Myxobacterial secondary metabolites: bioactivities and modes-of-action

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The myxobacteria, long a source of fascination due to their sophisticated, social lifestyles, are now increasingly recognized as multi-producers of promising natural products. Here we provide an overview of the bioactivities and modes-of-action of these secondary metabolites, with an emphasis on potential clinical applications.

1 Introduction to myxobacteria and their secondary metabolism

Myxobacteria are Gram-negative δ-proteobacteria, which occupy a wide range of habitats including soil, the bark of trees, decaying plant materials, herbivore dung, and the marine environment.[1–3] These intriguing microbes display several behavioral features that distinguish them from many other bacteria. For example, they move about on solid surfaces by gliding or creeping, much like amoebas.[4] They secrete exo-enzymes which allow them to use a range of biological macromolecules (*e.g.*, cellulose) as food sources, but are also able to prey actively on whole microorganisms such as fungi and bacteria.[5,6] In addition, under starvation conditions, they implement a cooperative developmental program involving hundreds of thousands of cells:[7] the cells aggregate to form a pseudoplasmodium-like slimy mass, which ultimately transforms into a complex, multi-cellular fruiting body harboring propagative spores.

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Another notable characteristic of the myxobacteria is their rich secondary metabolism, which places them among the best known natural product producers (*i.e.*, actinomycetes, *Bacillus* species, pseudomonads, and fungi).[8,9] To date, the approximately 7500 identified myxobacterial strains have yielded at least 100 distinct core structures (although only 67 have been reported in the primary literature to date) and some 500 derivatives.[3] The majority of these compounds are polyketides, non-ribosomal polypeptides, and their hybrids, while other structural types include terpenoids, phenyl-propanoids, and alkaloids.[10] Many myxobacterial strains produce metabolites belonging to multiple structural classes, as well as a number of chemical variants on each basic scaffold. In addition, many of the natural products exhibit unique structural features relative to compounds known from other microorganisms. Furthermore, whole-genome sequencing of several strains (refs. 11 and 12, and unpublished data) has revealed that the secondary metabolic depth of the myxobacteria is far greater than that suggested by fermentation under standard laboratory conditions. The sequenced genomes include the largest yet known from any bacterium (13.0 MBp for *Sorangium cellulosum* So ce56[12]), consistent with the strengthening correlation between genome size and the extent of secondary metabolism.

From the perspective of this review, however, the most important properties of the compounds are their rare or wholly novel modes-of-action, features which make them attractive as lead structures for drug discovery. We aim here to summarize the current state of knowledge on these promising and diverse bioactivities, highlighting specific cases where there is a potential therapeutic application.

2 Overview of the bioactivities of myxobacterial compounds

All published myxobacterial compounds (excluding four metabolites reported only in patents), their bioactivities and modes-of-action (if known) are shown in Table 1. Note: the names in the table refer to the complete family of metabolites, which in most cases encompasses multiple members (*e.g.* aurachins A–D); for drawing purposes, each compound family has been represented by the most abundant

metabolite, unless specific reference has been made to an additional compound.

2.1 Anti-fungal

The most commonly observed bioactivity among myxobacterial metabolites is anti-yeast/fungal (approximately 54%).[13] This anti-microbial activity arises from a number of different mechanisms, the most common being inhibition of electron flow within the mitochondrial respiratory chain. Interestingly, although many myxobacterial compounds exhibit this mode-of-action, it is rarely observed with natural products sourced from other bacteria.[14] The function of the mitochondrial electron transport chain is to allow the energy stored in electron carriers (NADH and $FADH_2$) to be converted into ATP. The chain, which is located in the mitochondrial inner membrane, consists of five protein complexes: NADH dehydrogenase (complex I), succinate-coenzyme Q reductase (complex II), cytochrome bc_1 (complex III), cytochrome c oxidase (complex IV) and ATP synthase. Myxobacterial compounds target one of two sites within the chain, complex I (*e.g.*, ajudazol **1**,[15,16] myxalamid **2**,[17] phenoxan **3**,[18] pyrrolnitrin **4**,[19,20] and thiangazole **5**[21]), or complex III (*e.g.* crocacin **6**,[22,23] cyrmenin **7**,[24] cystothiazole **8**,[25] haliangicin **9**,[26] melithiazol **10**,[27,28] miuraenamide **11**,[29] myxothiazol **12**[30,31] and stigmatellin **13**[32,33]), although certain metabolites exhibit cross-complex reactivity.[34] Interestingly, two members of the aurachin **14** family[35] interact with complex I, while the other two target complex III.[36,37] Inhibition of respiration may account for the cytotoxic effects of many of these metabolites towards mammalian cells, which has generally limited their development as anti-fungal drugs. Nonetheless, crocacin, melithiazol,[38] and miuraenamide[39] have all been the object of structure–activity relationship (SAR) studies, directed towards advancing the compounds as agricultural anti-fungal agents.[40] For example, a panel of simplified variants of crocacin D **15** was designed on the basis of crystallographic data and molecular modeling.[41] Among the analogues were structures which were active both in a respiration assay and against certain pathogenic fungi growing on plants, while showing significantly greater photostability than the natural compound. The other notable exception is pyrrolnitrin, which was licensed shortly after its discovery from *Pseudomonas pyrrocinia* for the treatment for fungal infections of the skin.[42] It also served as a lead structure for synthesis of several more light-stable analogues, fenpiclonil **16** and fludioxonil, which are presently in use as agrochemical fungicides. Interestingly, pyrrolnitrin is one of the few myxobacterial natural products to have a structure identical to a compound produced by other bacterial groups, including *Serratia* sp. and Pseudomonads.

Undoubtedly, myxobacterial respiration inhibitors have found the greatest utility in 'chemical genetics'[43,44] experiments to elucidate the detailed mechanisms of electron transport. For example, the discovery that different metabolites target distinct sub-sites within cytochrome bc_1 was instrumental in the development of the 'Q cycle hypothesis', which explains the coupled electron and proton transfers achieved by this protein complex.[45] The specific binding patterns to bc_1 can at least partially be rationalized in terms of chemical structure: cyrmenin **7**, cystothiazole **8**, haliangicin **9**, melithiazol **10**, miuraenamide **11** and myxothiazol **12** all interact with the same sub-site, consistent with the presence of a common β-methoxyacrylate (MOA) pharmacophore in these structures, stigmatellin **13** binds to a second sub-site, and crocacin **6**, which shares a C_6-fragment with stigmatellin, occupies both sites simultaneously.[41]

Several myxobacterial compounds disrupt fungal macromolecule synthesis: the unusual, mixed polyketide-nonribosomal peptide-terpenoid metabolite leupyrrin **17** interrupts DNA replication and transcription, while both leupyrrin and gephyronic acid **18** inhibit translation.[46,47] Cell membrane integrity is the target of three different metabolites, ambruticin **19**,[48,49] jerangolid **20**[50] and pedein **21**.[51] Ambruticin, the polyketide product

Kira J. Weissman

Kira J. Weissman obtained her BS in Chemistry from Stanford University, California, in 1995. Receipt of a Churchill Fellowship allowed her to relocate to the United Kingdom, where she carried out MPhil (1996), PhD (2000) and post-doctoral research at the University of Cambridge with Professors Jim Staunton and Peter Leadlay. Her work at Cambridge was supported by several fellowships, including a Royal Society Dorothy Hodgkin Fellowship. She is presently a Senior Scientist at the University of Saarland, Saarbrücken, Germany, working with Prof. Rolf Müller. Kira's research interests include the mechanistic enzymology and structural biology of modular PKS and NRPS systems.

Rolf Müller

Rolf Müller studied pharmacy in Bonn and received his PhD in 1994 with Eckhard Leistner. After postdoctoral work with Heinz Floss he moved to the German Research Center for Biotechnology in Braunschweig as a junior research group leader. The group moved to Saarland University in 2003 (Chair of Pharmaceutical Biotechnology). In 2006, Rolf also assumed a position at the Helmholtz Center for Infection Research (HZI) in Braunschweig. In 2009, Saarland University and HZI founded the Helmholtz Institute for Pharmaceutical Research Saarland, where Rolf now serves as founding director. He coordinates efforts to develop an integrated and multidisciplinary approach to studying myxobacteria.

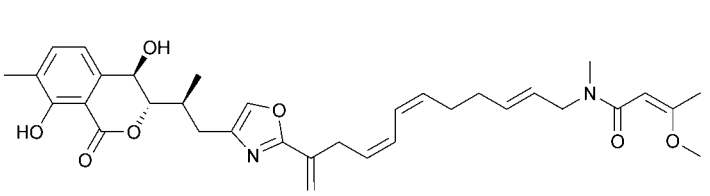
Table 1 Complete list of known myxobacterial metabolites and their activities[a]

