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REVIEW PAPER

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Drug discovery from natural products

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Abstract Natural product compounds are the source of numerous therapeutic agents. Recent progress to discover drugs from natural product sources has resulted in compounds that are being developed to treat cancer, resistant bacteria and viruses and immunosuppressive disorders. Many of these compounds were discovered by applying recent advances in understanding the genetics of secondary metabolism in actinomycetes, exploring the marine environment and applying new screening technologies. In many instances, the discovery of a novel natural product serves as a tool to better understand targets and pathways in the disease process. This review describes recent progress in drug discovery from natural sources including several examples of compounds that inhibit novel drug targets.

Keywords Drug discovery · Natural products · Drug targets · Screening · Secondary metabolism

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Introduction

Natural products continue as a source for innovation in drug discovery by playing a significant role in the discovery and understanding of cellular pathways that are an essential component in the drug discovery process. In many cases, natural products provide compounds as clinical/marketed drugs, or as biochemical tools that demonstrate the role of specific pathways in disease and the potential of finding drugs. Numerous reviews have been written that describe the importance of compounds derived from microbes, plants and animal sources to treat human diseases [3, 4, 14, 23, 24]. In the areas of cancer and infectious disease, 60 and 75%, respectively, of new drugs, originate from natural sources. This review will focus on current strategies employed to discover new natural products using recent advances in the understanding of genetic pathways for secondary metabolite production, under-explored sources of natural products and novel screening technologies. Examples of the essential role that natural product compounds play in the understanding of the basic science and development of novel therapeutics are described. This review summarizes presentations from a symposium, Drug Discovery from Natural Products, held at the SIM Annual Meeting 2005.

Genetic pathways of secondary metabolism

In order to discover novel chemical entities, Ecopia Biosciences is applying genomics and bioinformatics to actinomycetes. By identifying gene clusters that represent potential biosynthetic pathways and applying bioinformatics, partial or complete structures of compounds can be predicted. Their database consists of 55,000 genes and over 1,400 biosynthetic loci. Generally 10–20 gene clusters that code for secondary metabolites are present per actinomycete strain. After identifying gene clusters that code for potential compounds of

interest, the actinomycete is grown in a wide variety of media and conditions to express the predicted compounds, which can be identified in crude extracts by virtue of the predicted structure. The compound or compounds are isolated and evaluated for biological activity. Fermentation conditions are optimized to express larger quantities of compound when desirable activity is discovered. Ecopia has successfully used this approach to identify ECO-4601, diazepinomicin.

ECO-4601 is a novel farnesylated dibenzodiazepinone produced by *Micromonospora* strains (Fig. 1) [5]. This compound, containing a unique dibenzodiazepinone, has antibacterial, anti-inflammatory and antitumor activity. The structure of ECO-4601 is closely related to the natural product anthramycin. Biosynthetic studies of anthramycin demonstrated that tryptophan by conversion to 3-hydroxy-anthranilate, methionine and tyrosine are biosynthetic precursors [26]. Analysis of the biosynthetic locus for ECO-4601 predicts that this compound is derived from 3-hydroxy-anthranilate, 5-amino-benzene-1,3-diol and a non-cyclic terpenoid chain. Feeding experiments with isotopic 3-hydroxy-anthranilate resulted in the expected biosynthetic precursor incorporation, confirming this as a biosynthetic precursor.

ECO-4601 is in preclinical development as an oncology drug. The compound has broad spectrum cytotoxic activity and has demonstrated in vivo activity against glioma, breast and prostate cancer mouse models. Fermentation production yield has been optimized from initial titers of 1–3 mg/l to >300 mg/l through media optimization, UV mutation and selection of streptomycin resistant clones. Purification is straightforward and GLP/GMP compound has been produced. ECO-4601 is scheduled for IND filing later this year.

Under-explored sources of natural products

The marine environment is a largely untapped source of chemical diversity. The opportunity to discover new species and therefore, new chemical diversity has increased through the application of new tools to explore the marine environment. This new chemical diversity combined with our current understanding of disease processes improves the ability to discover compounds with therapeutic utility. Nereus Pharmaceuticals is exploring marine microbes for novel therapeutic compounds and has discovered the novel anticancer compounds, NPI-0052 [9, 20] and NPI-2358 [25], and the antibacterial compounds, NPI-3114 and NPI-3304.

NPI-0052, salinosporamide A, (Fig. 2) is a novel β-lactone-γ-lactam isolated from the fermentation broth of *Salinispora tropica*, a new marine actinomycete. NPI-0052 demonstrates potent cytotoxicity in the NCI 60 tumor cell line panel with a mean GI_{50} of <10 nM by inhibiting chymotrypsin-, trypsin-, and caspase-like proteasome activities [6]. Proteasomes regulate the levels of proteins that are important for cell-cycle control and apoptosis such as cyclins, NF-kB, BCL2 and caspases. Inhibition of proteasome activities has been demonstrated to be an effective cancer target with the introduction of Bortezomib/Velcade(/PS-341 for the treatment of multiple myeloma. NPI-0052 has demonstrated activity against Velcade-resistant multiple myeloma cells, is less toxic to normal cells and has demonstrated oral and intravenous (i.v.) activity in several tumor models [6]. An IND has been filed in 2005.

NPI-0052 is not amenable to commercial synthesis and is being prepared by large scale fermentation. The original fermentation titer for NPI-0052 in shake flasks was 4 mg/l. Media optimization, resin addition and single colony isolation has optimized yields to 350 mg/l in a laboratory fermentor. A 1,500 l GMP fermentation has achieved a titer of 240 mg/l demonstrating a successful industrial-scale saline fermentation to produce NPI-0052 for preclinical and clinical studies.

