David and Goliath: chemical perturbation of eukaryotes by bacteria

Louis K. Ho · Justin R. Nodwell

Received: 6 August 2015 / Accepted: 9 September 2015 / Published online: 3 October 2015
© The Author(s) 2015. This article is published with open access at Springerlink.com

Abstract Environmental microbes produce biologically active small molecules that have been mined extensively as antibiotics and a smaller number of drugs that act on eukaryotic cells. It is known that there are additional bioactives to be discovered from this source. While the discovery of new antibiotics is challenged by the frequent discovery of known compounds, we contend that the eukaryote-active compounds may be less saturated. Indeed, despite there being far fewer eukaryotic-active natural products these molecules interact with a far richer diversity of molecular and cellular targets.

Keywords Actinomycetes · Eukaryotes · Target diversity

Introduction

Actinobacteria are gram-positive bacteria that are ubiquitous in soil and marine sediments. There are an exceptionally diverse number of genera that include *Streptomyces*, *Micromonospora*, *Amycolatopsis*, *Salinospora*, *Saccharopolyspora*, *Actinomycetes* and many others. These organisms have evolved striking developmental and physiological adaptations that allow them to compete and survive in crowded environments. One notable feature is their ability to produce biologically active small molecules, referred to variously as ‘natural products’, ‘secondary metabolites’, and ‘specialized metabolites’ that have been isolated and used as antibiotics and other therapeutics.

Drug discovery based on mining metabolites from actinobacteria, based on enormous screens of culture supernatants against pathogenic bacteria, was successful from 1950 to 1970 and generated many of the antibacterial drugs we now have at our disposal. However, the repeated re-discovery of known antibacterials from this source led to the abandonment of this approach during the 1990s. The prevailing view by the mid-1990s was that there was no new chemical diversity left to discover from this source. However, the advent of genome sequencing revealed that the reservoir of biosynthetic genes for these compounds, including polyketides, non-ribosomal peptides and other classes, is much larger than had been previously appreciated [19, 90, 138]. We now know that each actinomycete genome encodes 20–50 biosynthetic gene clusters for secondary metabolites [134]. It is not currently possible to assign a product structure or biological activity to most of these biosynthetic pathways. Indeed, many of the secondary metabolites produced by well-characterized model strains such as *Streptomyces coelicolor*, *Streptomyces griseus* and *Streptomyces avermitilis* are still unknown. As a result, there has been renewed emphasis on the discovery and characterization of these cryptic metabolites through the use of new bioinformatic approaches, innovative culture techniques, genetic manipulation, chemical manipulation and new screening regimens [40, 41, 69, 194, 103, 109, 117, 127, 136, 151, 165, 173].

There are several explanations for why so many secondary metabolites have eluded discovery. One view is that many secondary metabolic genes are expressed at low levels in the laboratory and that their products cannot therefore be easily detected. Another is that there may be a ‘screening bias’ in the existing discovery regimens. For example, the vast majority of screening has been for antibiotics—it is
possible that some of the uncharacterized chemical matter act on other targets.

The primary focus in this field since its inception has been on the discovery of new antibiotics. This charge has been renewed most recently due to a pressing need for new approaches to treating resistant pathogens [15, 16]. Nevertheless, we wonder whether some of the diversity of natural products is being overlooked. It is known for example,

| Drug                  | Producer                  | Primary target             |
|-----------------------|---------------------------|----------------------------|
| Nucleotide synthesis  |                           |                            |
| Actinomycin D         | *Streptomyces spp.*      | DNA                        |
| Bleomycin             | *Streptomyces verticillus*| DNA, RNA                   |
| Calicheamicin         | *Micromonospora echinospora* | DNA                     |
| Doxorubicin           | *Streptomyces peucetius*  | DNA                        |
| Mitomycin             | *Streptomyces spp.*      | DNA                        |
| Sterols               |                           |                            |
| Amphotericin B        | *Streptomyces nodosus*    | Ergosterol                 |
| Candidin              | *Streptomyces griseus*    | Ergosterol                 |
| Natamycin             | *Streptomyces natalensis* | Ergosterol                 |
| Nystatin              | *Streptomyces noursei*    | Ergosterol                 |
| Immunosuppression     |                           |                            |
| Ascomycin             | *Streptomyces hygroscopicus* | FKBP12, Calcineurin         |
| FK506                 | *Streptomyces tsukubaensis* | FKBP12, Calcineurin         |
| Rapamycin             | *Streptomyces hygroscopicus* | FKBP12, mTOR               |
| Mitochondrial function|                           |                            |
| Antimycin A           | *Streptomyces spp.*      | Cytochrome C reductase     |
| Oligomycin            | *Streptomyces distatochromogenes* | ATP synthase               |
| Protein degradation   |                           |                            |
| Epoxomicin            | *Streptomyces hygroscopicus* | 20S proteasome             |
| Salinosporamide A     | *Salinospora spp.*       | 20S proteasome             |
| Neurotransmission     |                           |                            |
| Avermectin            | *Streptomyces avermectinius* | GluCl channel             |
| Milbemycin            | *Streptomyces hygroscopicus* | GluCl channel             |
| Spinosyn              | *Saccharopolyspora spinosa* | nACh receptor             |
| Membrane              |                           |                            |
| Ionomycin             | *Streptomyces conglobatus* | Lipid bilayer             |
| Nigiricin             | *Streptomyces hygroscopicus* | Lipid bilayer             |
| Valinomycin           | *Streptomyces spp.*      | Lipid bilayer             |
| Vacuolar pH           |                           |                            |
| Bafilomycin           | *Streptomyces griseus*    | V-ATPase                   |
| Concanamycin          | *Streptomyces neyagawaensis* | V-ATPase                 |
| Signaling             |                           |                            |
| Lavendustin A         | *Streptomyces griseolavendus* | Tyrosine kinase           |
| Sangivamycin          | *Streptomyces rimosus*    | Protein kinase C          |
| Staurosporine         | *Streptomyces staurosporeus* | Protein kinase C          |
| Other                 |                           |                            |
| Borrelidin            | *Streptomyces parvalus*   | Threonyl-tRNA synthetase   |
| Cycloheximide         | *Streptomyces griseus*    | 60S ribosome               |
| Geldanamycin          | *Streptomyces hygroscopicus* | Hsp90                    |
| Leptomycin B          | *Streptomyces spp.*      | CRM1 (exportin)           |
| Rebeccamycin          | *Streptomyces spp.*      | Topoisomerase I            |
| Trichostatin A        | *Streptomyces spp.*      | HDAC (class I and II)      |
| Tunicamycin           | *Streptomyces spp.*      | UDP-HexNAc                 |
that there are many secondary metabolites that interact with eukaryotic cells (Table 1). These include known secondary metabolites commonly used as clinical antifungal, anticancer, immunosuppressive, antiangiogenic, and antiprotozoal drugs [20]. As we will describe in this review, the target diversity of these eukaryote-directed compounds exceeds that of the antibacterials. Indeed, another explanation for the failure to discover some of this diversity could be that the screening bias towards finding antibacterials has caused compounds that are expressed in the lab to go undetected due to the fact that the wrong assay was employed. Our intent, therefore, is to examine a selection of known eukaryote-directed secondary metabolites in the interest of stimulating the discovery of secondary metabolites that act on eukaryotic targets. In addition to providing new probes of intricate biological pathways, such molecules could provide leads for new therapeutics against many diseases.

**Targeting DNA synthesis: doxorubicin**

One of the mainstays of cancer chemotherapy involves the use of the anthracycline drugs epirubicin, pirarubicin, aclorubicin and idarubicin, all of which are derived from the foundational drug doxorubicin. These drugs are routinely used against malignancies such as adult acute leukemia, breast carcinoma, non-Hodgkin’s lymphoma and ovarian carcinoma [39, 89]. Indeed, the first clinically approved nano-drug (Doxil®) was a liposomally encapsulated form of doxorubicin used for the treatment of AIDS-induced Kaposi’s sarcoma and solid tumours [17].