Compound family	No. of metabolites in family	Structural class	Biological activity; mode-of-action	Refs.
Ajudazol **1**	2	Isochromanone	Anti-fungal, anti-bacterial (w); not reported	15,16
Althiomycin **40**	1	Cyclic peptide	Anti-bacterial; inhibits the peptidyltransferase reaction	75,78
Ambruticin **19**, **23**	6	Lactone	Anti-fungal; interferes with osmoregulation *via* the HOG pathway	49,53,54
Angiolam **39**	≥1	Macrolactone	Anti-bacterial; inhibits protein synthesis	74
Apicularen **45**	2	Benzolactone enamide	Cytotoxic, anti-bacterial (w); inhibits vacuolar-type ATPases, disrupts microtubule architecture, induces mitochondria-independent apoptosis	91,101,102,130
Archazolid **55**	2	Macrolactone	Cytotoxic; inhibits vacuolar-type ATPases	125,130
Argyrin **24**	8	Peptolide	Cytotoxic, immunosuppressant, anti-bacterial (w), anti-fungal (w); proteasome inhibitor, inhibits antibody formation	57,137
Aurachin **14**	4	Quinolone	Anti-fungal, anti-bacterial; inhibits respiration (complexes I, III)	35
Aurafuron **25**	2	Furanone	Anti-fungal, anti-bacterial (w), cytotoxic; not reported	58
Carolactone **46**	1	Macrolactone	Anti-bacterial (anti-biofilm); not reported	92
Chivosazol **26**	7	Macrolactone	Cytotoxic; inhibits actin polymerization	59,100,122
Chlorotonil **68**	1	Macrolactone	None reported	155
Chondramide **27**	4	Depsipeptide	Cytotoxic, anti-fungal; inhibits actin polymerization	60,115,118
Chondrochloren **28**	2	Styrene	Anti-bacterial (w), anti-fungal (w); not reported	61
Corallopyronin **35**	3	α-Pyrone	Anti-bacterial; inhibits RNAP	69,183
Crocacin **6**	4	*N*-Acylpeptide	Anti-fungal, cytotoxic; inhibits respiration	22,23
Cruentaren **56**	2	Benzolactone enamide	Cytotoxic, anti-fungal; inhibits mitochondrial F_0F_1-ATPase	133
Cyrmenin **7**	3	*N*-Acylpeptide	Anti-fungal, cytotoxic (relatively w); inhibits respiration (complex IIII)	24
Cystothiazole **8**	2	Bithiazole	Anti-fungal, cytotoxic; inhibits respiration (complex III)	25
Dawenol **69**	1	Polyene	None reported	156
Disorazol **51**, **72**	29	Macrodilactone	Cytotoxic, anti-fungal; inhibits tubulin polymerization	97,189,192
Dkxanthene **66**	13	Polyene	Pheromone-like role in myxospore formation	153
Epothilone **53**	4	Macrolactone	Cytotoxic, anti-fungal; promotes tubulin polymerization	206–208
Etnangien **36**	1	Macrolactone	Anti-bacterial, antiviral (HIV-1); not reported	70
Gephyronic acid **18**	1	Aliphatic acid	Anti-fungal, cytotoxic; inhibits protein synthesis	47
Haliangicin **9**	4	Polyene	Anti-fungal, cytotoxic; inhibits respiration (complex III)	26
Jerangolid **20**	1	Lactone	Anti-fungal; alters membrane permeability	50
Leupyrrin **17**	6	Macrodilactone	Anti-fungal, cytotoxic; inhibits DNA, RNA and protein synthesis	46
Maracin **47**/maracen **48**	2	Vinyl ether	Anti-bacterial (mycobacteria)	93
Melithiazol **10**	13	Bithiazole	Anti-fungal, cytotoxic; inhibits respiration (complex III)	27,28
Miuraenamide **11**	6	Depsipeptide	Anti-fungal; inhibits respiration (complex III)	29,39
Myxalamid **2**	4	Polyene	Anti-fungal; inhibits respiration (complex I)	17
Myxochelin **49**	2	Catechol		94,147,149

Table 1 (*Contd.*)

Compound family	No. of metabolites in family	Structural class	Biological activity; mode-of-action	Refs.
			Anti-bacterial, antiviral (cytomegalovirus), inhibition of tumor invasion; siderophore	
Myxochromide **58**	7	Lipopeptide	Cytotoxic (w); not reported	141
Myxopyronin **37**	2	α-Pyrone	Anti-bacterial; inhibits RNAP	71,183
Myxothiazol **12**	1	Bithiazole	Anti-fungal, cytotoxic; inhibits respiration (complex III)	31,95,209
Myxotyroside **59**	2	Rhamnoside	Cytotoxic (w), antiplasmodial (w); not reported	142
Myxovalargin **41**	4	Peptide	Anti-bacterial, cytotoxic (w), antiplasmodial (w); inhibits protein synthesis, damages cell membranes	76,77,80
Myxovirescin **44**	≥12	Macrolide	Anti-bacterial; interferes with cell wall synthesis	83,86
Nannochelin **29**	3	Hydroxamate	Anti-bacterial, anti-fungal; siderophore	62
Pedein **21**	2	Cyclic peptide	Anti-fungal, cytotoxic (w); disrupts membrane integrity	51
Phenalamide **63** (stipiamide)	5	Polyene	Anti-viral (HIV-1), reverses multi-drug resistance; not reported	146,150
Phenoxan **3**	1	γ-Pyrone	Anti-fungal; inhibits respiration (complex I)	18
Phenylnannolone **64**	3	Polyene	Reverses daunorubicin resistance; not reported	151
Phoxalone **60**	1	Macrolide	Cytotoxic; none reported	143
Pyrrolnitrin **4**	1	Phenylpyrrole	Anti-fungal, anti-bacterial; inhibits respiration (complex I)	19,20
Ratjadon **57**	4	α-Pyrone	Cytotoxic; inhibits formation of the nuclear export complex	138,139
Rhizopodin **30**	1	Macrodiolide	Cytotoxic, anti-fungal (w); inhibits actin polymerization	63,120,121
Ripostatin **31**	2	Macrolactone	Anti-bacterial, anti-fungal (w); inhibits bacterial RNAP	64
Saframycin Mx1 **50**	1	Isoquinoline	Anti-bacterial, cytotoxic; binds DNA	96
Sorangiadenosine **32**	1	Nucleoside	Anti-bacterial; not reported	65
Sorangicin **38**	2	Macrolactone	Anti-bacterial; inhibits RNAP	72,188
Sorangiolid **42**	1	Macrolactone	Anti-bacterial; disrupts membrane integrity	81
Soraphen **23**	≥50	Macrolactone	Anti-fungal, anticancer, anti-metabolic syndrome; inhibition of acetyl-CoA carboxylase (ACC), BC domain	159,160,175,177,178
Soraphinol **65**	3	Indole alkaloid	Radical scavenger	152
Spirangien **61**	2	Spiroketal	Cytotoxic, anti-fungal; not reported	144
Spirodienal **62**	2	Spiroketal	Cytotoxic; not reported	145
Stigmatellin **13**	1	γ-Chromone	Anti-fungal; inhibits respiration (complex I)	32,33
Stigmolone **67**	1	Ketone	Pheromone-like role in fruiting body formation	154
Tartrolon **43**	2	Macrodiolide	Anti-bacterial, cytotoxic; disrupts membrane integrity	82
Thiangazole **5**	1	Tris-thiazolonine	Cytotoxic; inhibits respiration (complex I)	21
Thuggacin **34**	6	Macrolide	Anti-bacterial; inhibits respiration	67,68
Tubulysin **52**	33	Peptide	Cytotoxic; inhibits tubulin polymerization	210,211
Tuscolid **70**	1	Macrolide	None reported	157
Tuscoron **71**	2	Furanone	None reported	157
Vioprolide **33**	4	Peptolide	Anti-fungal, cytotoxic; not reported	66

[a] Survey of all secondary metabolites produced by myxobacteria, and published in the scientific literature (excluding patents) up to the present. w = weak.



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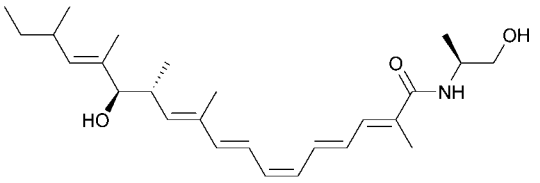

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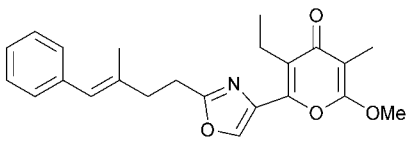

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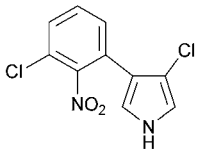

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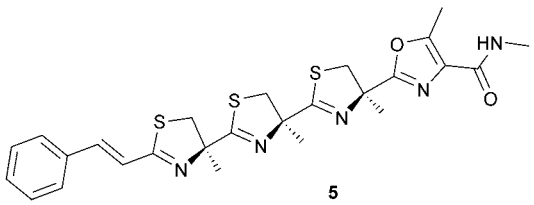

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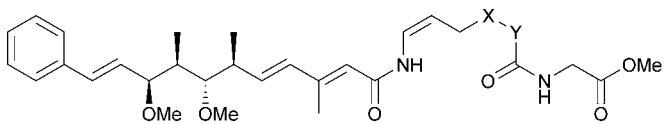
of an unusual biosynthesis involving a carbon excision,[52] interferes with the ability of cells to osmoregulate. It acts by hyperstimulating the high-osmolarity glycerol (HOG) protein kinase signaling pathway, leading to overproduction of glycerol.[53] In the absence of high external osmolarity, the elevated intracellular glycerol results in the leakage of cellular contents, and ultimately, in cell death. Ambruticin S and two analogues of ambruticin VS3[54] were shown recently to cure mice of acute coccidodiomycosis and histoplasmosis,[55] while administration of VS3 **22** itself improved survival in a murine model of invasive pulmonary aspergillosis.[56] These promising initial results argue for continued development of the ambruticins as anti-fungal agents. Ambruticin's close structural relative jerangolid **20**, and the 24-membered cyclic hexapeptide pedein **21**, appear to cause more direct changes in membrane permeability. These effects may reflect an underlying disturbance to membrane synthesis as proposed for jerangolid,[50] but more studies will be required to fully elucidate the mechanisms. Pyrrolnitrin **4** exhibits cross-resistance with both ambruticin and jerangolid,[50] and therefore it may also target the cell membrane.



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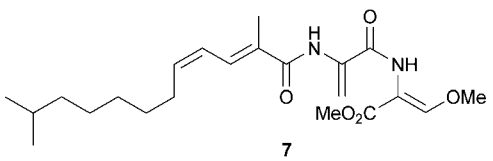

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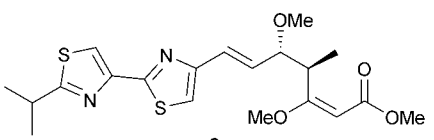

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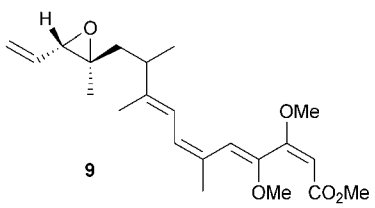

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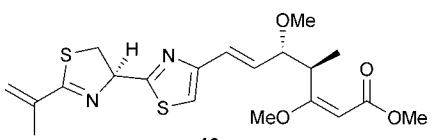

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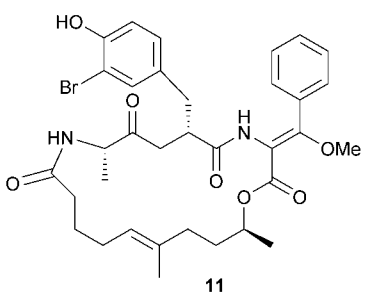

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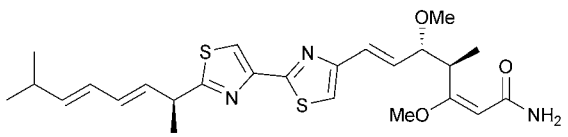

