quinone group to a hydroquinone by the NAD(P)H quinone oxidoreductase 1 (NQO1)[8]. Although hydroquinone analogues can be made synthetically, they are readily oxidised back to the quinone form in solution.

In macbecin biosynthesis, the quinone group is produced by post-PKS hydroxylation and oxidation of the phenol precursor. Biotica created a mutant version of their macbecin producer strain which lacked the post-PKS gene responsible for converting the phenol precursor to the quinone product. This resulted in a small library of macbecin compounds without the quinone moiety whose activity is independent of NQO1. One of the compounds was more than 300 times as potent as 17-AAG in Hsp90 inhibition and much better tolerated *in vitro* and *in vivo* than 17-AAG.

EntreChem

EntreChem was spun out of the University of Oviedo in 2005. It has a dual business strategy, with a service arm offering biotransformation and metabolite factories in addition to its drug discovery activities and has recently announced lead optimisation collaborations with Laboratoire Servier (2006) and Biomar SA (2007).

Francisco Moris, EntreChem co-founder and CEO, believes that natural products are especially good starting points for drug discovery because of their high potency, and that bioengineering can be used to eliminate toxicity and improve DMPK. He describes combinatorial biosynthesis as doing "what nature hasn't done yet" to create a pharmaceutical NCE.

EntreChem's internal programmes are focused on cancer drug discovery. The most advanced is based on an approved but now unused cancer treatment, mithramycin, an aureolic acid which inhibits SP-1 transcription factor and, thereby, inhibits c-myc and VEGF. Being a polyketide,

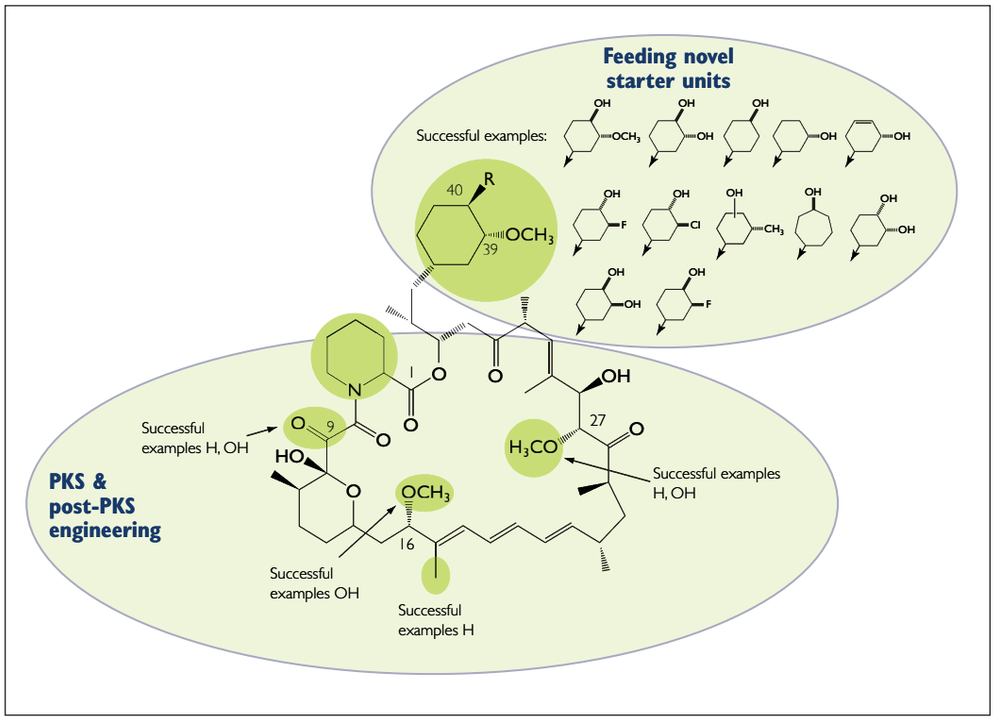

Figure 2
Transformations carried out by Biotica on rapamycin prior to the agreement with Wyeth. Any individual transformation can be combined with any or all of the others to build a substantial compound library

mithramycin is produced through sequential condensation of CoAs, then cyclised and glycosylated. Entrechem applied combinatorial biosynthesis to generate a library of analogue compounds with a variety of different glycosylations. The improved analogues include compounds with greater activity and 10-fold reduced toxicity. Early xenograft data show excellent activity, with tumour regression at doses well below MTD.

In its second programme, Entrechem started with staurosporine, an indolocarbazole NP which inhibits protein kinase C, but is too promiscuous for clinical use. Entrechem deciphered the gene cluster responsible for producing staurosporine and a related compound rebeccamycin. It then engineered a series of NCEs using a range of bioengineering techniques, including altering glycosylation patterns[9]. The result was a library of compounds with far higher selectivity and specificity for a range of disease-associated kinases. Potential lead compounds include an IKKb inhibitor with picomolar activity, for use in inflammation and cancer, and a highly selective JAK2 inhibitor for use in myeloproliferative disorders.