NPI-2350, (-) halimide, (Fig. 3) is a novel diketopiperazine isolated from a marine *Aspergillus sp*. The IC_{50} of NPI-2350 in the HT-29 cytotoxicity assay is 440 nM. Synthesis of 110 analogues of this compound

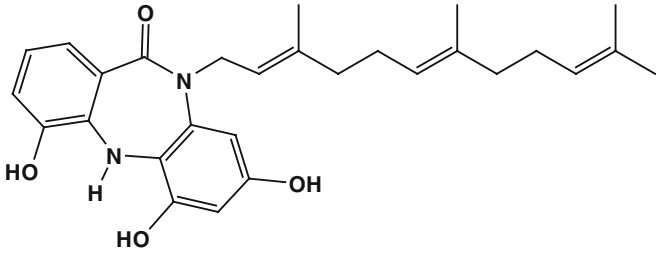


Fig. 1 Structure of ECO-4601, diazepinomicin, a novel antitumor compound from an actinomycete

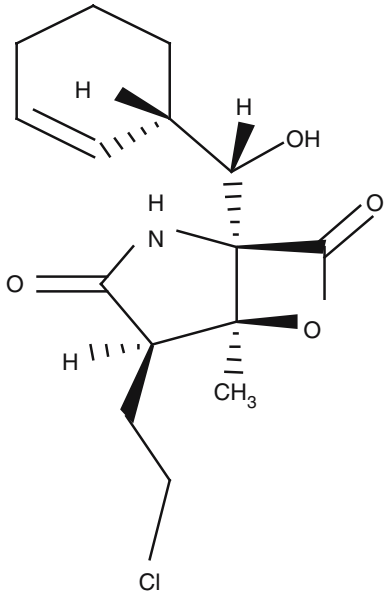


Fig. 2 Structure of NPI-0052, salinosporamide A, a novel proteasome inhibitor from a marine actinomycete

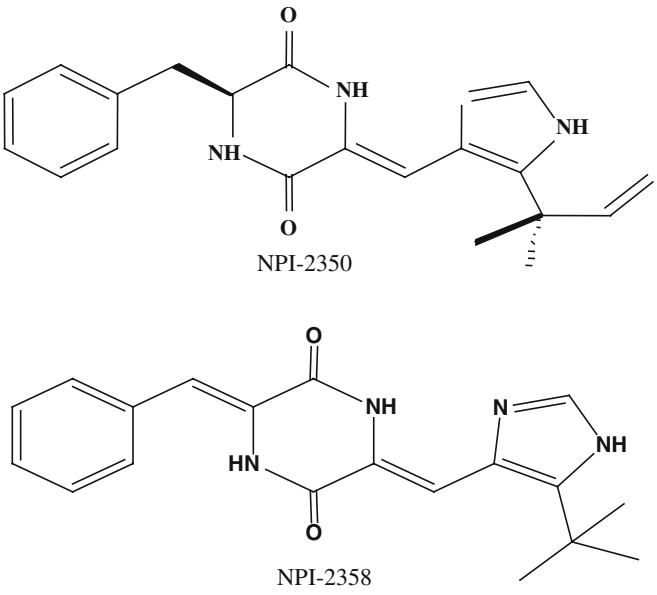


Fig. 3 Structures of NPI-2350 [(-) halimide] and synthetic analogue, NPI-2358, antitumor compounds originating from a marine fungus

yielded a novel analogue, NPI-2358, (Fig. 3) with an IC50 of 18 nM. NPI-2358 causes tubulin depolymerization by binding to the colchicine binding site, disrupting the structural integrity of the tumor vasculature and tumor cells. This mechanism results in necrosis of tumors due to loss of blood supply and apoptosis due to a direct effect on tumor cells. NPI-2358 has demonstrated tumor regression in a breast tumor (N202) dorsal skinflap model. An IND has been filed in 2006.

NPI-3114 (Fig. 4), a chlorinated bisindole pyrrole, and NPI-3304 (Fig. 4), a 4-oxazolidinone are antibacterial compounds isolated from novel marine actinomycetes. The three dimensional structure of NPI-3114 is distinct from the classic indolocarbazole, staurosporine, and therefore has distinct biological properties. NPI-3304 is structurally related to the antibiotic, linezolid (Fig. 4), an antibacterial compound previously discovered and developed directly from a synthetic chemistry program [11]. NPI-3114 and NPI-3304 have broad Gram positive antibacterial activity, including activity against methicillin resistant S*taphylococcus aureus*, and no hemolytic or cytotoxicity against normal fibroblasts. The compounds are in early preclinical studies.