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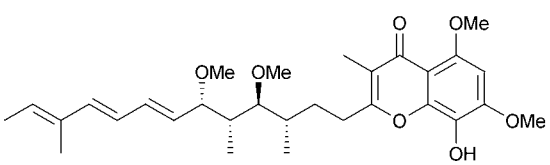

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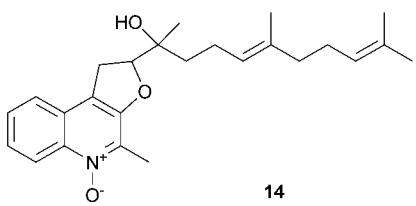

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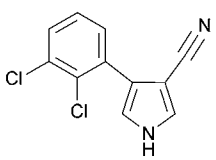

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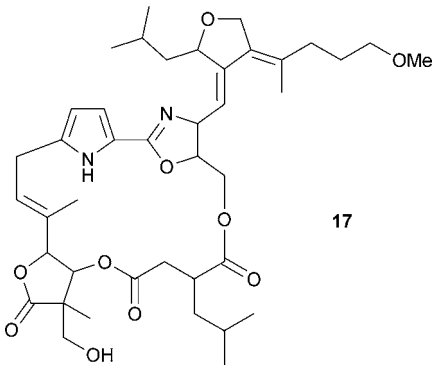

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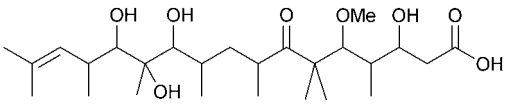

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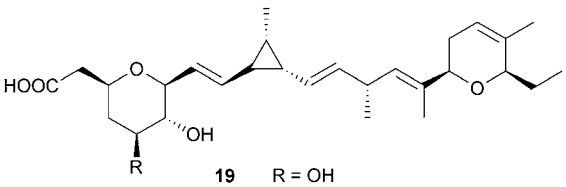

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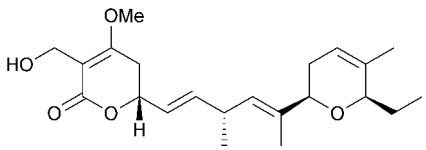

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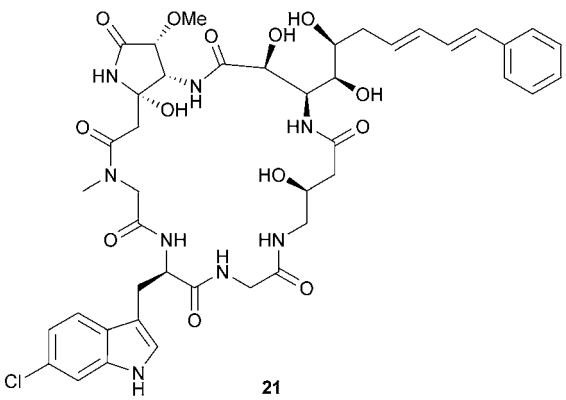

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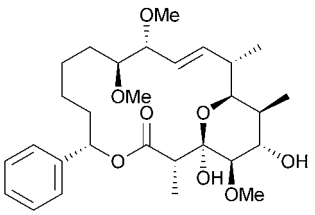
The polyketide metabolite soraphen **23** exhibits an apparently unique mode-of-action, targeting the enzyme acetyl-CoA carboxylase (ACC). The story of soraphen's discovery and development will be related more comprehensively in section 3.1. Finally, a number of myxobacterial natural products exhibit some level of anti-fungal activity, but nothing is yet known about their specific modes-of-action. These include argyrin **24** (weak),[57] aurafuron **25**,[58] chivosazol **26**,[59] chondramide **27**,[60] chondrochloren **28** (w),[61] nannochelin **29**,[62] rhizopodin **30** (w),[63] ripostatin **31** (w),[64] sorangiadenosine **32**[65] and vioprolide **33**.[66] In the case of argyrin, chivosazol and chondramide, the targets of the molecules in mammalian cells are known (see section 2.3), and the same mode-of-action may explain their effects on fungi, while nannochelin's anti-fungal activity may arise from chelation or iron.



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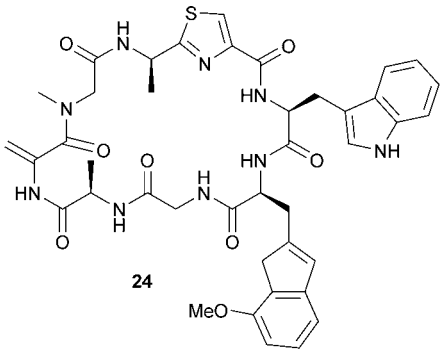
2.2 Anti-bacterial

Some 29% of myxobacterial metabolites exhibit anti-bacterial activity.[13] Electron transport is so far known to be the target of a single metabolite, thuggacin **34**,[67,68] though the precise step at which the compound interferes with respiration has not yet been determined. Notably, thuggacin shows good activity against clinical isolates of *Mycobacterium tuberculosis*, the causative agent of tuberculosis.[67]

Five compounds, corallopyronin **35**,[69] etnangien **36**,[70] myxopyronin **37**,[71] ripostatin **31**[64] and sorangicin **38**,[72] act through inhibition of the eubacterial RNA polymerase. The detailed mode-of-action for all metabolites with the exception of etnangien has been elucidated (see section 3.2). Etnangien **36**, a complex lactone bearing a C_{21} carboxylic acid side chain, additionally disrupts the function of bacterial DNA polymerase. Its potency against a range of Gram-positive bacteria, notably including mycobacteria, makes it an interesting lead molecule for further study.[70] Indeed, etnangien has already been successfully modified to mitigate the inherent acid sensitivity of the polyunsaturated side chain, by direct esterification of the parent compound in a crude extract, followed by purification.[73] The resulting methyl ester retained good activity against a broad panel of Gram-positive bacteria, including a rifampicin-resistant strain of *Staphylococcus aureus*.

A number of myxobacterial compounds inhibit protein synthesis, including angiolam **39**, a branched, macrocyclic mixed polyketide-nonribosomal peptide,[74] althiomycin **40**[75] and myxovalargin **41**.[76,77] Althiomycin, which is also produced by several *Streptomyces*, disrupts translation at the peptidyltransfer stage.[78] It is a broad-spectrum agent, with low cytoxicity and good selectivity towards prokaryotic cells. Taking advantage of the relative simplicity of its structure, some 50 althiomycin analogues have been generated using a combination of semi-synthesis and total synthesis.[79] However, only one of the new compounds showed appreciable antibiotic activity, demonstrating the very restricted SAR of the metabolite. Myxovalargin, the first true peptide to be isolated from myxobacteria, disrupts the binding of the aminoacyl-tRNA to the A site.[80] Myxovalargin is also one of three myxobacterial natural products which cause damage to cell membranes. This activity, observed at more elevated concentrations, leads to



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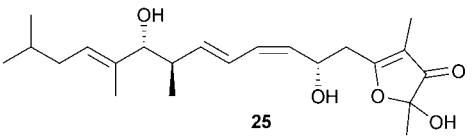

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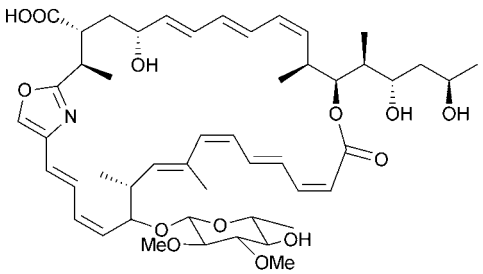

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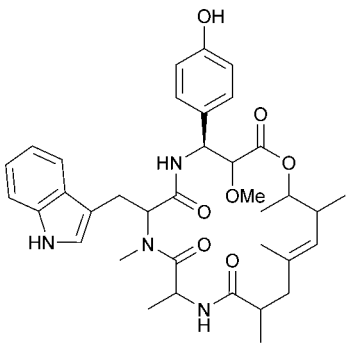

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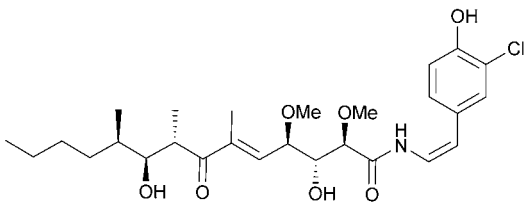

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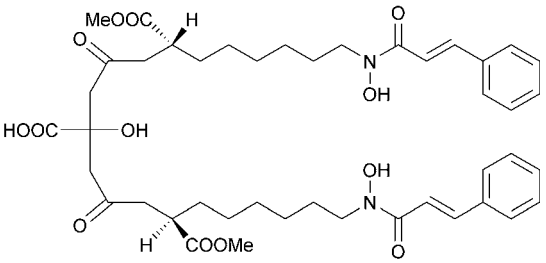

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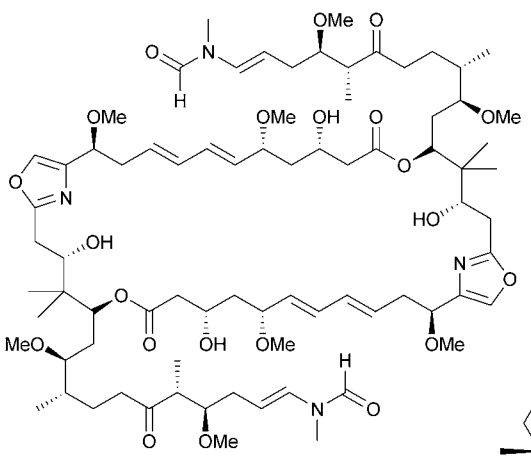
several secondary effects such as decreased O_2 consumption and the breakdown of RNA synthesis, perhaps explaining the high toxicity of myxovalargin towards animal cells.[80] Membrane integrity is also affected by the macrocylic polyketide sorangiolid **42**,[81] and the unusual boron-containing polyketide, tartrolon **43**,[82] although the detailed mechanism in each case remains to be elucidated.