The first member of this class, daunorubicin, was isolated from *Streptomyces peucetius* in 1963 and found to be effective against murine tumours [48]. However, clinical trials revealed severe cardiotoxicity so the compound was abandoned [170]. In an effort to find a more therapeutically favourable analogue, Arcamone et al. mutagenized *S. peucetius* and isolated strains that produced an altered, and more clinically favourable form of the drug that was named doxorubicin [4]. Doxorubicin is still toxic however it can be dosed so as to maximize its anticancer activity and minimize damage to normal tissue. Both compounds are planar tetracyclic structures attached to an amino sugar moiety: doxorubicin differs from daunorubicin by a single hydroxyl group (Fig. 1a).

The earliest indication of doxorubicin’s mechanism of action came from in vivo assays showing reduced RNA synthesis in HeLa cells [49]. In that same year, Calendi et al. observed distinct changes in the physical properties of DNA when incubated with the drug in vitro [33]. Indeed, crystal structures [63] and NMR spectroscopy [195] of doxorubicin-DNA complexes show that the drug intercalates between the nitrogenous base pairs by planar insertion (Fig. 1b).

Doxorubicin was found to induce double strand breaks in the DNA of leukemic cells where the ends of the broken strands were associated with a protein complex. The protein was subsequently identified as topoisomerase II, the homodimeric enzyme responsible for relieving positive supercoiling by a double-strand cleavage and rejoining mechanism [172]. This and other work led to a model where doxorubicin intercalates DNA causing topoisomerase II to become trapped resulting in a ternary complex and a double-strand break [119]. The exact molecular mechanism of this process is not fully understood, however several mutagenesis studies in yeast implicate the CAP-like DNA-binding domain of topoisomerase II as a direct target [130, 144].

This model is widely recognized as doxorubicin’s primary mechanism of targeting proliferative cancerous cells in vivo. However there is support for alternative mechanisms in the literature. This includes most notably the generation of reactive oxygen species (ROS) [101] and genespecific damage [35, 93]. It is possible that these alternative mechanisms occur simultaneously and are concentration dependant [66].
Targeting fungal membranes: amphotericin B

Many antibiotics produced by actinomycetes target fungal cells [20]. The most clinically relevant class in this category are the polyene macrolides, particularly amphotericin B, isolated in 1953 at the Squibb Institute from the fermentation of \textit{S. nodosus} [52]. Amphotericin B is a mainstay for managing systemic fungal infections. Amphotericin B is active against many fungal pathogen species in vitro including \textit{Candida albicans} [10, 145], \textit{Aspergillus fumigatus} [8, 55], \textit{Cryptococcus neoformans} [10, 44], \textit{Blastomyces dermatitidis} [114, 166], \textit{Histoplasma capsulatum} [114], \textit{Rhizopus sp.} [55, 56] and \textit{Mucorales sp.} [156]. Despite amphotericin B’s long-standing monotherapeutic use over the last 50 years, few resistant strains have emerged.

This is due to a tradeoff between tolerability and the fitness that is believed to limit resistance from developing [179]. However, a number of amphotericin B-resistant strains of \textit{Aspergillus} [167], \textit{Cryptococcus} [123] and \textit{Candida} [190] have emerged in the clinic in recent years. In addition, amphotericin B treatment is often associated with adverse effects including nephrotoxicity [186] and anemia [116, 121, 193]. Interestingly, amphotericin B-induced anemia has been shown to occur through the inhibition of the transcription factor hypoxia-inducible factor-1 (HIF-1), thereby reducing the expression of erythropoietin (EPO) which controls red blood cell proliferation [121, 193].

Amphotericin B is comprised of an amphipathic macrolactone ring with a mycosamine attachment (Fig. 2a). These molecular features work together to bind fungal-specific sterols such as ergosterol in the membrane, causing ion-leakage [11, 28]. Hydrogen bonds formed between the mycosamine of amphotericin B and the hydroxyl group present in both ergosterol (fungal) and cholesterol (human) are essential for binding to occur [141]. Amphotericin B’s selective toxicity towards fungi is due to a more stable interaction between its seven conjugated double bonds with ergosterol [150, 177], presumed to be the result of reduced conformational flexibility [12].

An early model of amphotericin B’s mechanism of action was the ‘barrel-stave’ [125, 176]. In this model,
eight amphotericin B-sterol complexes are aligned perpen-
dicularly to the lipid bilayer forming a channel with the hydrophobic face on the exterior and the hydrophilic face pointing towards the interior (Fig. 2b). K⁺ ions would then leak out of the cell resulting in membrane depolarization and eventually cell death [5, 108]. Furthermore, amphotericin B forms ion channels more easily in the presence of ergosterol [88]. Recent studies by Gray et al. have challenged the notion that ion leakage by pore formation is the sole biochemical feature in its mechanism of action [70]. In particular, they found that a chemically modified analogue, C35deOAmB, lacking the ability to form pores, retained its antifungal potency. Likewise, the related polyene natamycin possesses antifungal activity despite its inability to form pores in the membrane [184]. An alternative model therefore, is that amphotericin B binds to the membrane monomerically, parallel to the lipid moieties (adsorption) [46, 131] or in aggregates (sponge) [2] to sequester ergosterol thereby causing a global reduction of sterol levels in the membrane. This in turn could limit the sterol’s function in maintaining the structural integrity and fluidity of the lipid bilayer as well as enabling the function of membrane-bound enzymes that influence a wide range of diverse signalling cascades [115, 185]. While it is likely that monomeric, aggregated and pore-forming states of amphotericin B occur simultaneously, the ratios at which these formations exist at various concentrations remain unknown.

Ongoing debate about its mechanism of action and toxicity suggests that modification of this drug, or the isolation and investigation of new congeners from other actinomyces could drive the development of better antifungal drugs. More recently, novel derivatization of amphotericin B using diphenylphosphoryl azide (DPPA) led to two analogues: AmBMU and AmBAU, which were shown to be effective in evading resistance to Candida while having greater selectivity for ergosterol and were thus less toxic to human blood cells [45]. Notably, this study also revealed that amphotericin B-resistant strains of Candida are non-pathogenic in mice suggesting that minor changes in ergosterol significantly reduces pathogenicity. In addition, robust methods of synthesizing less toxic analogues of amphotericin B have been developed using the iterative cross-coupling of polyene building blocks which could potentially provide more potential candidates for improving drug efficacy [113].

It is widely agreed that additional antifungal drugs are needed to combat resistant strains and improve therapeutic outcomes associated with opportunistic mycoses including candidiasis, cryptococcal meningitis and aspergillosis which often do not respond well to a limited number of current drug regimens [54, 146]. Indeed, fungal infections that were previously treated successfully with this drug are showing increasing resistance [67].

Targeting cell growth (mTOR): rapamycin

The macrocyclic lactone antibiotic rapamycin has had an enormous impact on medicine and on our understanding of eukaryotic cells. Its story began in 1964 when a Canadian expedition team collected soil samples from Easter Island in the southeastern point of the Polynesian Triangle in the Pacific Ocean. This soil sample was then investigated at Ayerst Laboratories in Montreal where the molecule, rapamycin (from Rapa Nui, the indigenous name for Easter Island), later derived from the isolated strain S. hygroscopicus, showed remarkable antifungal activity against Candida [178]. Persistent efforts led to rapamycin’s rise to acclaim where it was found to possess potent immunosuppressive and antiproliferative properties [86, 124], leading in turn to further investigation of its mode of action.

Initially, the structurally related immunosuppressant FK506 was found to target the 12-kDa FK506-binding protein (FKBP12), a peptidylprolyl romtase [81, 164]. The complex then acquires a gain-of-function ability to suppress the activation of T-cells in the immune system through a third target, calcineurin [104]. Similarly, rapamycin also binds to FKBP12 however mounting evidence suggested that FK506 and rapamycin varied in their mechanism of immunosuppression in murine T-cells [23, 50, 51], suggesting that the tertiary target of the rapamycin-FKBP12 complex was not the same as FK506. A landmark study by Heitman et al. was carried out in the budding yeast Saccharomyces cerevisiae in which genetic screens led to the identification of dominant mutations in TOR1 and TOR2 that were shown to confer rapamycin resistance [83]. This suggested that the encoded TOR (target of rapamycin) proteins—paralogous serine/threonine kinase subunits—were the targets of the FKBP-rapamycin complex that ultimately resulted in immunosuppression and growth reduction. It was subsequently found that rapamycin binds proteins in a mammalian cells that shared extensive sequence similarity to the TOR1, providing not only direct evidence of the rapamycin-FKBP binding targets but also showing that the mechanistic targets are highly conserved in lower and higher eukaryotes [29, 42, 153, 154]. X-ray crystallography further elucidated the drug’s mode of action showing that rapamycin has two binding sites [13, 37] (Fig. 3a). Most eukaryotic organisms possess one TOR protein. The mTOR (mammalian target of rapamycin) is a large (289 kDa) protein that belongs to the phosphoinositide kinase-related kinase (PIKK) family. It associates with other proteins to form two functionally distinct complexes: mTORC1 and mTORC2.