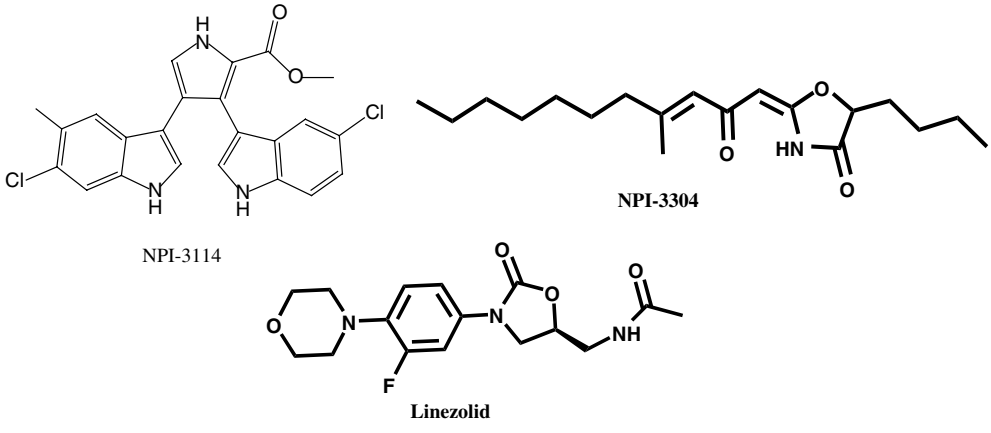
Novel screening technologies

The ability to discover natural products for drug discovery requires sensitive and robust assays that can be run in high throughput. Cetek Corporation and Cubist have developed novel high throughput screening technologies that optimize natural product drug discovery.

Cetek has developed a capillary electrophoresis (CE)-based screening platform that allows access to challenging and conventional drug targets and interfaces with all small molecule drug sources [1, 21, 28]. Due to its high-resolution separation component, CE is able to identify active compounds in natural product extracts containing background fluorescence that interferes with other assay formats. Assays can be developed that measure binding (affinity) of compounds to targeted proteins, functional activity or displacement of a known ligand. In its affinity-based binding format, CE can detect a shift in protein mobility when a ligand binds to a protein due to a change in its conformation and surface charge. CE can distinguish between weak and strong binding compounds in extracts prior to determining their concentration. This is particularly important for prioritizing extracts for isolation chemistry early in the screening process.

This sensitive and robust high throughput screening CE technology has been applied to Cetek's internal drug discovery programs in cancer and infectious disease. The cancer target, HSP90 is a molecular chaperonin that is responsible for maintaining the correct folding and stability of over 100 client proteins including many proteins in cancer survival, such as the estrogen receptor, androgen receptor, HIF-1(, Raf-1, Akt/PKB, Cdk4, and ErbB2 [22, 29]. Inhibition of HSP90 leads to degradation of the client proteins resulting in cell death (apoptosis).

Fig. 4 Structures of NPI-3114, NPI-3304 and linezolid, antibacterial compounds



HSP90 exists in a high affinity, activated protein complex in cancer cells. This activated complex is less prevalent in normal cells and therefore, can yield a therapeutic window for inhibitors. The natural products geldanamycin, an ansamycin type compound, and the macrolide radicicol, are known inhibitors of HSP90. NCI/Kosan have progressed 17-allylaminogeldanamycin to Phase II clinical trials. In Phase I clinical trials dose limiting hepatotoxicity has been observed. Several other analogues of geldanamycin are also in preclinical evaluation including 17-dimethylaminoethylamino-17-demethoxygeldanamycin, IPI-504 and CNF-1010. The radicicol analogues, cycloproparadicicol and radicicol oximes are in preclinical development. Several synthetic inhibitors, including PFU24FCl and CCT-018159 are also in preclinical development.

A competitive CE assay was developed which identified hits by monitoring the displacement of fluoresceinated geldanamycin (Fl-GM) from a Fl-GM/HSP90 complex (Fig. 5). Using this assay a natural product compound unrelated to geldanamycin or radicicol was identified that inhibited HSP90 with an $IC_{50} = 800$ nM, demonstrated potent cytotoxicity against several tumor cell lines and showed tumor growth delay in ovarian cancer and leukemia mouse models. A synthetic program is underway to improve the biological properties of this compound.

For a cellular HIV target, a CE assay was developed in the binding format to search for natural product ligands (Fig. 6). A compound was identified that potently inhibits the target with an $IC_{50} = 5$ nM and is active against a broad range of HIV isolates (including a multidrug resistant isolate) with IC50s ranging between 1 and 133 nM and low cytotoxicity of 30 uM in peripheral blood mononuclear cells (PBMCs). Analogue synthesis is in progress to optimize this compound.

Natural product derived compounds represent 78% of commercial antibacterials. There is a continuing need to discover novel antibacterials since antibiotic resistance by pathogens will ultimately limit the utility of marketed antibiotics [18, 19, 27]. The difficulty in discovering new natural product antibacterials by directly screening for antibacterial activity as opposed to molecular targets results from the high frequency and high titer of known compounds and the low frequency and/or titers of novel compounds. This problem is difficult to overcome by current chemical dereplication processes. Cubist is addressing this problem by the development of a modified Gram negative strain that incorporates multiple resistance markers (CM400) to