The mixed polyketide-nonribosomal polypeptide myxovirescin **44** (antibiotic TA),[83–85] acts at an earlier stage, interfering with cell wall synthesis by inhibiting the incorporation of



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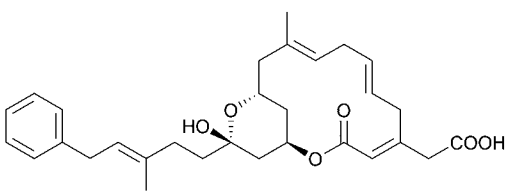

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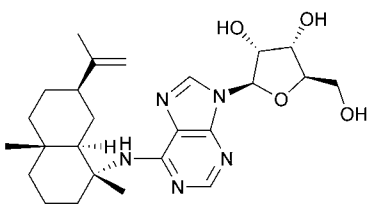

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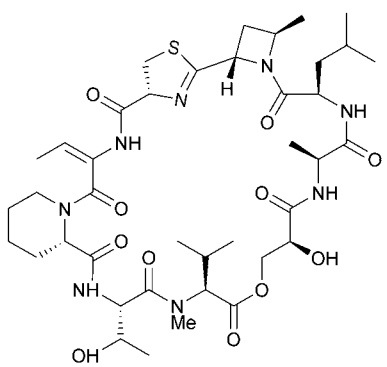

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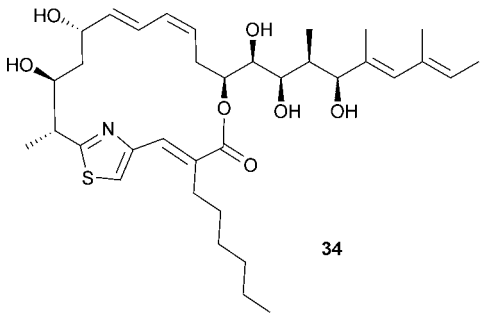

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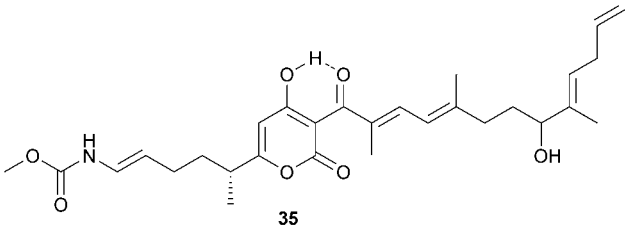

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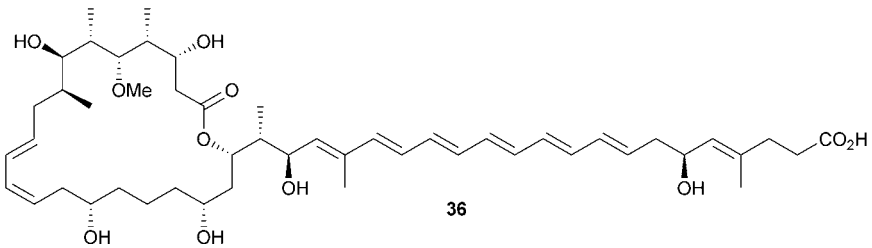

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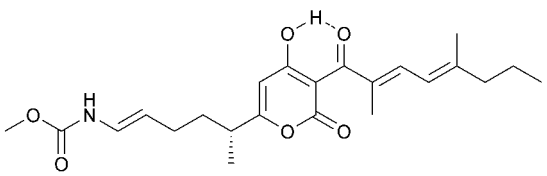

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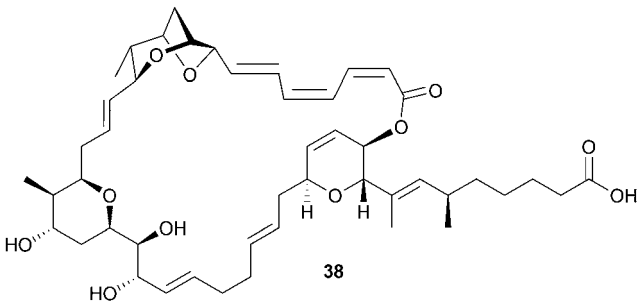

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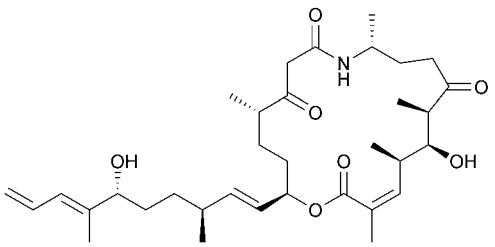

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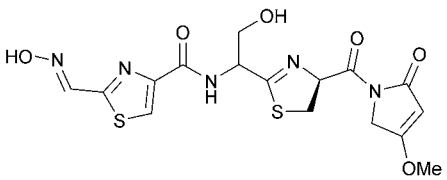

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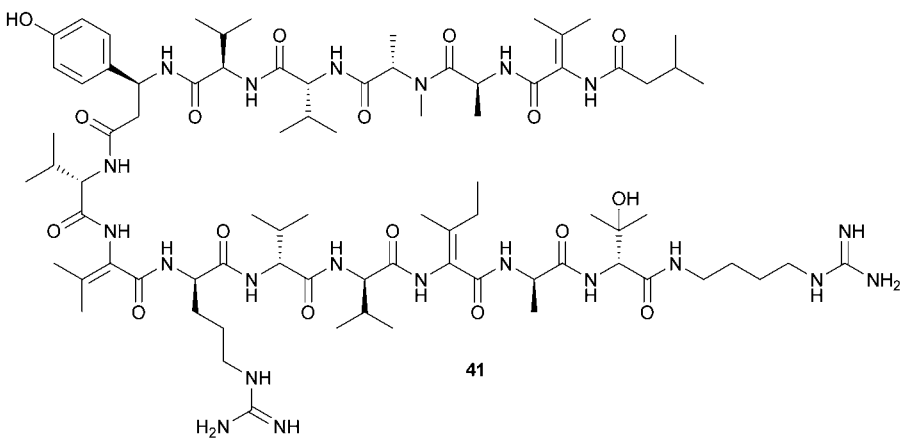

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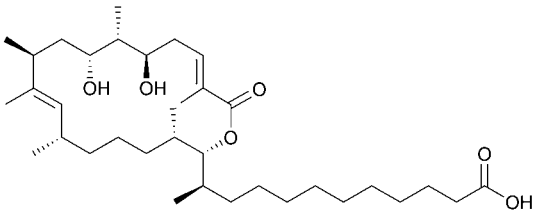

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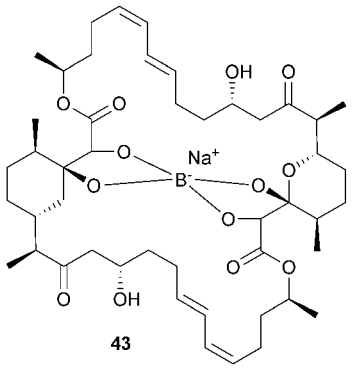

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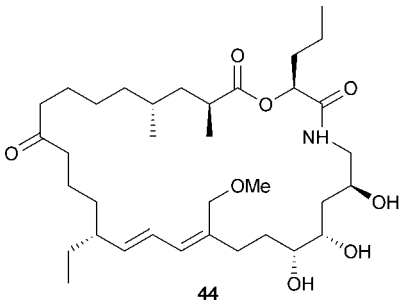

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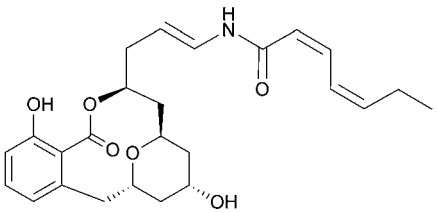

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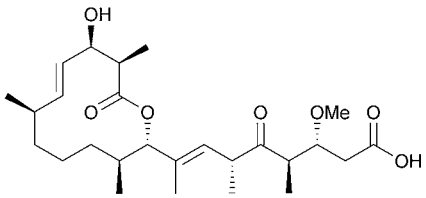

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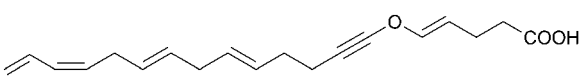
N-acetyl-glucosamine.[86] Myxovirescin exhibits a second useful property which is strong adherence to a variety of surfaces, including dental tissues and rubber, while retaining its anti-microbial activity. This observation has led to experiments to investigate its applicability for the treatment of plaque and gingivitis,[87–89] as well as for catheter-associated urinary tract infections.[90]

Many other myxobacterial natural products exhibit some level of anti-bacterial activity by an as yet unidentified mechanism. These include ajudazol **1** (w),[15] apicularen **45** (w),[91] argyrin **24** (w),[57] aurachin **14**,[35] aurafuron **25**,[58] carolactone **46**,[92] chondrochloren **28** (w),[61] maracin **47**/maracen **48**,[93] myxochelin **49**,[94] myxothiazol **12** (w),[95] nannochelin **29**,[62] saframycin Mx1 **50**,[96] and sorangiolid **42**.[81]

2.3 Cytotoxic towards mammalian cells

One of the most promising bioactivities exhibited by myxobacterial substances is cytotoxicity towards mammalian cells, due to the potential to deploy these compounds in cancer chemotherapy. Multiple metabolites have been discovered to date which interfere with the eukaryotic cytoskeleton: disorazol **51**,[97] tubulysin **52**[98] and epothilone **53**[99] all target tubulin, while chivosazol **26**,[59,100] chondramide **27**,[60] and rhizopodin **30**[63] interact with actin. From a mechanistic perspective, the compounds can be divided into two groups: chivosazol, disorazol, tubulysin, and rhizopodin destablize their polymeric targets, while chondramide and epothilone stabilize them. Despite shared molecular targets and/or biological effects, each compound belongs to a distinct class of secondary metabolite.