Proteins that act upstream of mTORC1 mediate intracellular responses to a variety of intra- and extracellular cues: growth factors [61], oxygen levels [9], energy [27, 102], mitogens [38, 57] and amino acids [10, 25]. TSC 1/2
(tuberous sclerosis 1 and 2) are key upstream regulators that inhibit mTORC1 by repressing the formation of the GTP-bound state of Rheb (Ras homolog enriched in brain) [91, 171]. Two downstream effectors phosphorylated by mTORC1 are the eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) and S6 kinase (S6K) [21, 30, 32, 80]. The current model posits that mTORC1 senses environmental cues and works to positively regulate downstream signals of protein synthesis by controlling components within the translation machinery (Fig. 3b).

The mTORC2 signalling network was initially thought to be rapamycin insensitive [95, 157] however, recent studies suggest that mTORC2 does respond to rapamycin in certain cell types after prolonged exposure to the drug [147, 158]. Less is known about the mTORC2 pathway however it has been shown to associate with the ribosome and is required for activation [196]. mTORC2 has been shown to regulate three kinases: Akt [159], serum- and glucocorticoid-induced protein kinase 1 (SKG1) [65] and protein kinase C-α (PKCα) [157]. Akt works to phosphorylate downstream processes of survival, apoptosis, growth and proliferation as well as directly feedback into mTORC1 signaling through the inhibition of TSC [72, 92, 94], SKG1 affects ion transport and growth [162] and PKCs affects the remodelling of the actin cytoskeleton [87, 95, 157].

In addition to mediating rapamycin’s clinical use for preventing graft rejection after organ transplantation and for treating autoimmune disorders, the components of the mTOR pathway have also been implicated in many other conditions including obesity related type 2 diabetes [105, 112, 148, 174, 175] and cancer [73, 182, 189]. Indeed, mutations in negative and positive regulators of mTOR signaling are among the most common tumour suppressors and oncogenes that arise in cancer patients. As a result, a number of rapamycin derivatives (rapalogues) have been approved for the treatment of various cancers [18]. More recently, rapamycin has been explored as a treatment for age related diseases after the drug remarkably was shown to increase the lifespan of yeast [149], nematodes [152], fruit flies [24] and mice [3, 82, 129].

Rapamycin exemplifies the enormous value that eukaryotic targeting compounds can have through the exploration of the drug’s mode of action. In addition to the drug and its derivatives being useful therapeutics with a variety of applications, they serve as chemical probes that can be used to elucidate the inner workings of complex biological pathways.

**Targeting neurotransmission: avermectin**

The avermectins are a class of macrocyclic lactones that have broad-spectrum activity against nematodes and insects, but that lack antimicrobial activity. In the 1970s researchers at the Kitasato Institute isolated *S. avermitilis* (also referred to as *S. avermectinii*) from a soil sample on a golf course in Shizuoka Prefecture, Japan. The fermentation of this microbe was found to have potent activity against helminth parasitic worm *Nematospiroides dubius* and remarkably cured the worm-infected mice with little to no toxicity [31]. Soon following, these compounds were identified as a mixture of eight isomers of which avermectins B1a and B1b were found to be the most potent derivatives [139] (Fig. 4a).
Avermectin disrupts glutamate-gated chloride channels (GluCls) in nematodes [6, 7] and insects [43, 99] that play a critical role in muscle contraction required for locomotion and feeding. The GluCls channel belongs to a Cys-loop receptor family and is comprised of five subunits. These respond to glutamate to allow the influx of chloride ions to transmit an action potential from the presynaptic to the postsynaptic neuron (Fig. 4b). Avermectin disrupts this process by irreversibly inserting itself between the transmembrane domains thereby causing ions to constitutively leak through the compromised channel [84]. This results in the hyperpolarization of the neuromuscular synapses causing paralysis and subsequent death. It is selective for nematode parasites because mammals do not have GluCls but instead have the evolutionarily related gamma-aminobutyric acid (GABA) receptor channels [188]. While avermectin can bind GABA receptors in the mammalian central nervous system, the pharmacological effectiveness of the drug is owed to its inability to cross the blood–brain barrier [160, 161].

Initially, avermectin was studied for use in veterinary medicine and animal husbandry. The medical formulation of the drug, ivermectin, became useful in agriculture, saving livestock affected by ectoparasitic arthropods and endoparasitic helminth nematodes [34]. But the most significant contribution that this drug has had was its use to treat river blindness, a disease caused by the parasite *Onocerca volvulus* that is transmitted by the black fly. Ivermectin is credited for significantly reducing morbidity and transmission of onchocercal infections in the endemic regions of sub-Saharan Africa and Latin America preventing an estimated 600,000 cases of river blindness [26].

### Targeting nucleo-cytoplasmic transport: leptomycin B

Like many compounds that target the eukaryotic cell, leptomycin B and its derivatives were originally identified in screens for antifungal and antitumor antibiotics [77–79, 110]. In 1994, Nishi et al. identified a mutant of the *crm1* (chromosome region maintenance) gene that conferred leptomycin B resistance in fission yeast [135]. This gene, previously reported by Adachi and Yanagida [1], affected higher order chromosomal structure and resulted in an identical phenotype when mutated compared to leptomycin B-treated cells [135]. This provided strong evidence that the molecular target of leptomycin B was CRM1, a protein that belongs to the importin-β-like family of nuclear transport machinery that mediates the export of proteins and RNAs out of the nucleus [192].

In order to understand how leptomycin B works, it is important to first recognize the role that the nuclear envelope plays in the cell. That is, the physical separation of the genome and cytoplasm, a central feature of eukaryotic cells. The trafficking of proteins and RNA is a highly coordinated process that takes place across the nuclear envelope which is contiguous with the endoplasmic reticulum and contains anywhere between 200 and 2000 nuclear pore complexes (NPC) that facilitate bi-directional transport between the nuclear and cytoplasmic compartments.

Later, in a screen carried out by Wolff et al. leptomycin B was identified as an inhibitor of the nuclear export of Rev, a protein required for trafficking of HIV-1 mRNA from the nucleus to the cytoplasm [187]. This coincided well with the fact that leptomycin B prevents the cargo-loading of
proteins that carry leucine-rich nuclear export signals (NES) that are to be transported to the cytoplasm through the nuclear pore [62] (Fig. 5b). It does so by forming a covalent bond with CRM1 where inactivation is thought to occur by a Michael-type addition between the α,β-unsaturated lactone terminus of the compound and a key cysteine residue that is essential for leptomycin B sensitivity [111] (Fig. 5a).

The specificity of leptomycin B has been used to validate the CRM1-dependent export of many NES-containing proteins including actin [181], cytokines [140], tyrosine kinases [169], cyclin-CDK [75, 191], MDM2/p53 [64], inhibitors of NF-κB transcription [155] and MHC class II complexes [36]. Inhibition by this drug results in the accumulation of these key regulatory proteins which eventually leads to cell death.

Efforts have been made to improve the therapeutic efficacy through the synthesis of leptomycin B semi-synthetic derivatives [132]. However, in contrast to many of the well-known actinomycete-derived molecules that target eukaryotic organisms, leptomycin B has gained most of its notoriety as a powerful experimental tool to probe biological complexity.

**Targeting the proteasome: epoxomicin**

The 20S proteasome is found in all eukaryotic cells where it serves to degrade proteins during their natural turn-over cycle or proteins that have been misfolded or have sustained other damage. One way that proteins are targeted for proteolysis is via a post-translational modification called ‘ubiquitination’. This involves the ligation of a small regulatory protein called ubiquitin to the protein; ubiquitin is then recognized by the proteasome resulting in targeting of the modified adduct for degradation [68].