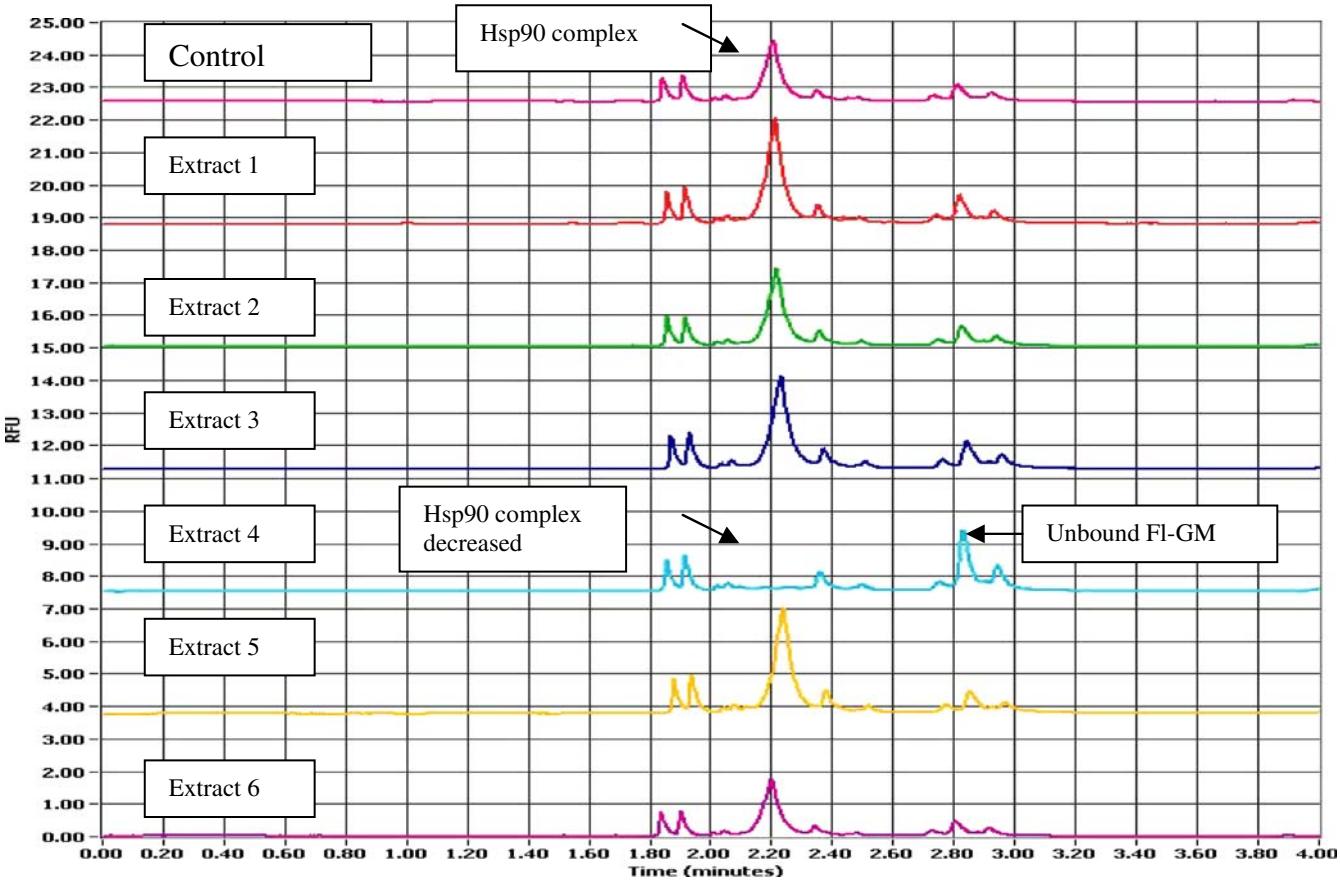


Fig. 5 Capillary electrophoresis electropherograms showing the HSP90 fluoresceinated geldanamycin complex and inhibition of the complex by a compound in extract 4. Extracts 1–3, 5 and 6 are inactive