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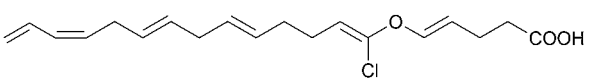

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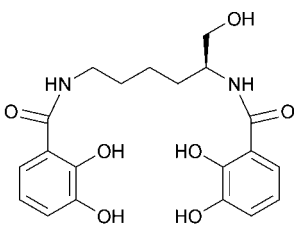

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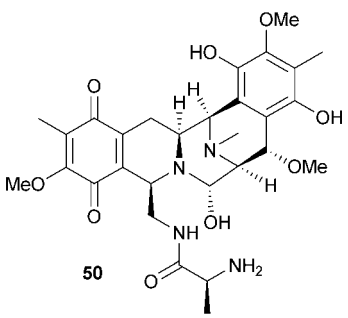

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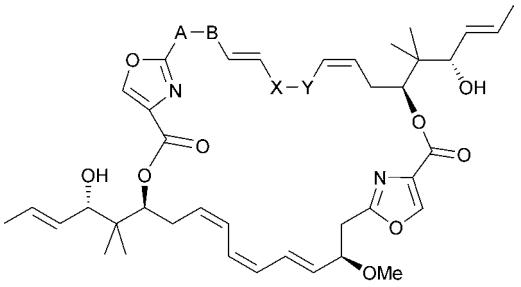
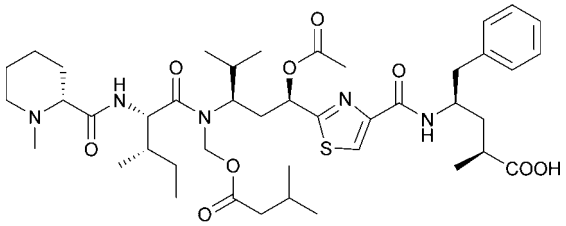
The benzolactone enamide apicularen **45**[101] also disrupts microtubule architecture but by a distinct mechanism, down-regulation of tubulin synthesis.[102]



51 A–B = ═ X–Y = 

(cf. structure **72**)



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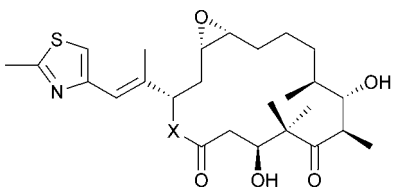


53 X = O

54 X = NH

Microtubules, structural proteins found in nearly all eukaryotic cells, are polymers of α- and β-tubulin dimers. In conjunction with other cytoskeletal components including actin microfilaments and intermediate filaments, these structures participate in multiple, basic cellular processes such as the segregation of genetic material, intracellular transport, maintenance of cell shape, positioning of organelles, and the movement of cells by means of flagella and cilia. Compounds which modulate microtubule dynamics are promising anti-neoplastic agents, as interference with microtubule function during mitotic spindle formation causes cell cycle arrest in mitosis, and ultimately, the induction of apoptosis (programmed cell death).[103] Clinical agents with this mode-of-action include the taxanes (promote polymerization) and the *Vinca* alkaloids (inhibit polymerization).[104]

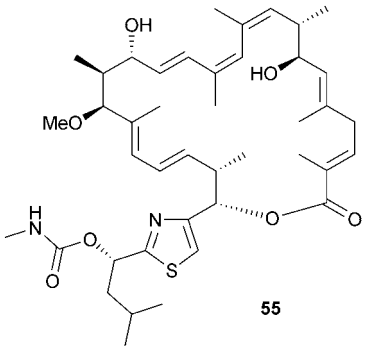
Both epothilone and tubulysin have been the target of concerted programs based on total synthesis, semi-synthesis, and biosynthetic approaches, to establish comprehensive structure–activity relationships and to improve their physico-chemical and pharmacological properties. These efforts have been summarized in a number of recent reviews (the reader is referred to refs. 105–110 for epothilone, and refs. 106 and 111 for tubulysin), and so the subject will not be covered further here. However, it must be highlighted that a semi-synthetic analogue of epothilone B, ixabepilone **54**,[112] was the first natural product made exclusively by myxobacteria to be approved for clinical use in the US for the treatment of aggressive breast cancer. A number of additional modified epothilones are progressing through clinical trials,[105] while pre-clinical evaluation of tubulysin has also been encouraging.[113] Development of disorazol **51** as an anti-cancer drug is also on-going (discussed in more detail in section 3.3).

The mixed polyketide-nonribosomal peptide macrolide chondramide **27** induces actin polymerization at low doses.[114,115] Molecular docking experiments suggest that the metabolite adopts a binding mode similar to the established actin-imaging agent phalloidin,[116] at the point of contact between three actin protein monomers in the polymeric filament, a model consistent with initial SAR data.[117,118] Two other mixed metabolites, rhizopodin **30** and chivosazol **26**, cause the opposite effect, actin depolymerization. Rhizopodin has a particularly dramatic impact on cells, provoking the formation of long, branching and reticular runners, which resemble the rhizopodia of certain protozoa (hence the compound's name).[63,119] Recently, the original structure elucidation was revised to reveal that rhizopodin is a unique symmetrical, dimeric bis-lactone, incorporating two side chains which terminate in methylvinylformamide groups.[120] This symmetry explains its function as a bivalent inhibitor, forming a ternary complex with two monomers of actin.[121] Although chivosazol also promotes actin disassembly, its detailed mode-of-action is distinct from that of rhizopodin, as both compounds give rise to characteristic, yet different phenotypic changes.[122] Further detailed studies should shed light on the molecular basis for this discrepancy.

As small molecules which interfere with the dynamic assembly and disassembly of actin have proven value as probes of its function, chivosazol, chrondramide and rhizopodin are likely to be useful additions to this chemical biology 'toolbox'. All three compounds also exhibit anti-proliferative activity towards cancer cells, which makes them interesting candidates for drug development. Chondramide appears particularly attractive in this respect, as it is cell-permeable. However, as remodeling of actin as a strategy for chemotherapeutic drug development has received scant attention to date,[123,124] the real medicinal potential of the compounds remains uncertain.

The cytotoxic effects of both apicularen **45**[101] and the macrolactone archazolid **55**[125] arise from inhibition of vacuolar ATPases (V-ATPases). V-ATPases are a family of hetero-multimeric, proton-translocating proteins, which consist of a proton-translocating complex (V_o), and a catalytic complex (V_i). These machineries occur in the endomembrane system of all eukaryotic cells, and in the plasma membrane of many animal cells. According to their cellular location, they energize various transport processes or regulate the pH of the corresponding compartments. The centrality of their function is reflected in the fact that aberrant V-ATPase function has been implicated in a wide range of disease states, including diabetes, osteoporosis, Alzheimer's, and cancer.[126–128] The potential to target V-ATPases in clinical therapy has motivated efforts to identify binding sites on the enzyme for both archazolid and apicularen,[129,130] as well as SAR studies[129,131,132] to uncover crucial binding elements on these molecules. In addition to its effects on V-ATPases and tubulin

synthesis, apicularen has also been shown to induce apoptosis in cancer cell lines *via* the mitochondria-independent Fas-L/caspase-8/caspase-3 cascade.[102]



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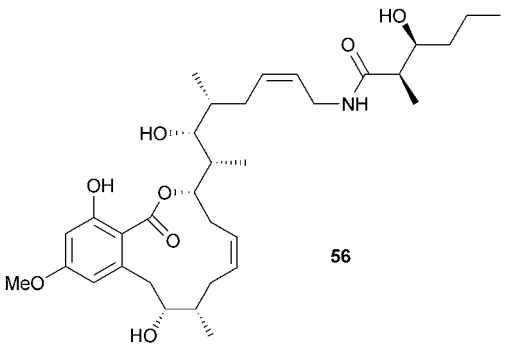
Although a close structural relative of apicularen, cruentaren **56**[133] does not affect V-ATPases. Instead, its extremely high cytotoxicity (IC_{50} of 1.2 ng/mL against mouse fibroblast cell line L929) arises from inhibition of the evolutionarily-related F_oF_1ATPases, through interaction with the F_1 complex.[134] As mitochondrial ATPases also play crucial roles in the pathology of several human disorders including cancer, cruentaren and its synthetic derivatives may form the basis for future therapeutic strategies. Initial SAR studies have already been performed, although most modifications resulted in reduced potency.[135,136]



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A number of modes-of-action have to date been documented for only a single myxobacterial metabolite. These include inhibition of the proteasome (argyrin **24** (see section 3.4)[57,137]), protein synthesis (gephyronic acid **18**[47]), and nuclear export complex formation (ratjadon **57**[138,139]), disruption of membranes (sorangiolid **42**[81]), and binding to DNA (saframycin Mx1 **49**[96]). For the remaining compounds in this category, the molecular basis for cytotoxicity remains unknown. This is the case for aurafuron **25**,[58] myxochromide **58** (w),[140,141] myxotyroside **59** (w),[142] phoxalone **60**,[143] spirangien **61**,[144] spirodienal **62**[145] and vioprolide **33**.[66]

2.4 Additional activities

A wide variety of additional bioactivities have been noted. These include immunosuppressive (argyrin),[57] anti-viral (anti-HIV-1: etnangien **36**,[70] phenalamide **63**,[146] phenoxan **3**[18] and thiangazole **5**;[21] anti-cytomegalovirus: myxochelin **49**[147]), antiplasmodial/antimalarial (aurachin **14**,[148] myxotyroside **59** (w),[142] myxovalargin[76]), inhibition of tumor invasion (myxochelin[149]) and the ability to reverse P-glycoprotein-mediated multi-drug resistance, a phenomenon common to many cancer cell lines (phenalamide **63**,[150] and phenylnannolone **64**[151]). A number of other compounds exhibit properties of less clinical value, but more obvious importance to the microorganisms themselves. These include the iron-chelating activities of myxochelin[94] and nannochelin **29**,[62] the antioxidant properties of soraphinol **65**,[152] and the pheromone-like roles of DKxanthene **66**[153] and stigmolone **67**[154] in fruiting body formation. On the other hand, no biological role of any sort has yet been identified for a small subset of myxobacterial compounds, including chlorotonil **68**,[155] dawenol **69**,[156] and tuscolid **70**/tuscoron **71**.[157]

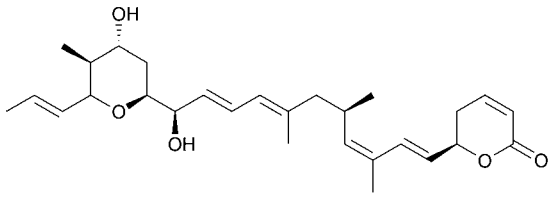
3 Highlights of metabolite modes-of-action: case studies

In this section we relate four case-studies, which serve to highlight the future promise of myxobacterial metabolites in clinical therapy, but at the same time, the pitfalls that all compounds face as they progress through the lengthy drug development process. As indicated above, two of the most promising candidate compound classes (epothilones and tubulysins) have recently been reviewed elsewhere.[105,106–111]

3.1 Soraphen: an inhibitor of acetyl-CoA carboxylase

Soraphen has been aptly christened 'an almost success story'.[158,159] Soraphen A **23**, the most abundant metabolite, was isolated from culture broths of *Sorangium cellulosum* So ce26 in 1994 by the natural products group at the Helmholtz Zentrum für Infektionsforschung (Helmholtz Center for Infection Research (HZI), formerly the Gesellschaft für Biotechnologische Forschung (Society for Biotechnological Research (GBF)).[160] The compound is a polyketide macrolactone, which incorporates an unusual, unsubstituted phenyl ring derived from benzoate.[161] Initial bioactivity studies revealed that soraphen A exhibited potent activity against yeast and molds (MIC, 0.03–4 μg/mL), and only moderate cytotoxicity towards mouse fibroblasts (10 μg/mL). The high, broad-spectrum anti-fungal activity of soraphen A fueled an intensive developmental program, carried out in collaboration by academic groups from five countries and an industry partner, Ciba-Geigy.[158] Early results against phytopathogenic fungi on plants in greenhouse tests, and more critically, in the field, were encouraging, and so soraphen was targeted for development as a plant protective agent – an attractive biological alternative to chemically-synthesized compounds. The efforts focused on several related aspects, including strain improvement by mutagenesis and selection, as well as growth optimization, ultimately yielding a strain of So ce26 capable of producing 2 g/L soraphen A (a 4000-fold increase over the parent strain). Large-scale fermentation also led to the discovery of some 50 chemical variants of soraphen A, making the soraphens the largest known family of myxobacterial secondary metabolites to date.