The α’,β’-epoxyketone epoxomicin specifically targets proteasomes, a key protease of intracellular protein degradation. Epoxomicin was discovered in 1992 through a screening programme at Bristol-Myers Squibb in Tokyo, Japan and is produced by the unidentified actinomycete strain Q996-17 where it was initially reported having antitumor activity against B16 melanoma cells in mice [76].

The chemical structure of epoxomicin consists of four linked peptides with an unusual terminal epoxy ketone group (Fig. 6a). This chemical moiety is highly reactive and is therefore considered the ‘warhead’ or ‘pharmacophore’ of the drug due to the triangular epoxy ring having highly strained 60° bond angles which are more stable once decyclized by nucleophilic attack. This inherent instability led to the near abandonment of further development of the drug [100]. However, efforts to understand the epoxomicin’s mode of action were continued hoping to gain a better understanding of its antitumor activity.

The peptidic nature of epoxomicin allowed Meng et al. to synthesize the drug with ease which was then biotinylated to chemically attach and immobilize the drug to an affinity column [128]. This, remarkably, led to the epoxomicin-binding proteins being identified as components of the catalytic β subunits of the 20S proteasome: low-molecular mass polypeptide-7 (LMP7, β5i), subunit X (PSMB5), which confer chymotrypsin-like activity and multicatalytic endopeptidase complex like 1 (MECL1, β2i), subunit Z (β2), which confer trypsin-like activity to the proteasome. Consistent with the fact that epoxomicin preferentially inhibits the β5 subunit of the core proteasomal particle, epoxomicin is highly selective against chymotrypsin-like activity—that is the inhibition of protein cleavage after aromatic and hydrophobic amino acid residues such as tyrosine, tryptophan and phenylalanine [53, 128] (Fig. 6b).

Groll et al. then co-crystallized epoxomicin bound to the yeast 20S proteasome to elucidate the exact molecular mechanism of the epoxomicin-proteasome interaction. The three-dimensional molecular interaction between the drug and the proteasomal catalytic subunit revealed that a covalent linkage with the N-terminal threonine of the proteasome forms a six-membered morpholino ring [71, 183]. Also showing that epoxomicin fits well into the pocket surrounding the threonine residue within the active site, preferentially binding to the chymotrypsin-like pocket, and at higher concentration than the trypsin-like pocket.

---

**Fig. 5**

a The chemical structure of leptomycin B.

b Nucleo-cytoplasmic transport of protein cargo with a leucine-rich NES (nuclear export signal) by exportin/CRM1. Leptomycin B inhibits the loading of exportin with the cargo and Ran-GTP by alkylation
To understand epoxomicin’s activity on a cellular level, we will shortly recapitulate the function of the proteasome, the key protease for short-lived proteins regulating a broad variety of cellular processes such as cell cycle progression, gene expression, protein quality control and stress response. Well known proteasomal substrates include cyclins [14, 22], caspases [133, 168], p53 [122], p27 [120], BCL2 [118] and nuclear factor κB (NF-κB) [142]. The inhibition of their proteolysis triggers apoptosis. Thus, chemically induced apoptosis by proteasome inhibitors such as Bortezomib (Velcade®) are successfully used to combat the progression of certain cancer cells.

Several lines of evidence suggest a heightened dependency on protein quality-control mechanisms mediated by the ubiquitin–proteasome system in cancer cells [74, 85, 120]. Because of this, epoxomicin in combination with proteasome inhibitors are exceptional candidates as antineoplastic therapeutics that can have a very potent cytotoxic effects in cancer cells. In phase I and II clinical trials, inhibition of the 20S proteasome is highly cytotoxic to plasma cell cancer multiple myeloma [163] and mantle cell lymphoma [137]. The high expression of proteasomes in proliferative blood cells also suggests that proteasome inhibitors are potentially suited to haematopoietic malignancies [97]. The drug form of epoxomicin (Carfilzomib) is now released as an FDA-approved treatment for relapsed multiple myeloma and is currently undergoing phase III clinical trials [96, 100, 126, 180]. Presumably, higher expression of proteasomes in blood cells compared to peripheral tissues may diminish the drug’s access to solid tumors which may limit proteasome inhibitors to blood cancers [47].

Epoxomicin is a rare compound that specifically targets a unique process in eukaryotic organisms, namely chymotrypsin-like activity of the proteasome. Similarly, some other actinomycete-derived proteasome inhibitors: lactacystin [58] and salinosporamide [59], also inhibit the β5 catalytic subunit of the 20S proteasome which suggests that the proteasome may be a common target for natural products of microbial-origin. There are likely a number of natural products that inhibit the proteasome yet to be discovered.

Eukaryotic targets are more diverse than prokaryotic targets

Target diversity

The targets of antibacterials are conspicuously concentrated in four pathways: DNA synthesis, RNA synthesis, protein synthesis and cell wall synthesis [106]. Indeed, there are often multiple targetable proteins in each pathway. For example, tetracycline targets the small 30S ribosomal subunit, chloramphenicol targets the large ribosomal subunit, and kirromycin targets EF-Tu. Aside from a few minor antibiotics and antibiotic targets (e.g., platensimycin inhibits fatty acid biosynthesis and daptomycin disrupts the cell membrane) these central components of macromolecular synthesis are the targets of virtually all naturally occurring antibiotics that are known at this time.

To date we have identified far fewer eukaryote-active compounds than prokaryote-active compounds among the secondary metabolites produced by actinobacteria. However, the contrast in target diversity could not be greater (Fig. 7). Indeed, Table 1 reveals at least 20 distinct molecular targets in most of the major organelles of the eukaryotic
cell. In fact, the common antibacterial targets (DNA synthesis, RNA synthesis and cell wall synthesis) are underrepresented relative to the high number of other identified molecular targets in eukaryotic cells.

There are many biosynthetic processes for which there are no natural product inhibitor, and others for which there are only one or two that are known. This, again, is in marked contrast with the antibiotics where dozens of distinct compounds are known that inhibit common targets within bacteria. This suggests that we have yet to reach saturation of possible eukaryotic targets and that additional compounds of interest await discovery. We wonder in particular, whether there are bacterially produced inhibitors of peroxisome biogenesis and function, centrosomes, or even components of key signaling pathways like the Janus kinase/signal transducers (JAK-STAT) pathway, the mitogen-activated protein kinase (MAPK) cascade or regulators of the cell cycle cascade.

How might these questions be addressed? We suggest, as a foundational concept, that any eukaryotic pathway that has been in contact with actinobacteria since its appearance in evolutionary time has a potential biochemical target for a secondary metabolite. The remarkable (though far from exhaustive) description of compound/target interactions that we have provided certainly supports this. Therefore, we propose that more concerted screening campaigns of actinobacterial metabolites against model eukaryotes is a timely and exciting response to this question. These screens should harness more than just live/dead screening. In other words, we should look for interesting developmental and behavioural phenotypes using the well-developed model systems. And in designing screens, we should harness the known molecular biology of the various pathways of interest. In doing so, and by avoiding simple live/dead screens only against microbes, we could avoid the rediscovery of known compounds such as daunorubicin, bafilomycin and cycloheximide and focus our attention on novel chemical scaffolds.

For example, the roundworm *Caenorhabditis elegans* could be screened for compounds that act via components

![Diagram](https://example.com/diagram.png)

**Fig. 7** The highly diverse eukaryotic targets of actinomycete metabolites
the nervous system and confer motility defects [98]. The fly Drosophila melanogaster could be screened for compounds that interfere with a myriad of developmental pathways, most of which are conserved in humans [143]. Fruiting body formation in the amoeboid organism Dictyostelium discoideum could serve as a reporter for cell adhesion and cell sorting [60]. The simple mustard plant Arabidopsis thaliana offers numerous possibilities for the identification of compounds that interact with the photosynthetic apparatus or other conserved pathways in the plant Kingdom [107]. Indeed, chemical perturbation of the life cycles of many well-characterized eukaryotic organisms also offers the potential for unique insights into both morphogenesis and hidden mechanistic details of eukaryotic cell biology.