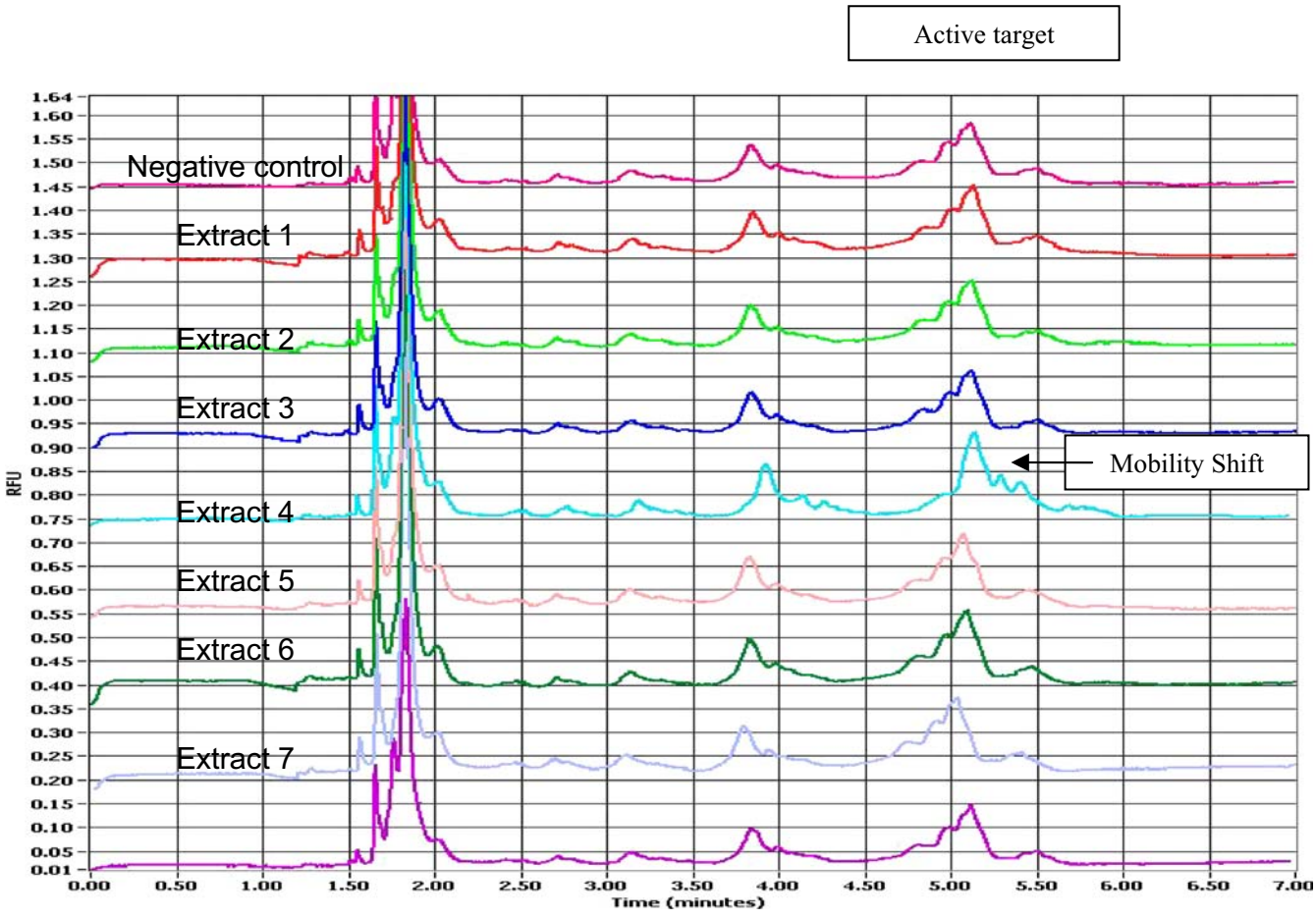


Fig. 6 Capillary electrophoresis electropherograms showing a fluoresceinated HIV target protein (negative control) and a mobility shift observed when a compound binds to the protein in extract 4. Extracts 1–3 and 5–7 are inactive

reduce detection of previously identified compounds (Fig. 7). In addition, a strain with increased permeability (CM435) was derived from CM400 to maximize sensitivity to antibacterial compounds (Fig. 7). These unique strains can be utilized to screen diverse environmental samples at higher rates than other technologies which allows for screening of rarer organisms.

To achieve higher screening rates, Cubist has designed a protocol that encapsulates individual environmental microorganisms, actinomycetes, in macrodroplets (alginate beads), cultivates and tests for antibacterial activity of metabolites against CM400 or CM435. A routine production run is approximately 250,000 macrodroplets; therefore throughput can exceed 10 million screening events per year. This process has resulted in the screening of over 8 million actinomycetes for antibacterial activity. Optimized microbial isolation and physiology, fermentation, bioactivity and chemical profiling have resulted in an overall chemical follow-up rate of 0.001%. This has resulted in a discovery rate of 17:1, known to novel compounds. Based on these early results it is clear that natural product sources, specifically the actinomycetes, will continue to provide interesting, biologically active chemical scaffolds for clinical development.

Novel drug targets and natural product inhibitors

Novartis has remained committed to natural product innovation in their drug discovery efforts. This commitment has lead to the discovery of new targets and pathways in the disease process as well as useful clinical compounds. Some results are highlighted below.

The US market for antibacterials is dominated by six antibacterials, the β-lactams (Rocephin®, Augmentin®), macrolides (Zithromax®, Biaxin®), and fluoroquinolones (Cipro®, Levoquin®). This limited number of structural classes in combination with ineffective management of drug usage makes this therapeutic area vulnerable to the emergence of resistant organisms. For example, resistance against glycopeptide antibiotics like vancomycin or teicoplanin is observed with increasing frequency and resistance has emerged within one year for the recently introduced oxazolidinones (WHO Report on Infectious Disease 2001). Novartis and Vicuron

Fig. 7 The sensitivity of the parent strain, MG-1655, and the modified strains CM400 and CM435 to a broad range of antibiotics. (D. Ritz, C. Monahan, C. Murphy, J. Silverman, D. Baker and R.H. Baltz, unpublished data)

		MIC (μg/mL)			
Genetic Mutation Conferring Resistance	**Compound**	**MG1655**	**CM400**	**CM435**	**Target/MOA**
dhfr1	Trimethoprim	0.13	> 400	> 400	DHFR
aph	Tobramycin	≤ 0.25	> 256	ND	protein synthesis
aph	Dibekacin	0.5	> 256	ND	protein synthesis
aph	Netilmycin	≤ 0.25	128	ND	protein synthesis
aph	Gentamicin	0.5	128	128	protein synthesis
cat	Chloramphenicol	2	256	256	protein synthesis
aph	Kanamycin	2	> 256	256	protein synthesis
rpsL	Streptomycin	2	> 256	256	protein synthesis
sat	Streptothricin	2	256	256	protein synthesis
tetA	Tetracycline	2	> 256	64	protein synthesis
ble	Bleomycin A2	1	> 64	8	DNA interaction
bla	Ampicillin	8	> 256	> 256	cell wall
gyrA	Nalidixic Acid	8	> 256	> 256	gyrase
rpoB	Rifampin	8	> 256	16	RNA Polymerase
aadA	Spectinomycin	8	> 256	> 256	protein synthesis
aac	Apramycin	2	64	64	protein synthesis
fhuA	Albomycin	low	high	high	

are developing novel compounds active against a new target, peptide deformylase, an essential protein that is unique to bacteria and conserved across several bacterial species [12].