These efforts also led to the elucidation of soraphen A's mode-of-action, which remains entirely novel. The first insights derived from the observation that growth inhibition of animal cells by soraphen A could be reversed by addition of fatty acids to the medium.[162] This finding suggested that soraphen



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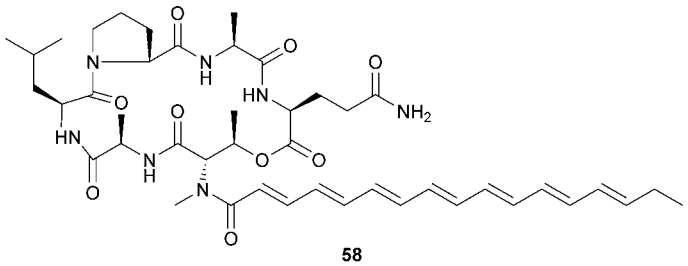

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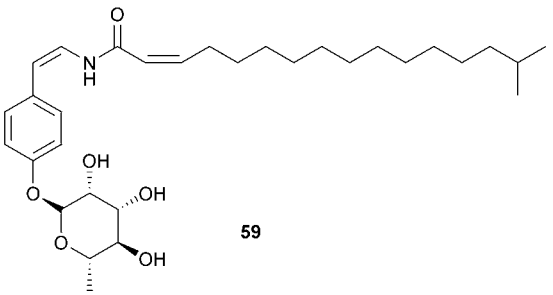

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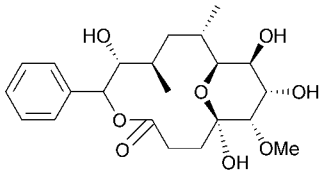

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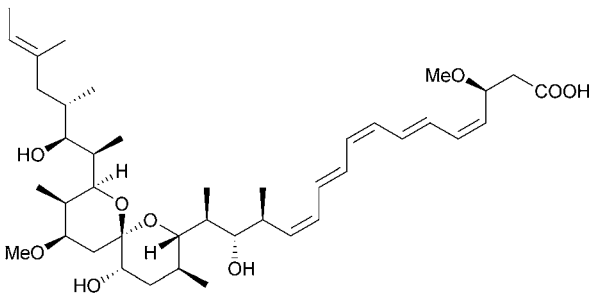

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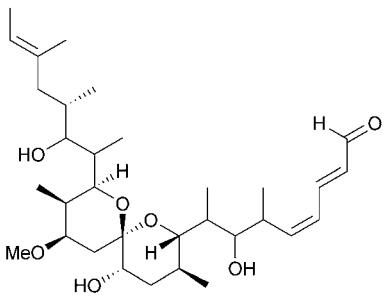

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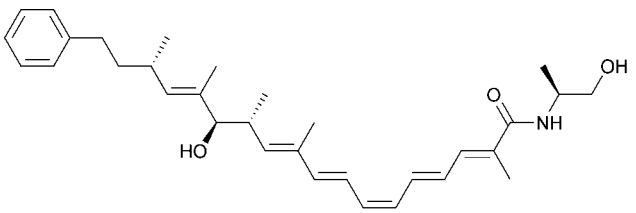

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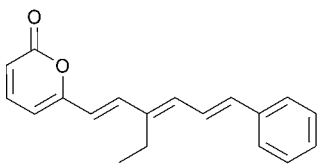

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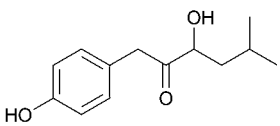

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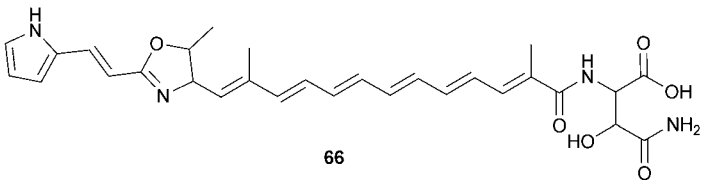

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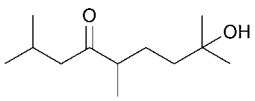

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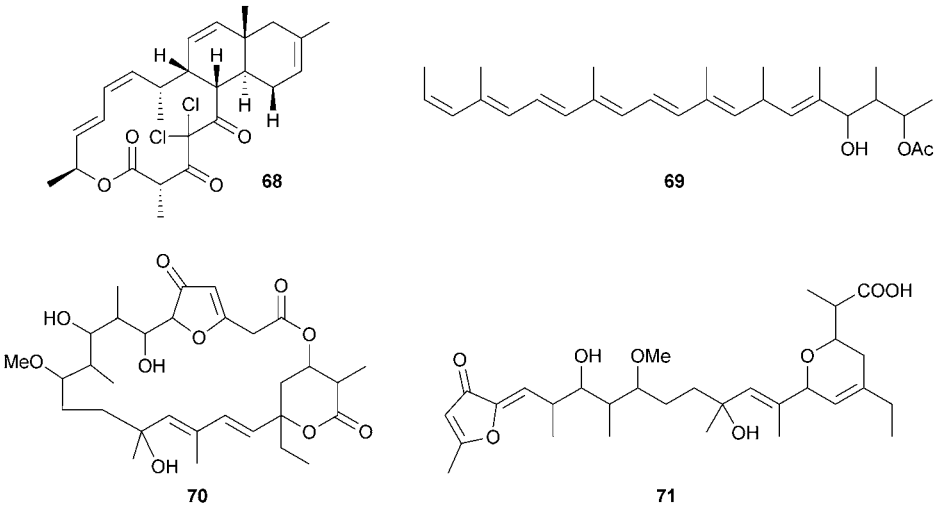
interferes with a step in the biosynthesis of fatty acids. This expectation was then confirmed by *in vitro* experiments using extracts of the causative agent of corn smut, *Ustilago maydis*, which demonstrated that soraphen A inhibits acetyl-CoA carboxylase (ACC). ACC is a biotin-dependent enzyme that catalyzes the carboxylation of acetyl-CoA to produce the malonyl-CoA building block of fatty acids, using two catalytic activities, biotin carboxylase (BC) and carboxyltransferase (CT).[163] *In vivo* evidence for the same inhibitory action was subsequently provided by isolation of soraphen-resistant mutants of the yeast *Saccharomyces cerevisiae*, followed by mapping of the mutations to a specific locus in the genome. All mutations were localized to the *acc1* gene, suggesting that ACC is the only major biochemical target of soraphen A in yeast.[159] Inhibition by soraphen A of the *S. cerevisiae* ACC was again directly confirmed *in vitro* using partially-purified enzyme, while the corresponding enzymes of plants and bacteria were insensitive to the antibiotic.[160]



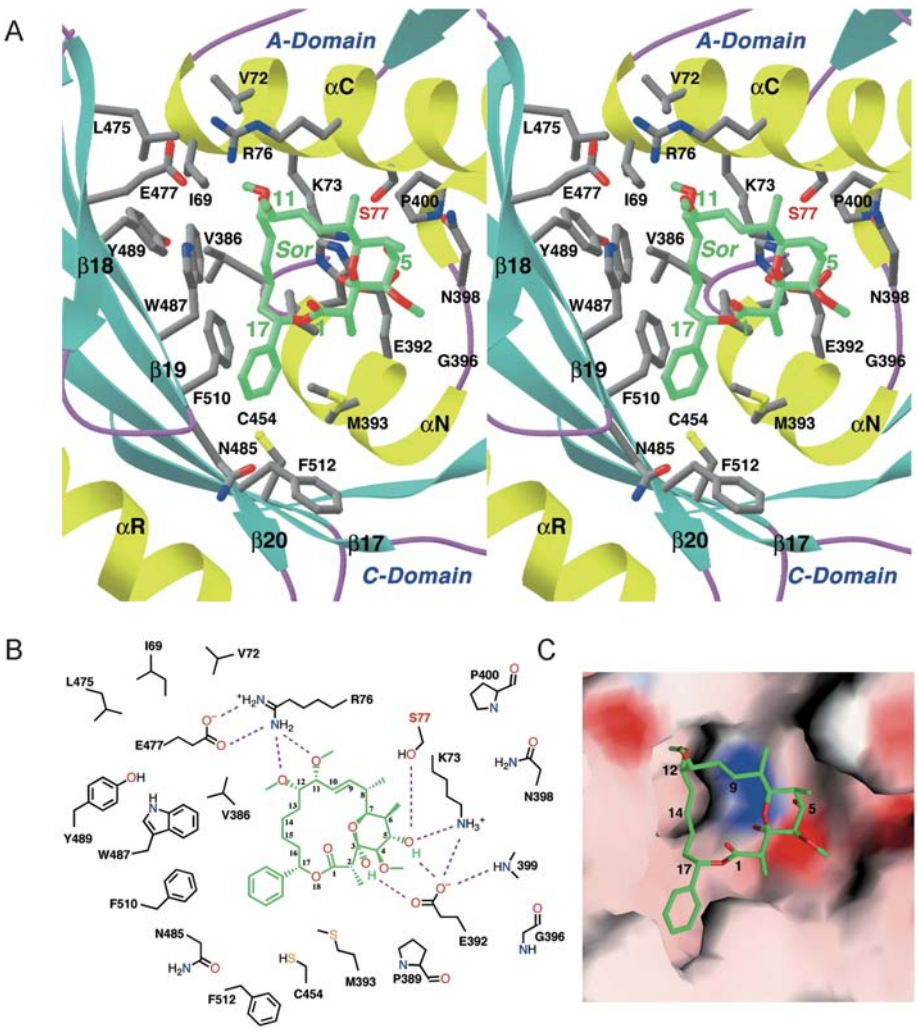


Fig. 1 The binding mode of soraphen. A: Stereographic drawing showing the binding site for soraphen A within the BC domain of yeast acetyl-CoA carboxylase (ACC). B: Interactions between soraphen A and the BC domain shown in schematic form. C: Molecular surface of the BC domain in the soraphen binding site. Reprinted from Y. Shen *et al.*, 'A mechanism for the potent inhibition of eukaryotic acetyl-coenzyme A carboxylase by soraphen A, a macrocyclic polyketide natural product', *Mol. Cell*, 2004, **16**, 881–891 (copyright 2004), with permission from Elsevier.[175]