Novacta

Novacta is a UK biotech founded in 2003, with substantial bioengineering expertise. It specialises in lantibiotics, a novel class of ribosomal peptide anti-infectives. To bioengineer these peptides, Novacta generates libraries of plasmids containing different variants of the relevant gene. These are inserted into a knock-out version of the producer strain, generating a library of up to 19 analogues per substituted residue. Dr Jesus Cortez, who is now a scientist at Novacta, believes that ribosomal peptides have advantages over compounds like polyketides because a new compound will always be formed, even if it is incorrectly processed.

At Novacta, the focus of the bioengineering work is on optimising activity. Work begins with identifying the active regions of the peptide, which are not altered. The aim is to remove any barriers to target-binding in other areas. If the resultant bioengineered peptide has any DMPK issues, such as poor solubility, Novacta addresses them using medicinal chemistry.

Novacta's lead programme, NVB302 is a lantibiotic in development for the important emerging indication of *Clostridium difficile*. The lead candidate is currently undergoing pre-IND studies, supported by a Strategic Investment Grant from the Wellcome Trust. It is administered orally but is not absorbed, targeting the bacteria in the digestive tract without any risk of side effects. Lack of oral bioavailability, which would normally be a significant issue for an antibiotic has, in this case, been turned to advantage.

Competitiveness of NP drug discovery

Chemical space

One of the major benefits of using bioengineering to optimise NP leads is that it allows access to new chemical space. NPs offer a range of structures quite unlike those resulted from fragment-based approaches in that they offer much wider structural space for analogue preparation. Small synthetic molecules have more limited room for novel derivatisation, thus jamming the IP space. Bioengineering allows changes to be made in areas that are not practically achievable by semi-synthesis. For example, in Biotica's rapamycin programme, replacing the 6-membered cyclohexane ring with a 7-membered cycloheptane ring could not have been done semi-synthetically, so a compound containing that change would be both novel and patentable.

Bioengineering and semi-synthesis can be combined to expand structural diversity still further. For example, bioengineering can be used to add to otherwise untractable compounds a 'hook' for semi-synthetic derivatisation, forming the basis for an entire series of semi-synthetic derivatives.

The number of compound libraries generated by bioengineering may be smaller than those created through synthetic medicinal chemistry, but EntreChem's Dr Moris points out that the size of a compound library is not the key factor determining its value. He says: "Libraries may contain dozens of compounds rather than hundreds. But the diversity is very great, and the percentage of active compounds is very much higher than for a combinatorial synthesis or fragment based programme, so we can be much more confident of finding a clinical candidate."

Dr Moris acknowledges that it may take longer to start generating new compounds using bioengineering than for a medicinal chemistry drug discovery programme. He estimates that new compounds may be expected in a one to two year timeframe from project initiation. From this point on, new compounds can be generated at a multiplicative rate, through combinations of bioengineering techniques.

Dr Ming Zhang, Senior Vice President of R&D at Biotica, estimates that a fully staffed bioengineering project requires approximately six FTEs. It takes about 6-12 months to generate a set of genetic mutants of a producer strain with targeted changes. Dependent on bioengineering techniques employed, the first set of new analogues can be made approximately one to two months later. From that point, the analogue library expands rapidly, through combining PKS changes and feeding novel starter acids. "Quite distinct from a conventional medicinal chemistry lead optimisation project wherein typically 300-500 analogues are made before a development candidate is selected, we expect to select a candidate within a library of 40-50 analogues over an approximately 12-18 month period."

Conclusions

NPs are very valuable pharmaceutical leads due to their potency against often difficult targets, but they can suffer from issues such as poor PK, and their intractability to synthetic chemistry has hindered their use. The rapid advancement in molecular biology, such as rapid gene sequencing, has facilitated the wider application of bioengineering as a viable approach for NP lead optimisation to a development candidate. Due to their intrinsic structural diversity and assembly-line-like biosynthesis, polyketides are particularly appropriate for this approach, but other NPs, such as peptides, are also suitable.

NP lead optimisation can result in a substantial library of diverse compounds, engineered for improved selectivity, safety or physico-chemical properties. This opens the ability to revisit well-studied natural products for which optimisation programmes have in the past been unsuccessful. The aim can be either to find improved versions of compounds already being developed or to develop broader applications for analogues of known molecules, like Biotica's rapamycin analogues. Moreover, it is should be interesting to optimise novel NPs discovered through bioprospecting much earlier using bioengineering in parallel to using synthetic medicinal chemistry, and this could result in discovery of a new cohort of clinically and commercially valuable NPs. DDW

Dr Melanie McCullagh *is currently Business Development Director of Biotica Technology Ltd, a position she has held since April 2008. Dr McCullagh was previously at Antisoma, where she was instrumental in the 2007 licensing of ASA404 to Novartis in a deal worth $890 million, and was involved in establishing the Roche collaboration in 2002. She was also responsible for bringing new investigational cancer drugs into the company through in-licensing and collaborations, and for company strategic analysis. Prior to Antisoma, Melanie led the Strategy Analysis group in Datamonitor Healthcare, providing insight to leading pharmaceutical and biotechnology clients into areas such as forecasting, licensing, M&A and portfolio management. Melanie has an MA from Cambridge University, a DPhil from Oxford University and an MBA from London Business School.*

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