Screening of natural product samples against this target has resulted in the discovery of a novel compound, NVP-LBM-415 (Fig. 8), a derivative of the natural product actinonin, having a Ki of 0.3 nM. The compound has potent antibacterial activity, is orally bioavailable and has good tissue distribution. Phase I studies are in progress.

Vascular cell adhesion molecule-1 (VCAM-1) plays a critical role in inflammatory conditions, regulating the leukocyte migration and cell–cell interactions. Proteins, including VCAM-1, are synthesized on the ribosome with a signal peptide sequence. This signal peptide sequence is recognized by the signal recognition particle (SRP) for translocation. A fungal metabolite, HUN-7293, has been identified at Novartis (Fig. 9) that selectively prevents translocation of VCAM-1 to the luminal side of the endoplasmic reticulum. This occurs via inhibiting the interaction of the signal peptide of VCAM-1 with Sec61β complex, a highly conserved protein-conducting channel [2, 7, 10]. Signal peptide dependent blocking of translocation is possible without affecting the translocation of other proteins, offering new therapeutic possibilities.

Although some natural products cannot be developed into drugs, natural products play a significant role as tools for pathway validation and are primary compound candidates to interrupt protein–protein interactions. For example, the WNT pathway plays a significant role in cell survival, proliferation and differentiation and mutations in B-catenin, a protein in the pathway, is associated with colon cancer. B-catenin interacts with T cell factor (Tcf) transcription factors. A screen for

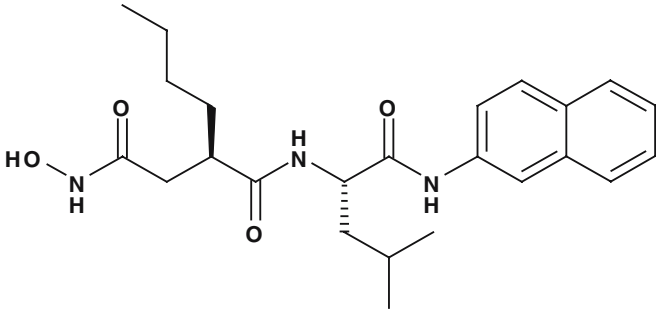


Fig. 8 Structure of NVP-LBM-415, a novel antibacterial with potent peptide deformylase activity

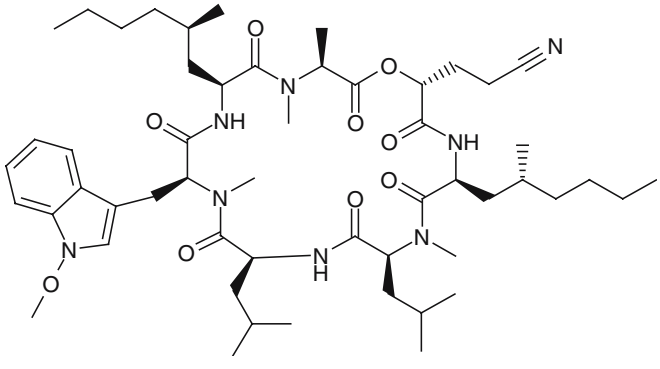


Fig. 9 Structure of HUN-7293, a novel anti-inflammatory that blocks VCAM-1 cellular translocation

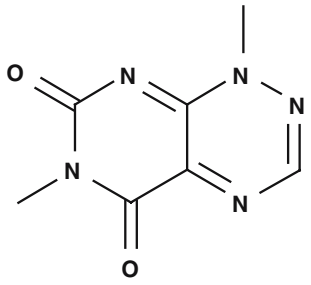


Fig. 10 Structure of toxoflavin, inhibitor of the B-catenin:T cell factor transcription factor complex

inhibitors at Novartis of this protein–protein interaction yielded the natural product toxoflavin (Fig. 10). Treatment of *Xenopus sp*. with mRNA of B-catenin produced an abnormal body type. This transformation was reversed with toxoflavin [17].

Hypoxic tumor cells are resistant to conventional chemotherapy and radiation. There is a positive link between hypoxia inducible factor-1 (HIF-1) levels and increased patient mortality. Screening for inhibitors at Dana-Farber Cancer Institute and Harvard Medical School of the protein–protein interaction of HIF-1 with the CH1 domain of p300 identified chetomin, a natural product inhibitor (Fig. 11). Systemic administration of chetomin inhibited hypoxia-inducible transcription within tumors and inhibited tumor growth [16], validating this cancer target.

Histones are key elements of gene expression via alteration of the chromatin architecture. The conformation of histones are regulated by acetylation. Dysregulation of histone deacetylases is associated with several cancers [15]. Inhibition of histone deacetylase results in hyperacetylation and increased transcription of genes that inhibit cell growth. Figure 12 shows several

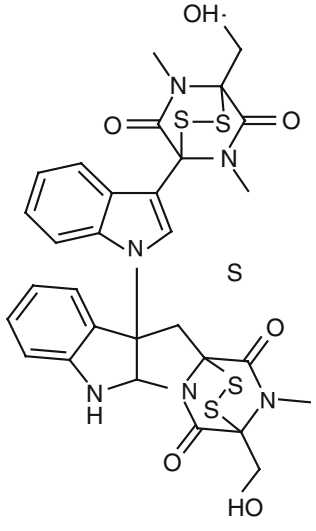


Fig. 11 Structure of chetomin, an antitumor compound which inhibits formation of the HIF-1:P300 complex

natural products discovered and several derivatives that are in preclinical and clinical development.