In parallel, the structure of the soraphen A was solved by X-ray crystallography, and the compound was prepared by total synthesis.[164] Comprehensive derivatization experiments were also performed in order to elucidate structure–activity relationships, as well as to evaluate whether less complicated structures might retain good bioactivity.[165–169] Unfortunately, even minor changes to the molecule led to a complete loss in potency. Synthetic analogues of the southern ring segment, which was thought to house the pharmacophore, were also entirely inactive.[170–173] However, the nail in the coffin for the project was toxicological tests carried out by Ciba-Geigy.[158] Although acute toxicity in mammals was negligible, experiments with rats revealed soraphen A's teratogenic activity (potential to cause malformation of embryos). In addition, soraphen provoked allergic reactions in some test animals, as well as inflammation of the skin and mucous membranes. Together, these possible hazards halted further development of the metabolite as a plant protective agent.

Despite the failure to progress soraphen into agricultural use, the project yielded valuable insights into the biochemistry, physiology and genetics of *S. cellulosum* as a producing organism, information which was ultimately applied to optimizing production of epothilone by a related strain, *S. cellulosum* So ce90. In addition, identification of ACC as the protein target enabled more detailed study of soraphen's mode-of-action, including the determination of its binding site within the ACC, the catalytic BC domain.[174] Furthermore, the crystal structures of soraphen A bound to the BC domain of yeast[175] (Fig. 1) and human ACC[176] strongly suggests that the inhibitory effect of the metabolite arises from binding in the BC dimer interface, interrupting its oligomerization – thus soraphen is a protein–protein interaction inhibitor. The structure of the complex also elegantly accounts for the SAR results, as the entire macrocycle is involved in binding to the domain. Equally, it explains the lack of soraphen's activity towards bacterial ACC, as there are large structural differences between the eukaryotic and bacterial BC domains in the binding site. In future, these insights may enable the structure-based design of new inhibitors against ACC enzymes, which do not suffer from soraphen's undesirable, toxic side-effects. These efforts have gained added impetus from the recent finding that small-molecule inhibitors of human ACCs have potential in the treatment of both the metabolic syndrome[177] and cancer.[178]

3.2 Corallopyronin, myxopyronin, ripostatin, and sorangicin: inhibitors of bacterial RNA polymerase

Myxopyronin **37**[71] and its close chemical cousin corallopyronin **35**[69] were discovered in the early 1980s, and both were found to exhibit anti-bacterial activity through inhibition of RNA polymerase (RNAP). Further myxobacterial inhibitors of RNAP followed (sorangicin **38** (1987),[72] ripostatin **31** (1995)[64] and etnangien **36** (2007)[70]), though none bore any strong structural resemblance to myxopyronin/corallopyronin or to each other. These metabolites are of significant interest, because selective inhibition of bacterial RNAP forms the basis for the antibiotic treatment of tuberculosis, the most widespread and persistent bacterial infection on the planet.[179] All told, the disease kills approximately 1.7 million people each year (2006 figure),[180] largely in developing countries. The rifamycin group of antibiotics (rifampicin, rifapentine, and rifabutin) has been the gold-standard in tuberculosis chemotherapy for decades, as these agents selectively inhibit the activity of bacterial RNAP, and not its mammalian equivalent.[181] However, the emergence of mycobacterial strains resistant to these medicines has considerably hampered TB treatment, prompting the search for new and more effective drugs that target RNAP.

One essential criterion of any novel drug candidate designed to replace rifampicin is that it shows activity against rifampicin-resistant mutants. This requirement is met by myxopyronin,

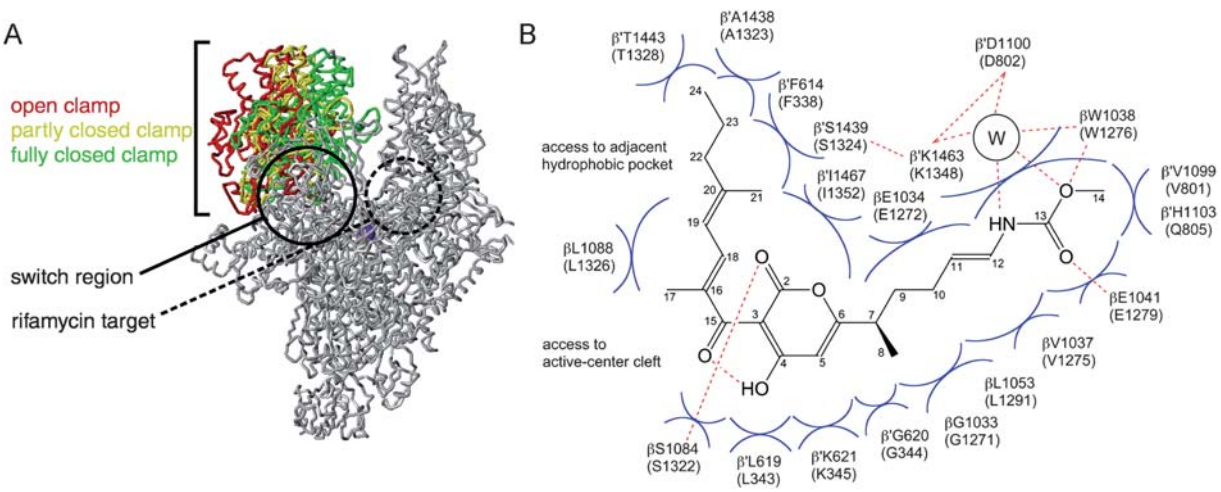


Fig. 2 Structure of the bacterial RNA polymerase (RNAP) and the mechanism of its inhibition by myxopyronin, corallopyronin and ripostatin. A: Structure of the RNAP clamp, showing open (red), partly closed (yellow) and fully closed (green) conformations. The solid circle indicates the switch region where the myxobacterial metabolites bind, and the dashed circle shows the binding site for the rifamycin-type antibiotics. The violet sphere indicates the active site Mg^{2+}. B: Schematic of the set of contacts between RNAP and myxopyronin. W represents an ordered bound water, while dashed lines represent H-bonds. Reprinted from J. Mukhopadhyay, *et al.*, 'The RNA polymerase "switch region" is a target for inhibitors', *Cell*, 2008, **135**, 295–307 (copyright 2008), with permission from Elsevier.[183]

corallopyronin and ripostatin, but resistance to the rifamycins confers cross-resistance to sorangicin.[182] The structural basis for the lack of cross-resistance to the three metabolites was recently revealed by an elegant study carried out by Arnold, Ebright and colleagues,[183] in which they elucidated in detail the binding interaction between myxopyronin and bacterial RNAP. The overall shape of both bacterial and eukaryotic RNAPs is reminiscent of a crab claw, wherein the two pincers define the active site cleft (Fig. 2). The largest subunit of RNAP makes up one pincer (the 'clamp') and a portion of the active site cleft, while the second largest subunit forms the other pincer, and also contributes to the active site. The clamp can exist in a range of conformational states, from a fully open configuration which permits the entry and exit of DNA, to a fully closed conformation, which prevents these translocations. In the early stages of transcription, the clamp must open to allow DNA to access the active site, and then must close again to retain the DNA during transcription initiation and elongation. Underlying these conformational transitions is a 'switch region' at the base of the clamp, which acts as hinge on which the clamp swings relative to the remainder of the enzyme.

Using a combination of genetic, biochemical and structural approaches, the authors showed that myxopyronin allosterically inhibits RNAP by 'jamming' the hinge (Fig. 2). Binding of myxopyronin to the switch region appears to lock the clamp in one conformation (partly closed or partly-to-fully closed), thereby preventing opening of the RNAP active site cleft to permit entry of double-stranded DNA. The residues involved in the interaction are remote from the binding site with the rifamycins, explaining the lack of cross-resistance between the antibiotics. This study additionally provided evidence that both corallopyronin and ripostatin interact with the same site on RNAP, despite the fact that the only similarity between ripostatin and the other metabolites is its size and overall hydrophobicity.[180] Thus, it appears that two distinct myxobacterial chemotypes have evolved to function through a shared mechanism. In 2009, Artsimovitch, Vassylyev and co-workers[184] independently reported binding of a desmethyl myxopyronin to the RNAP switch region. However, in the resulting inhibition model, this interaction does not impede entry of DNA into the active site cleft, but instead interferes with formation of the pre-catalytic transcription initiation complex. Presumably, structural analysis at higher resolution will resolve this discrepancy.

Together, these studies have revealed the RNAP switch region as an attractive, new target for drug discovery of antibacterials. The site comprises amino acids which are conserved among Gram-positive and Gram-negative bacteria, providing the basis for broad-spectrum activity of single drugs, but which diverge in eukaryotic RNAPs, allowing for therapeutic selectivity. In addition, the binding site is an enclosed, largely hydrophobic pocket, features which favor its 'druggability' by multiple chemotypes. However, none of myxopyronin, corallopyronin or ripostatin is suitable as a medicine in its native form.[179] The spectrum of activity of these compounds is largely restricted to Gram-positive bacteria and does not cover *Pseudomonas aeruginosa*, limited membrane permeability limits their effectiveness against Streptococci and Enterococci, and their chemical stability is low due to their inherent photosensitivity. The scaffolds nonetheless represent attractive leads for structure-guided drug design,[185] although previous SAR studies suggest that simple modifications will be unlikely to satisfy all therapeutic requirements.[186,187]

Despite the issues of cross-resistance, sorangicin too may provide instructive lessons for future drug discovery efforts directed at RNAP. Structural, functional and genetic analysis of the shared rifamycin/sorangicin binding site within the DNA/RNA channel revealed that rifamycin binding is highly sensitive to mutations expected to change the shape of the pocket, whereas sorangicin is not (Fig. 3).[188] In other words, fewer mutations conferred strong or partial resistance to sorangicin. The authors suggest that the inherent conformational flexibility of sorangicin allows it to adapt to changes in the architecture of the binding pocket, while the more rigid rifamycin cannot. This principle may be useful for the design of novel inhibitors against the RNAP rifamycin binding pocket, and other rapidly mutating targets.