We note that the road blocks encountered in antibacterial screening will also be encountered in these searches. Low levels of expression of many secondary metabolic compounds would necessitate strategies for the search for cryptic metabolites, many of which now exist. And the rediscovery of known compounds will also confound some screens. However, this confluence of technologies, the rapidly expanding database of actinobacterial genomes, and the wide-spread interest in chemical inhibitors of eukaryotic life suggests that the time has never been better for a concerted search for new eukaryote-active secondary metabolites.

Acknowledgements We would like to thank Cordula Enenkel, Alexander Palazzo, Leah Cowen, Fiona Smaill, and Anthony Grillo for providing insight and expertise for this review.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Adachi Y, Yanagida M (1989) Higher order chromosome structure is affected by cold-sensitive mutations in a Schizosaccharomyces pombe gene crm1· which encodes a 115-kD protein preferentially localized in the nucleus and its periphery. J Cell Biol 108(4):1195–1207
2. Anderson TM et al (2014) Amphotericin forms an extramembranous and fungicidal sterol sponge. Nat Chem Biol 10(5):400–406
3. Anisimov VN, Zabershinski MA, Popovich IG et al (2011) Rapamycin increases lifespan and inhibits spontaneous tumorigenesis in inbred female mice. Cell Cycle 10(24):4230–4236
4. Arcamone F, Cassinelli G, Fantini G, Grein A, Orezzi P, Pol C, Spalla C (1969) Adriamycin, 14-hydroxydaunomycin, a new antitumor antibiotic from S. peucetius var. caesius. Biotechnol Bioeng 11(6):1101–1110
5. Arczewska M, Gagos M (2011) Molecular organization of anti-biotic amphotericin B in dipalmitoylphosphatidylcholine monolayers induced by K(+) and Na(+): ions: the Langmuir technique study. Biochim Biophys Acta 11:2706–2713
6. Arena JP, Liu KK, Paress PS, Cully DF (1991) Avermectin-sensitive chloride currents induced by Caenorhabditis elegans RNA in Xenopus oocytes. Mol Pharmacol 40(3):368–374
7. Arena JP, Liu KK, Paress PS, Schaeffer JM, Cully DF (1992) Expression of a glutamate-activated chloride current in Xenopus oocytes injected with Caenorhabditis elegans RNA: evidence for modulation by avermectin. Brain Res Mol Brain Res 15(3–4):339–348
8. Arikian S, Lozano-Chiu M, Paetznick V, Nangia S, Rex JH (1999) Microdilution susceptibility testing of amphotericin B, itraconazole, and voriconazole against clinical isolates of Aspergillus and Fusarium species. J Clin Microbiol 37:3946–3951
9. Arsham AM, Howell JJ, Simon MC (2003) A novel hypoxia-inducible factor-independent hypoxic response regulating mammalian target of rapamycin and its targets. J Biol Chem 278(32):29655–29660
10. Arthニングton-Skaggs BA, Motley M, Warrick DW, Morrison CJ (2000) Comparative evaluation of PASC0 and national committee for clinical laboratory standards M27-A broth microdilution methods for antifungal drug susceptibility testing of yeasts. J Clin Microbiol 38(6):2254–2260
11. Baginski M, Resat H, Borowski E (2002) Comparative molecular dynamics simulations of amphotericin B-cholesterol/ergosterol membrane channels. Biochim Biophys Acta 1567(1–2):63–78
12. Baginski M, Tempczyk A, Borowski E (1989) Comparative conformational analysis of cholesterol and ergosterol by molecular mechanics. Eur Biophys J 17(3):159–166
13. Banaэszynski LA, Liu CW, Wandless TJ (2005) Characterization of the FKBP, rapamycin. FRB ternary complex. J Am Chem Soc 127(13):4715–4721
14. Baldin V, Cans C, Knibiehler M, Doucommun B (1997) Phosphorylation of human CDC25B phosphatase by CDK1-cyclin A triggers its proteasome-dependent degradation. J Biol Chem 272(52):32731–32734
15. Baltz RH (2006) Marcel Faber roundtable: is our antibiotic pipeline unproductive because of starvation, constipation or lack of inspiration? J Ind Microbiol Biotechnol 33:507–513
16. Baltz RH (2008) Renaissance in antibacterial discovery from actinomycetes. Curr Opin Pharmacol 8:557–563
17. Barenholz Y (2012) Doxil®–the first FDA-approved nano-drug: lessons learned. J Control Release 160(2):117–134
18. Benjamin D, Colombi M, Moroni C, Hall MN (2011) Rapamycin passes the torch: a new generation of mTOR inhibitors. Nat Rev Drug Discov 10:868–880
19. Bentley SD, Chater KF, Cerdeno-Tarrago AM et al (2002) Complete genome sequence of the model actinomycete Streptomyces coelicolor A3 (2). Nature 417(6885):141–147
20. Berdy J (2005) Bioactive microbial metabolites. J Antibiot 58(1):1–26
21. Beretta L, Grein A, Orezzi P, Pol C, Spalla C (1969) Adriamycin, 14-hydroxydaunomycin, a new antitumor antibiotic from S. peucetius var. caesius. Biotechnol Bioeng 11(6):1101–1110
formed between an immunophilin and either FK506 or rapamy-
cin. Proc Natl Acad Sci USA 87(23):9231–9235
24. Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, Foley A, Partridge L (2010) Mechanisms of life span extension by rapa-
ymycin in the fruit fly Drosophila melanogaster. Cell Metab 11(1):35–46
25. Blommaart EF, Luiken JJ, Blommaart PJ, van Woerkom GM, Meijer AJ (1995) Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes. J Biol Chem 270:2320–2326
26. Boatin B (2008) The onchocerciasis control programme in West
African regions. Philos Trans R Soc Lond B Biol Sci 363(1493):1787–1797
27. Bolster DR, Crozier SJ, Kimball SR, Jefferson LS (2002) AMP-activated protein kinase suppresses protein synthet-
ization in rat skeletal muscle through down-regulated maul- 

target of rapamycin (mTOR) signaling. J Biol Chem 277(27):23977–23980
28. Braitburg J, Powderly WG, Kobayashi G, Medoff G (1990) Amphotericin B: current understanding of mechanisms of 

action. Antimicrob Agents Chemother 34(2):183–188
29. Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL (1994) A mammalian protein tar-
geted by G1-arresting rapamycin-receptor complex. Nature 369(6483):756–758
30. Brunn GJ, Hudson CC, Sekulic A, Williams JM, Heso H, Houghton PJ, Lawrence JC Jr, Abraham RT (1997) Phosphory-
lization of the translational repressor PHAS-I by the mammalian target of rapamycin. Science 277(5322):99–101
31. Burger RW, Miller BM, Baker EE et al (1979) Avermectins, new 
family of potent anthelmintic agents: producing organism and 

requirements for DNA cleavage by mammalian topoisomerase 

II. Cell Metab 11(1):35–46
32. Burnett PE, Barrow RK, Cohen NA, Snyder SH, Sabatini DM (1998) RAFT1 phosphorylation of the translational regula-
tors p70 S6 kinase and 4E-BP1. Proc Natl Acad Sci USA 95(4):1432–1437
33. Calendi E, Di Marco A, Reggiani M, Scarpinato B, Valentini L L (1965) On physico-chemical interactions between daunomycin and nucleic acids. Biochim Biophys Acta 103:25–49
34. Campbell WC (2012) History of avermectin and ivermectin, 

and nucleic acids. Biochim Biophys Acta 103:25–49
35. Capranico G, Kohn KW, Pommier Y (1990) Local sequence 
demands for DNA cleavage by mammalian topoisomerase 