Natural product compounds in many cases have biological properties that lead to the discovery of unique biology. Myriocin (Fig. 13) is a metabolite of the ascomycete, *Isaria sinclairii*, with potent immunosuppressive and anti-proliferative activity. The utility of myriocin was limited because of severe GI side effects. An analogue, FTY720, was synthesized that removed the serine-palmitoyl-transferase activity retained the immunosuppressive activity and eliminated the GI toxicity [8, 13]. The mechanism of action involved sequestration of lymphocytes from the blood to lymphatics which represents an unprecedented mode of action. FTY720 is being developed by Novartis and in Phase II clinical trials has demonstrated the potential to be the first oral treatment for multiple sclerosis.

The discovery of FK-506, as a novel immunosuppressive agent with a new mode of action, has led to the introduction of several new drugs in the marketplace, Prograf® (tacrolimus) for transplant patients and recently, Elidel ® Cream. The striking property of Elidel ® Cream, containing pimecrolimus a derivative of FK-506, is the compounds remarkably high affinity for skin. Elidel ® Cream is used to treat atopic dermatitis, including the severe Netherton syndrome.

Conclusions

Natural products have played a significant role in drug discovery. Over the past 75 years, natural product derived compounds have led to the discovery of many drugs to treat human disease. This review describes recent enabling technologies and natural product compound discoveries in the therapeutic areas of cancer, antibacterials and antivirals, and immunosuppressives. These natural products are being developed to improve cancer therapy, to treat resistant bacterial and viral infections and to expand immunosuppressive therapy to diseases such as multiple sclerosis. The recent introduction of Elidel ® Cream for eczema in the marketplace and the antibiotics, Cubicin ® and Tygacil ®, are examples of how improved products derived from natural products can be successful.

Natural product compounds not only serve as drugs or templates for drugs directly, but in many instances lead to the discovery of novel biology that provides a better understanding of targets and pathways involved in the disease process. Compounds that interact with novel targets, such as, the protein–protein complexes, B-catenin in the WNT pathway and HIF-1/p300, have validated these anticancer targets and pathways. These compounds create opportunities for further drug targets to be identified and exploited in these pathways.

In order for natural product drug discovery to continue to be successful, new and innovative approaches are required. Some of these new approaches include the use of advances in genomics, searching for natural

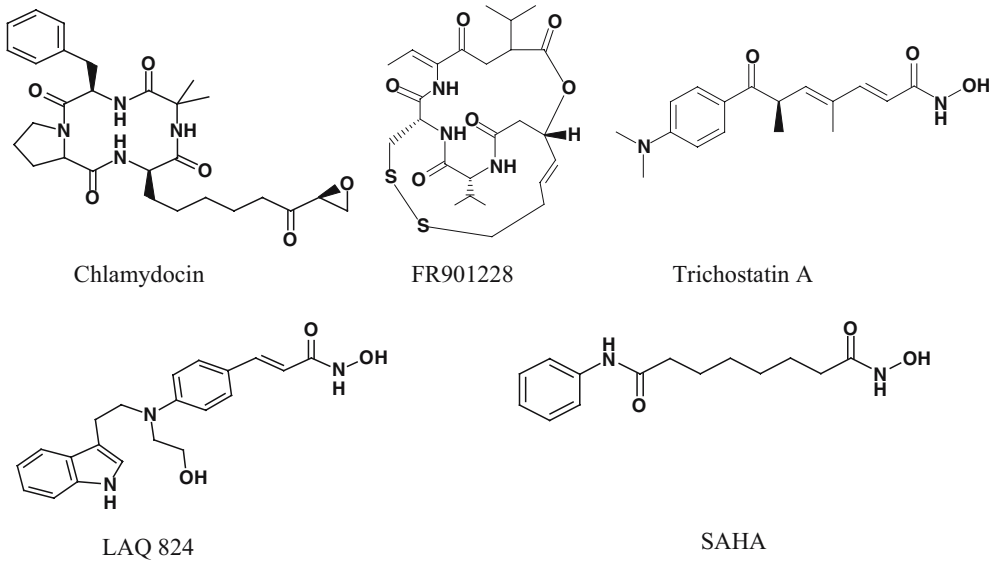


Fig. 12 Structures of natural product and derivatives that inhibit histone deacetylase

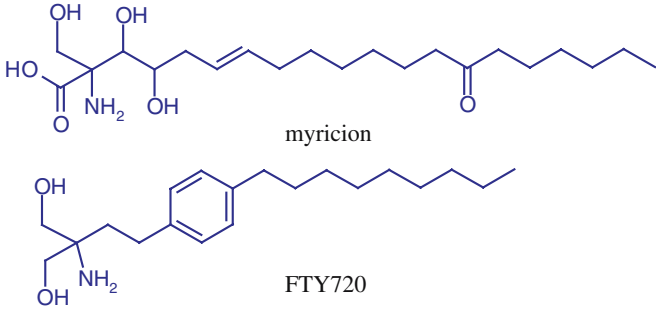


Fig. 13 Structures of myriocin and FTY720

product compounds in environments that have not been efficiently mined in the past and applying new screening technologies. This review illustrates numerous examples of recent discoveries that demonstrate the continuing innovation that can result from natural product drug discovery progress.