3.3 Disorazol: a potential anti-cancer agent

The myxobacterial strain *Sorangium cellulosum* So ce12 produces some 26 variants of the macrocyclic dilactone disorazol.[97] These metabolites exhibit some of the highest cytotoxicities towards

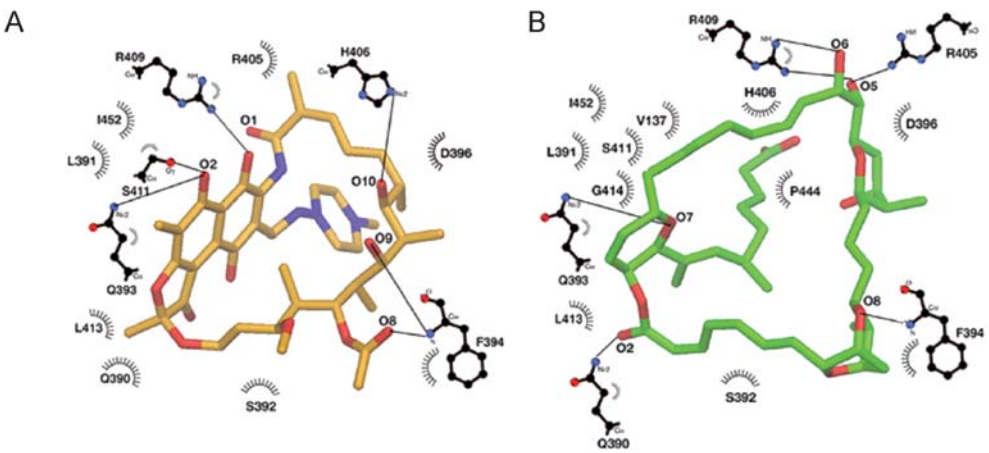
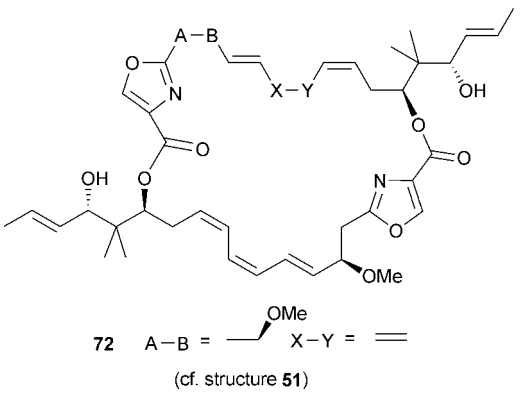


Fig. 3 Interactions between rifamycin, sorangicin and RNAP. A: Schematic drawing of RNAP interactions with rifamycin. B: Schematic drawing of RNAP interactions with sorangicin. Residues which participate in van der Waal's and hydrogen bond interactions are indicated, with the hydrogen bonds shown as lines. Reprinted from E. A. Campbell *et al.*, *EMBO J.*, 2005, **24**, 674–682 (copyright 2005), with permission from Macmillan Publishers Ltd.[188]

animal cells yet recorded (*e.g.*, IC_{50} for disorazol A_1 **51** = 3 pM against L929 mouse fibroblasts[189]). In contrast, antiviral and antibacterial properties have not been reported.[190] Although disorazol A_1 is the major product of fermentation,[191] the relatively simplified disorazol C_1 **72** appears to be the more promising candidate for therapeutic use as it lacks the reactive vinyl oxirane and tetraalkene moieties of disorazol A_1.[192] In a recent report, for example, disorazol C_1 was shown to exhibit antiproliferative activity against a wide range of tumor cells, and to cause both premature cellular senescence and apoptosis.[193] Mode-of-action studies have demonstrated that, uniquely among anti-mitotic agents, disorazol A_1 inhibits tubulin polymerization and leads to the depletion of microtubules in equal measure.[189] This perturbation to the microtubule network results in cell cycle arrest at the G2/M checkpoint, and induction of the apoptotic cell death cascade. Available evidence suggests that the precise site of binding within the so-called vinca domain of β-tubulin is different to that of tubulysin.[192,194]



72 (cf. structure **51**)

SAR studies to date have focused on disorazol A_1 **51**[191] and disorazol C_1 **72**.[193,195] Unfortunately, in the case of disorazol C_1, any truncation or alteration of the backbone was highly detrimental to the activity,[195] and indeed it has been suggested that disorazol C_1 represents the minimum pharmacophore of the metabolite family.[192] Complementary studies are ongoing, however, to develop specific targeting of the disorazols and their derivatives to cancer cells. A variety of tumors, including breast, ovarian and prostate, express receptors for the luteinizing hormone-releasing hormone (LHRH). Therefore, cytotoxic agents can be directed to cancer cells by covalently tethering the agents to an LHRH agonist peptide through a suitable, cleavable linker.[196,197] The conjugate is internalized into the cell, and the cytotoxic agent released spontaneously by hydrolysis. This strategy was recently applied to a natural disorazol derivative, disorazol Z.[198] The resulting conjugate showed higher tumor suppression activity *in vivo* than equimolar doses of disorazol Z itself (tumor volumes were decreased to 50% on day 18 after a single application), further supporting the promise of this type of approach in cancer therapy. For more information on disorazol, including its biosynthesis, antiproliferative activity and chemical synthesis, the reader is referred to a recent review in this journal by Hopkins and Wipf (ref. 192).

3.4 Argyrin: a proteasome inhibitor

Argyrin **24**, discovered in 2002, is a cyclic peptide, which was initially noted for its potent immunosuppressive activity:[57] it suppressed T cell-independent antibody formation by B cells, and showed activity in an *in vitro* model for alloantigen-mediated T cell activation and proliferation. Argyrin was also found to exhibit weak antibiotic activity, as well as cytotoxicity towards a number of mammalian cell lines. The likely basis for its cytotoxicity was recently revealed to be proteasome inhibition.[137]

The proteasome is an abundant, multi-subunit catalytic complex which plays a critical role in maintaining cellular homeostasis through the degradation of over 80% of cellular proteins.[199] In cancer, the balance between the production and destruction of malignancy factors including proteins which mediate proliferation, determines whether the cells live or die. Thus, following an understanding of the role of proteasome in protein recycling, it moved to center stage as a target for cancer therapy.[200,201] Indeed, despite some initial skepticism, this therapeutic approach was validated with the discovery of the peptide boronate bortezomib (Velcade, formerly PS-341), which was approved by the US Food and Drug Administration in 2003 for the treatment of multiple myeloma (MM) and mantle cell lymphoma. Although bortezomib is the standard of care for MM patients, its use is associated with serious side effects.[202] Interestingly, it was a recent study with argryin that revealed the likely cause of these undesirable properties, and swung the spotlight on argyrin as a candidate for further clinical development.[137]

Argyrin's unsuspected activity as a proteasome inhibitor was discovered through its ability to promote the intracellular accumulation of p27, a tumor suppressor protein which is frequently disregulated in human cancers. Subsequent studies *in vitro* with purified human proteasome demonstrated that argyrin inhibits all three of the major enzymatic activities with a potency equivalent to that of bortezomib. Furthermore, argyrin's biological effects (induction of apoptosis and inhibition of angiogenesis) were shown to be dependent on preventing the

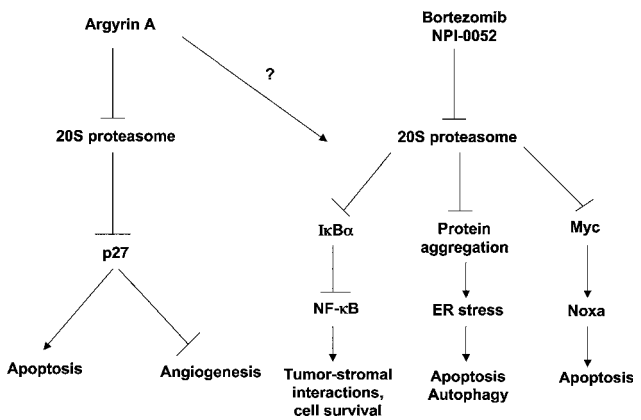


Fig. 4 Mechanisms to explain cell death induced by proteasome inhibitors. Argyrin has been shown to induce apoptosis and inhibit angiogenesis *via* p27-dependent mechanisms (left pathway). Studies with bortezomib (right pathway) have instead implicated inhibition of NF-κB, proteotoxic stress, and stabilization of Myc and downstream upregulation of Noxa, a BH3-only Bcl-2 family member (among other mechanisms). Argyrin appears to be the more specific of the two inhibitors, which implies that at least some of bortezomib's effects may be due to off-target activity. Reprinted from D. J. McConkey, 'A novel role for a familiar protein in apoptosis induced by proteasome inhibition', *Cancer Cell*, 2008, **14**, 1–2 (copyright 2008), with permission from Elsevier.[212]

destruction of p27. While siRNA-based knockdown of the proteasomal subunits also led to apoptosis *via* p27-dependent mechanisms, bortezomib's effects were found to be p27-independent; gene expression experiments further confirmed that argryin recapitulates the effects of proteasome knockdown, while bortezomib does not. Taken together, these data led the authors to conclude that argyrin is a more specific proteasome inhibitor than bortezomib. The stunning implication here is that bortezomib's off-target actions may account for at least some of its cytotoxic and side effects (Fig. 4), necessitating a re-evaluation of earlier thinking as to how proteasome inhibition causes cell killing.[203] Nonetheless, as the two agents have apparently different mechanisms, combining them may prove more effective than current therapies which all fail to cure multiple myeloma.

4 Outlook

Although only 100 basic structural types have been discovered from the myxobacteria to date, a myxobacterial secondary metabolite, epothilone, is already at work in the clinic, and a number of additional compounds are progressing through preclinical and clinical trials. The high hit-rate for these natural products, combined with the fact that we have likely only scratched the surface of myxobacterial secondary metabolism,[3,204,205] makes it likely that these microorganisms will provide us with many new drug leads in the future. In the meantime, the structures will continue to function as useful chemical genetics tools for unveiling the complex workings of biological processes in both prokaryotic and eukaryotic cells.

5 Acknowledgements

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