II. Cell Metab 11(1):35–46
36. Choi J, Chen J, Schreiber SL, Clardy J (1996) Structure of 

the FKBP12-rapamycin complex interacting with the binding 

domain of human FRAP. Science 273(5272):239–242
37. Citro S, Miccolo L, Meloni L, Chiocca S (2015) PI3K/mTOR 

mediated mitogen-dependent HDAC1 phosphorylation in breast cancer: a novel regulation of estrogen receptor expression. J Mol Cell Biol 7(2):132–142
38. Cortes-Funes H, Coronado C (2007) Role of anthracyclics in 

the era of targeted therapy. Cardiovasc Toxicol 7(2):56–60
39. Craney A, Ahmed S, Nodwell J (2013) Towards a new science of secondary metabolism. J Antibiott (Tokyo) 66(7):387–400
40. Craney A, Ozmok C, Pimentel-Eladio SM, Capretta A, Nod-
well JR (2012) Chemical perturbation of secondary metabolism demonstrates important links to primary metabolism. Chem Biol 19(8):1020–1027
41. Crespo JL, Hall MN (2002) Elucidating TOR signaling and rapamy-
cin action: lessons from Saccharomyces cerevisiae. Microbiol Mol Biol Rev 66(4):579–591
42. Cully DF, Paress PS, Liu KK, Schaeffer JM, Arena JP (1996) Identification of a Drosophila melanogaster glutamate-gated 

chloride channel sensitive to the antiparasitic agent avermectin. J Biol Chem 271(33):20187–20191
43. Davey KG, Holmes AD, Johnson EM, Szekely A, Warnock DW (1998) Comparative evaluation of FUGNITEST and 
broth microdilution methods for antifungal drug susceptibility testing of 

Candida species and Cryptococcus neoformans. J Clin Microbiol 36:926–930
44. Davis SA, Vincent BM, Endo MM, Whitesell L, Marchiklo K, Andes DR, Linquist S, Burke MD (2015) Nontoxic antimicro-

bials that evade drug resistance. Nat Chem Biol 11(7):481–487
45. de Kruijff B, Demel RA (1974) Polyene antibiotic-sterol inter-

actions in membranes of Acholoplasma laidlawii cells and leci-
thin liposomes. III. Molecular structure of polyene antibiotic-

cholesterol complexes. Biochim Biophys Acta 339:57–70
46. Deshayes RJ (2014) Proteotoxic crisis, the ubiquitin-proteasome 

system, and cancer therapy. BMC Biol 12:94
47. Di Marco A, Gaetani M, Dorigani M, Bellini O (1965) Studies on the history of other macrocyclic lactone antipara-

ticals that evade drug resistance. Nat Chem Biol 11(7):481–487
48. Di Marco A, Silvestrini R, Di Marco S, Dasdia T (1965) Inhibi-
tive effect of the new cytotoxic antibiotic daunomycin on 

nucleic acids and mitotic activity of HeLa cells. J Cell Biol 27(3):545–550
49. Dumont FJ, Melino MR, Staruch MJ, Kopral SL, Fischer PA, Sigal NH (1990) The immunosuppressive macrolides FK-506 

and rapamycin act as reciprocal antagonists in murine T cells. J 

Immunol 144(4):1418–1424
50. Dumont FJ, Staruch MJ, Kopral SL, Melino MR, Sigal NH (1990) Distinct mechanisms of suppression of murine T cell 

activation by the related macrolides FK-506 and rapamycin. J 

Immunol 144(1):251–258
51. Dutcher JD (1968) The discovery and development of amphi-

tericin B. Dis Chest 54(Suppl 1):296–298
52. Elofsson M, Splittergter M, Muynj M, Mohan R, Crews CM (1999) Towards subunit-specific proteasome inhibitors: synthe-
sis and evaluation of peptide alpha' , beta'-epoxyketones. Chem 

Biol 6(11):811–822
53. Enoch DA, Ludlam HA, Brown NM (2006) Invasive fungal 

infections: a review of epidemiology and management options. J Med Microbiol 55(7):809–818
54. Espinal-Ingroff A, Bartlett M, Bowden R, Chin NX, Cooper C Jr, Fothergill A et al (1997) Multicenter evaluation of proposed 

standardized procedure for antifungal susceptibility testing of 

filamentous fungi. J Clin Microbiol 35:139–143
55. Espinal-Ingroff A, Dawson K, Pfaller M, Analissse E, Breslin B 

and nucleic acids. Biochim Biophys Acta 103:25–49
56. Fenteany G, Standaert RF, Lane WS, Schreiber SL (1995) Inhibition of proteasome activities and 

immuno-suppressant. J Biol Chem 271(33):20187–20191
57. Felter RH, Buchanan GO, Mincer TJ, 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 