References

1. Belenky A, Hughes D, Korneev A, Dunayevskiy Y (2004) Capillary electrophoretic approach to screen for enzyme inhibitors in natural extracts. J Chromatogr A 1053(1–2):247–251
2. Besemer J, Harant H, Wang S, Oberhauser B, Marquardt K, Foster CA, Schreiner EP, de Vries JE, Dascher-Nadel C, Lindley IJ (2005) Selective inhibition of cotranslational translocation of vascular cell adhesion molecule 1. Nature 436(7048):290–293
3. Butler MS (2004) The role of natural product chemistry in drug discovery. J Nat Prod 67:2141–2153
4. Butler MS (2005) Natural products to drugs: natural product compounds in clinical trials. Nat Prod Rep 22:162–195
5. Charan RD, Schlinghann G, Janso J, Bernan V, Feng X, Carter GT (2004) Diazepinomicin, a new antimicrobial alkaloid from a marine *Micromonospora sp*. J Nat Prod 67(8):1431–1433
6. Chauhan D, Catley L, Li G, Podar K, Hideshima T, Velankar M, Mitsiades C, Mitsiades N, Yasui H, letai A, Ovaa H, Berkers C, Nicholson B, Chao T-H, Neuteboom STC, Richardson P, Palladino M, Anderson KC (2005) A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. Cancer Cell 8:407–419
7. Cheng Z, Jiang Y, Mandon EC, Gilmore R (2005) Identification of cytoplasmic residues of Sec61p involved in ribosome binding and cotranslational translocation. J Cell Biol 168(1):67–77
8. Chiba K (2005) FTY720, a new class of immunomodulator, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors. Pharmacol Ther 108(3):308–319
9. Feling RH, Buchanan GO, MincerTJ, Kauffman CA, Jensen PR, Fenical W (2003) Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus salinospora. Angew Chem Int Ed Engl 42(3):355–357
10. Garrison JL, Kunkel EJ, Hegde RS, Taunton J (2005) A substrate-specific inhibitor of protein translocation into the endoplasmic reticulum. Nature 436(7048):285–289
11. Gravestock MB (2005) Recent developments in the discovery of novel oxazolidinone antibacterials. Curr Opin Drug Discov Devel 8(4):469–477
12. Jain R, Chen D, White RJ, Patel DV, Yuan Z (2005) Bacterial Peptide deformylase inhibitors: a new class of antibacterial agents. Curr Med Chem 12(14):1607–1601
13. Kahan BD (2004) FTY720: from bench to bedside. Transplant Proc 36(2 Suppl):531S–543S
14. Koehn FE, Carter GT (2005) The evolving role of natural products drug discovery. Nat Rev 4:206–220
15. Kristeleit R, Stimson L, Workman P, Aherne W (2004) Histone modification enzymes: novel targets for cancer drugs. Expert Opin Emerg Drugs 9(1):135–154
16. Kung AL, Zabludoff SD, France DS, Freedman SJ, Tanner EA, Vieira A, Cornell-Kennon S, Lee J, Wang B, Wang J, Memmert K, Naegeli HU, Petersen F, Eck MJ, Bair KW, Wood AW, Livingston DM (2004) Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. Cancer Cell 6(1):33–43
17. Lepourcelet M, Chen YN, France DS, Wang H, Crews P, Petersen F, Bruseo C, Wood AW, Shivdasani RA (2004) Small-molecule antagonists of the oncogenic Tcf/beta–catenin protein complex. Cancer Cell 5(1):91–102

18. Levy SB, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10(12 Suppl):S122–S129
19. Livermore DM (2004) The need for new antibiotics. Clin Microbiol Infect 10(Suppl 4):1–9
20. Macherla VR, Mitchell SS, Manam RR, Reed KA, Chao TH, Nicholson B, Deyanat-Yazdi G, Mai B, Jensen PR, Fenical WF, Neuteboom ST, Lam KS, Palladino MA, Potts BC (2005) Structure–activity relationship studies of salinosporamide A (NPI-0052), a novel marine derived proteasome inhibitor. J Med Chem 48(11):3684–3687
21. Mullady EL et al (2004) A phthalide with in vitro growth inhibitory activity from an oidiodendron strain. J Nat Prod 67(12):2086–2099
22. Neckers L, Neckers K (2002) Heat-shock protein 90 inhibitors as novel cancer chemotherapeutic agents. Expert Opin Emerg Drugs 7(2):277–288
23. Newman DJ, Cragg GM, Snader KM (2000) The influence of natural products upon drug discovery. Nat Prod Rep 17:215–234
24. Newman DJ, Cragg GM, Snader KM (2003) Natural products as sources of new drugs over the period 1981–2002. J Nat Prod 66:1022–1037
25. Nicholson B, Lloyd GK, Miller BR, Palladino MA, Kiso Y, Hayashi Y, Neuteboom STC (2006) NPI-2358 is a tubulin-depolymerization agent: in vitro evidence for activity as a tumor vascular-disrupting agent. Anticancer Drugs 17(1):25–31
26. Ostrander JM, Hurley LH, McInnes AG, Smith DG, Walter JA, Wright JL (1980) Proof for the biosynthetic conversion of L-[indole-15 N]tryptophan to [10–15 N]anthramycin using (13C, 15 N) labelling in conjunction with 13C-NMR and mass spectral analysis. J Antibiot (Tokyo) 33(10):1167–1171
27. Overbye KM, Barrett JF (2005) Antibiotics: where did we go wrong? Drug Discov Today 10(1):45–52
28. Pierceall WE et al (2004) Affinity capillary electrophoresis analyses of protein–protein interactions in target-directed drug discovery. Methods Mol Biol 261:187–198
29. Whitesell L, Lindquist SL (2005) HSP90 and the chaperoning of cancer. Nat Rev Cancer 5(10):761–772