42(3):355–357
58. Firtel RA, Meili R (2000) Dictyostelium: a model for regulated 

cell movement during morphogenesis. Curr Opin Genet Dev 

10(4):421–427
61. Floyd S, Favre C, Lasorsa FM et al (2007) The insulin-like growth factor-I-mTOR signaling pathway induces the mitochondrial pyrimidine nucleotide carrier to promote cell growth. Mol Biol Cell 18(9):3545–3555
62. Fornerod M, Ohno M, Yoshida M, Mattaj IW (1997) CRM1 is an export receptor for leucine-rich nuclear export signals. Cell 90(6):1051–1060
63. Frederick CA, Williams LD, Ughetto G, van der Marel GA, van Boom JH, Rich A, Wang AH (1990) Structural comparison of anticancer drug-DNA complexes: adriamycin and daunomycin. Biochemistry 29(10):2538–2549
64. Freedman DA, Levine AJ (1998) Nuclear export is required for activation of serum- and glucocorticoid-induced protein kinase 1 (SKG1). Biochem J 416:375–385
65. Gerver DA (1999) A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochem Pharmacol 57(7):727–741
66. Ghannoum MA, Rice LB (1999) Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin Microbiol Rev 12(4):501–517
67. Glickman MH, Ciechanove A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 82(2):373–428
68. Gomez-Escribano JP, Bibb MJ (2011) Engineering antibiotic multiple myeloma. Clin Lymphoma Myeloma Leuk 12(5):310–318
69. Gray KC et al (2012) Amphotericin primarily kills yeast by simulations of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin Microbiol Rev 12(4):501–517
70. Groll M, Kim KB, Kairies N, Huber R, Crews CM (2000) Crystal structure of epoxomicin: 20S proteasome reveals a molecular basis for selectivity of β, β'-epoxyketone proteasome inhibitors. J Am Chem Soc 122(6):1237–1238
71. Guettin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY et al (2006) Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PAKCalpho, but not S6K1. Dev Cell 11:859–871
72. Guzman ML, Swiderski CF, Howard DS, Grimes BA, Rossi RM, Szilvassy SJ, Jorand CT (2002) Preferential induction of muscle cells in vitro. Proc Natl Acad Sci USA 99(25):16220–16225
73. Hagting A, Karlsson C, Clute P, Jackman M, Pines J (1998) MFp localization is controlled by nuclear export. EMBO J 17(4):4127–4138
74. Hanada M, Sugawara K, Kaneta K, Toda S, Nishiyama Y, Tomita K, Yamamoto H, Konishi M, Oki T (1992) Epoxomicin, a new antitumor agent of microbial origin. J Antibiot Tokyo 45(11):1746–1752
75. Hamamoto T, Gunji S, Tsuji H, Beppu T (1983) Leptomycins A and B, new antifungal antibiotics. I. Taxonomy of the producing strain and their fermentation purification and characterization. J Antibiot Tokyo 36(6):639–645
76. Hamamoto T, Seto H, Beppu T (1983) Leptomycins A and B, new antifungal antibiotics. II. Structure elucidation. J Antibiot Tokyo 36(6):646–650
77. Hamamoto T, Uozumi T, Beppu T (1985) Leptomycins A and B, new antifungal antibiotics. III. Mode of action of leptomycin B on Schizosaccharomyces pombe. J Antibiot Tokyo 38(11):1573–1580
78. Hara K, Yonezawa K, Weng QP, Kozlowski MT, Belham C, Avruch J (1998) Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4EBP1 through a common effector mechanism. J Biol Chem 273:14484–14494
79. Harding MW, Galat A, Uehling DE, Schreiber SL (1989) A receptor for the immunosuppressant FK506 is a cis–trans peptidyl-prolyl isomerase. Nature 341:758–760
80. Harrison DE, Strong R, Sharp ZD et al (2009) Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460(7253):392–395
81. Heitman J, Mowva NR, Hall MN (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science 253(5022):905–909
82. Hibbs RE, Gouaux E (2011) Principles of activation and permeation in an anion-selective Cys-loop receptor. Nature 474(7349):54–60
83. Hidestima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J, Anderson KC (2001) The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res 61(7):3071–3076
84. Houchens DP, Ovejera AA, Riblet SM, Slagel DE (1983) Human brain tumor xenografts in nude mice as a chemotherapy model. Eur J Cancer Clin Oncol 19(6):799–805
85. Hryciw DH, Pollock CA, Poronnik P (2005) PKC-alpha-mediated remodeling of the actin cytoskeleton is involved in constitutive albumin uptake by proximal tubule cells. Am J Physiol Renal Physiol 288(6):F1227–F1235
86. Huang W, Zhang Z, Han X, Tang J, Wang J, Dong S, Wang E (2002) Ion channel behaviour of amphotericin B in sterol-free and cholesterol- or ergosterol-containing supported phosphatidylcholine bilayer model membranes investigated by electrochemistry and spectroscopy. Biophys J 83(6):3245–3255
87. Hurley LH (2002) DNA and it associated processes as targets for cancer therapy. Nat Rev Cancer 2(3):188–200
88. Ikeda H, Ishikawa J, Hamamoto A et al (2003) Complete genome sequence and comparative analysis of the industrial microorganism Streptomyces avermitilis. Nat Biotechn 21:526–531
89. Inoki K, Li Y, Xu T, Guan KL (2003) Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev 17:1829–1834
90. Inoki K, Li Y, Zhu T, Wu J, Guan KL (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol 4:646–657
91. Ito H, Miller SC, Billingham ME, Akimoto H, Torti SV, Wade R, Gahlmann R, Lyons G, Kedes L, Torti FM (1990) Doxorubicin selectively inhibits muscle gene expression in cardiac muscle cells in vivo and in vitro. Proc Natl Acad Sci USA 87:4275–4279
92. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Huang Q, Qin J, Su B (2006) SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. Cell 127(1):125–137
93. Jacinto E, Loweth R, Schmidt A, Lin S, Ruegg MA, Hall A, Hall MN (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biol 6:1122–1128
94. Jagannath S, Vrij R, Stewart AK et al (2012) An open-label single-arm pilot phase II study (PX-171-003-A0) of low-dose, single-agent carfilzomib in patients with relapsed and refractory multiple myeloma. Clin Lymphoma Myeloma Leuk 12(5):310–318
114. Li RK, Tanaka K, Inamura N, Sone S, Ogura T, Matsumoto T, Tachikawa T, Shin S, Ichihara A (1990) Abnormally high expression of proteasomes in human leukemic cells. Proc Natl Acad Sci USA 87(18):7071–7075
115. Li X, Gianoulis TA, Yip KY, Gerstein M, Snyder M (2010) Extensive in vivo metabolism–protein interactions revealed by large-scale systematic analyses. Cell 143(4):639–650
116. Lin AC, Goldwasser E, Bernard EM, Chapman SW (1990) Amphotericin B blunts erythropoietin response to anemia. J Infect Dis 161(2):348–351
117. Ling LL, Schneider T, Peoples AJ et al (2015) A new antibiotic kills pathogens without detectable resistance. Nature 517(7535):455–459
118. Ling YH, Liebes L, Ng B, Buckley M, Elliott PJ, Adams J, Jiang JD, Muggia FM, Perez-Soler R (2002) PS-341, a novel proteasome inhibitor, induces Bcl-2 phosphorylation and cleavage in association with G2-M phase arrest and apoptosis. Mol Cancer Ther 1(10):841–849
119. Liu LF (1989) DNA topoisomerase poisons as antimutator drugs. Annu Rev Biochem 58:351–375
120. Loda M, Cukor B, Tam SW, Lavin P, Fiorentino M, Draetta G, Jessup JM, Pagano M (1997) Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressively colorectal cancers. Nat Med 3(2):231–234
121. MacGregor RR, Bennett JE, Erslev AJ (1978) Erythropoietin concentration in amphotericin B-induced anemia. Antimicrob Agents Chemother 14(2):270–273
122. Maki CG, Hubbrecht JS, Howley PM (1996) In vivo ubiquitination and proteasome-mediated degradation of p53. Cancer Res 56(11):2649–2654
123. Manfredi R, Fulgaro C, Sabbatani S, Legnani G, Fasulo G (2004) Interaction model for anthracycline activity against Streptomyces coelicolor gene absA1. J Antibiot Tokyo 53(4):177–192
124. Martel RR, Kliucis J, Galet S (1977) Inhibition of the immune response by rapamycin, a new antifungal antibiotic. Can J Pharmcol 55(1):48–51
125. Marty A, Finkelstein A (1975) Pores formed in lipid bilayer membranes by nystatin, differences in its one-sided and two-sided action. J Gen Physiol 65(4):515–526
126. McCormac PL (2012) Carbfilzimib: in relapsed, or relapsed and refractory, multiple myeloma. Drugs 72(15):2023–2032
127. McKenzie NL, Thaker M, Koteva K, Hughes DW, Wright GD, Nodwell JR (2010) Induction of antimicrobial activities in heterologous streptomyces using alleles of the Streptomyces coelicolor gene absA1. J Antimicrob Chemother 54(4):177–192
128. Meng L, Mohan R, Kwok BHH, Elfstrom M, Sin N, Crews CM (1999) Epoxomicin, a potent and selective proteasome inhibitor, exhibits in vivo anti-inflammatory activity. Proc Natl Acad Sci USA 96(18):10403–10408
129. Miller RA, Harrison DE, Astle CM et al (2010) Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterozygous mice. J Gerontol A Biol Sci Med Sci 66(2):191–201
130. Moro S, Beretta GL, Dal Ben D, Nittis J, Palumbo M, Caprancio G (2004) Interaction model for anthracycline activity against DNA topoisomerase II. Biochemistry 43(23):7503–7513
131. Mourti R, Konoki K, Matsumori N, Oishi T, Murata M (2008) Complex formation of amphotericin B in sterol-containing membranes as evidenced by surface plasmon resonance. Biochemistry 47:7807–7815
132. Mutka SC, Yang WQ, Dong SD, Ward SL, Craig DA, Timmermans PB, Murli S (2009) Identification of the nuclear export inhibitors with potent anticancer activity in vivo. Cancer Res 69(2):510–517
133. Naujokat C, Sezer O, Zinke H, Leclere A, Hauptmann S, Posinger K (2000) Proteasome inhibitors induced caspase-dependent apoptosis and accumulation of p21WAF1/Cip1 in human immature leukaemic cells. Eur J Haematol 65:221–236

Kumatori A, Tanaka K, Inamura N, Sone S, Ogura T, Matsumoto T, Tachikawa T, Shin S, Ichihara A (1990) Abnormally high expression of proteasomes in human leukemic cells. Proc Natl Acad Sci USA 87(18):7071–7075
114. Li RK, Tanaka K, Inamura N, Sone S, Ogura T, Matsumoto T, Tachikawa T, Shin S, Ichihara A (1990) Abnormally high expression of proteasomes in human leukemic cells. Proc Natl Acad Sci USA 87(18):7071–7075
115. Li X, Gianoulis TA, Yip KY, Gerstein M, Snyder M (2010) Extensive in vivo metabolism–protein interactions revealed by large-scale systematic analyses. Cell 143(4):639–650
116. Lin AC, Goldwasser E, Bernard EM, Chapman SW (1990) Amphotericin B blunts erythropoietin response to anemia. J Infect Dis 161(2):348–351
117. Ling LL, Schneider T, Peoples AJ et al (2015) A new antibiotic kills pathogens without detectable resistance. Nature 517(7535):455–459
118. Ling YH, Liebes L, Ng B, Buckley M, Elliott PJ, Adams J, Jiang JD, Muggia FM, Perez-Soler R (2002) PS-341, a novel proteasome inhibitor, induces Bcl-2 phosphorylation and cleavage in association with G2-M phase arrest and apoptosis. Mol Cancer Ther 1(10):841–849
119. Liu LF (1989) DNA topoisomerase poisons as antimutator drugs. Annu Rev Biochem 58:351–375
120. Loda M, Cukor B, Tam SW, Lavin P, Fiorentino M, Draetta G, Jessup JM, Pagano M (1997) Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressively colorectal cancers. Nat Med 3(2):231–234
121. MacGregor RR, Bennett JE, Erslev AJ (1978) Erythropoietin concentration in amphotericin B-induced anemia. Antimicrob Agents Chemother 14(2):270–273
122. Maki CG, Hubbrecht JS, Howley PM (1996) In vivo ubiquitination and proteasome-mediated degradation of p53. Cancer Res 56(11):2649–2654
123. Manfredi R, Fulgaro C, Sabbatani S, Legnani G, Fasulo G (2004) Interaction model for anthracycline activity against Streptomyces coelicolor gene absA1. J Antibiot Tokyo 53(4):177–192
124. Martel RR, Kliucis J, Galet S (1977) Inhibition of the immune response by rapamycin, a new antifungal antibiotic. Can J Pharmcol 55(1):48–51
125. Marty A, Finkelstein A (1975) Pores formed in lipid bilayer membranes by nystatin, differences in its one-sided and two-sided action. J Gen Physiol 65(4):515–526
126. McCormac PL (2012) Carbfilzimib: in relapsed, or relapsed and refractory, multiple myeloma. Drugs 72(15):2023–2032
127. McKenzie NL, Thaker M, Koteva K, Hughes DW, Wright GD, Nodwell JR (2010) Induction of antimicrobial activities in heterologous streptomyces using alleles of the Streptomyces coelicolor gene absA1. J Antimicrob Chemother 54(4):177–192
128. Meng L, Mohan R, Kwok BHH, Elfstrom M, Sin N, Crews CM (1999) Epoxomicin, a potent and selective proteasome inhibitor, exhibits in vivo anti-inflammatory activity. Proc Natl Acad Sci USA 96(18):10403–10408
129. Miller RA, Harrison DE, Astle CM et al (2010) Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterozygous mice. J Gerontol A Biol Sci Med Sci 66(2):191–201
130. Moro S, Beretta GL, Dal Ben D, Nittis J, Palumbo M, Caprancio G (2004) Interaction model for anthracycline activity against DNA topoisomerase II. Biochemistry 43(23):7503–7513
131. Mourti R, Konoki K, Matsumori N, Oishi T, Murata M (2008) Complex formation of amphotericin B in sterol-containing membranes as evidenced by surface plasmon resonance. Biochemistry 47:7807–7815
132. Mutka SC, Yang WQ, Dong SD, Ward SL, Craig DA, Timmermans PB, Murli S (2009) Identification of the nuclear export inhibitors with potent anticancer activity in vivo. Cancer Res 69(2):510–517
133. Naujokat C, Sezer O, Zinke H, Leclere A, Hauptmann S, Posinger K (2000) Proteasome inhibitors induced caspase-dependent apoptosis and accumulation of p21WAF1/Cip1 in human immature leukaemic cells. Eur J Haematol 65:221–236
170. Tan C, Tasaka H, Yu KP, Murphy ML, Kamofsky DA (1967) Daunomycin, an antitumor antibiotic, in the treatment of neoplastic disease. Clinical evaluation with special reference to childhood leukemia. Cancer 20(3):333–353

171. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J (2003) Tuberous sclerosis complex gene products, tuberin and hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toad Rheb. Curr Biol 13:1259–1268

172. Tewey KM, Rowe TC, Yang L, Halligan BD, Liu LF (1984) Adriamycin-induced DNA damage mediated by mammalian topoisomerase II. Science 226:466–468

173. Thaker MN, Walgechner N, Wright GD (2014) Antibiotic resistance-mediated isolation of scaffold-specific natural producers. Nat Protoc 9(6):1469–1479

174. Tremblay F, Brule S, Hee Um S, Li Y, Masuda K, Roden M, Sun XJ, Krebs M, Polakiewicz RD, Tomas G, Maretta A (2007) Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance. Proc Natl Acad Sci USA 104:14056–14061

175. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M et al (2004) Absence of S6 K protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature 431:200–205

176. van Hoogevest P, de Kruijff B (1978) Effect of amphotericin B on cholesterol-containing liposomes of egg phosphatidylcholine and didocosenoyl phosphatidylcholine. A refinement of the model for the formation of pores by amphotericin B in membranes. Biochim Biophys Acta 511(3):397–407

177. Vertut-Croquin A, Bolard J, Chabbert M, Gary-Bobo C (1983) Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. J Antibiot 28:721–726

178. Vincent BM, Lancaster AK, Scherz-Shouval R, Whitesell L, Lindquist S (2013) Fitness trade-offs restrict the evolution of resistance to amphotericin B. PLoS Biol 11(10):e1001692

179. Vij R, Wang M, Kaufman JL et al (2012) An open-label, single-arm, phase 2 (PX-171-004) study of single-agent carfilzomib in bortezomib-naive patients with relapsed and/or refractory multiple myeloma. Blood 199(24):5661–5670

180. Wada A, Fukuda M, Mishima M, Nishida E (1998) Nuclear export of actin: a novel mechanism regulating the subcellular localization of a major cytoskeletal protein. EMBO J 17(6):1635–1641

181. Wang S, Lloyd RV, Hutzler MJ, Rosenwald IB, Safran MS, Patwardhan NA, Khan A (2001) Expression of eukaryotic translation initiation factors 4E and 2 correlates with the progression of thyroid carcinoma. Thyroid 11(12):110–117

182. Wei D, Lei B, Tang M, Zhan CG (2012) Fundamental reaction pathway and free energy profile for inhibition of proteasome by epoxomicin. J Am Chem Soc 134(25):10436–10450

183. Welscher YM, Jones L, van Leeuwen MR, Dijkstra MR, de Kruijff B, Eitzen G, Breukink E (2010) Natamycin inhibits vacuole fusion at the priming phase via a specific interaction with ergosterol. Antimicrob Agents Chemother 54(6):2618–2625

184. White TC, Marr KA, Bowden RA (1998) Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev 11(2):382–402

185. Wingard JR, Kubilis P, Lee L, Yee G, White M, Walshe L, Bowden R, Anaissie E, Hienz L, Jister J (1999) Clinical significance of nephrotoxicity in patients treated with amphotericin B for suspected or proven aspergillosis. Clin Infect Dis 29(6):1402–1407

186. Wolff B, Sanglier JJ, Wang Y (1997) Leptomycin B is an inhibitor of nuclear export: inhibition of nucleo-cytoplasmic translocation of the human immunodeficiency virus type 1 (HIV-1) Rev protein and Rev-dependent mRNA. Chem Biol 4(2):139–147

187. Wolstenholme AJ (2012) Glutamate-gated chloride channels. J Biol Chem 287:40232–40238

188. Wong AS, Kim SO, Leung PC, Auersperg N, Pelech SL (2004) Profiling of protein kianses in the neoplastic transformation of human ovarian surface epithelium. Gynecol Oncol 82(2):305–311

189. Yang YL, Ho YA, Cheng HH, Ho M, Lo HJ (2004) Susceptibilities of Candida species to amphotericin B and fluconazole: the emergence of fluconazole resistance in Candida tropicalis. Infect Control Hosp Epidemiol 25(1):60–64

190. Yang J, Bardes ES, Moore JD, Brennan J, Powers MA, Kornbluth S (1998) Control of cyclin B1 localization through regulated binding of the nuclear export factor CRM1. Genes Dev 12(14):2131–2143

191. Yashiroda Y, Yoshida M (2003) Nucleo-cytoplasmic transport of proteins as a target of therapeutic drugs. Curr Med Chem 10:741–748

192. Yeo EJ, Ryu JH, Cho YS, Chun YS, Huang LE, Kim MS, Park JW (2006) Amphotericin B blunts erythropoietin response to hypoxia by reinforcing FIH-mediated repression of HIF1. J Biol Chem 281(5):757–768

193. Yoo V, Podwell JR (2014) Activating secondary metabolism with stress and chemicals. J Ind Microbiol Biotechnol 41(2):415–424

194. Zeman SM, Phillips DR, Cothren DM (1998) Characterization of covalent adriamycin-DNA adducts. Proc Natl Acad Sci USA 95(20):11561–11565

195. Zinzalla V, Stracka D, Oplinger W, Hall MN (2011) Activation of mTORC2 by association with the ribosome. Cell 144(5):757–768