

Mode of Action of Microbial Bioactive Metabolites

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ABSTRACT. Pathogenic microorganisms can be suppressed by cell wall destruction. Biosynthesis of peptidoglycans forming bacterial cell wall is interrupted by glycopeptides which inhibit polymerization of a disaccharide formed by *N*-acetylglucosamine and *N*-acetylmuramic acid, β -lactams and their derivatives inhibit peptidoglycan cross-linking. Antibiotics inhibiting protein synthesis bind to different sites on the rRNA and interfere with the formation of the polypeptide chain. Tumor cells resistant to chemotherapeutic drugs overproduce proteins transporting the drugs out of cells; these proteins eliminate substances which inhibit transcription of transport proteins. Some antitumor drugs (anthracyclines, fluoroquinolones, acridines *etc.*) act at topoisomerases which irreversibly bind to DNA and inhibit DNA synthesis. Immunosuppressants affect various components of the immune system such as T-helper, T-effector cell function, antigen presentation and B-cell function. Antiparasitics – avermectins – bind to a receptor of this Gab-gated chlorine channel in the nerve fiber of nematodes and anthropodes, increasing the permeability of the membrane for chloride ions; the increased transport of chloride ions into the cell causes the death of the parasite. Ionophores dissolve in phospholipid bilayers and enormously increase their ionic permeability. Respiration inhibitors block the transport of electrons at several places of the respiratory chain. Rifamycin binds to the β subunit of bacterial RNA polymerase, thereby blocking mRNA synthesis. Antiviral compounds inhibit the transcription of DNA by several mechanisms or by inhibition of viral entry into host cells.

Abbreviations

Dap *meso*-2,6-diaminopimelic acid

Gab 4-aminobutyric acid (Abu)

GlcNAc *N*-acetylglucosamine

MDR multidrug resistance

MRP(1) MDR protein (1)

MurNAc *N*-acetylmuramic acid

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1 INTRODUCTION

Microorganisms produce many substances which are widely used in human and animal medicine but the majority of microbial products are toxic or have side effects and cannot be used in clinical practice. When we want to eliminate the side effects of antibiotics and other bioactive metabolites we must study also their mode of action. Knowing the mode of action of an antibiotic, we can foresee the mode of damage to the macroorganisms. Several new bioactive substances were discovered by investigating metabolites which are active against targets of other bioactive substances. The knowledge of the mode of action of bioactive substances helped also to define the sequence of enzyme reactions in some metabolic pathways. Methods of molecular biology help us to estimate the mode of action of bioactive substances at molecular level.

2 INHIBITION OF CELL WALL AND CYTOPLASMIC MEMBRANE SYNTHESIS

Bacterial cell wall is the most suitable target for antibiotics since similar structures and biosynthetic pathways are absent from mammalian hosts. The structure of bacterial cell wall is formed by peptidoglycan,

composed of D-amino acids which makes cell wall resistant to proteolytic degradation. Peptidoglycan forms the murein sacculus, a giant macromolecule that surrounds the cell as a single, flexible meshwork (Rohrer and Berger-Baechli 2003). Peptidoglycan backbone is made up of alternating molecules of GlcNAc and MurNAc connected by a 1,4- β -D-glycosidic bond (Fig. 1). The 3-carbon of MurNAc is substituted with a lactyl ether group derived from pyruvate. This group connects the glycan backbone to a peptide side chain containing L-alanine, D-glutamate, Dap or L-lysine, and D-alanine. Polymerization of GlcNAc–MurNAc disaccharide is inhibited by glycopeptides (Fig. 2). β -Lactams and their derivatives bind to penicillin-binding proteins, the serine peptidases, and inhibit peptidoglycan cross-linking. Inhibition of peptidoglycan synthesis disrupts the delicate balance between the synthesis and degradation and autolytic enzymes trigger cell death. Cycloserine targets the peptidoglycan biosynthesis enzymes D-alanine racemase and D-alanyl-D-alanine ligase in *Mycobacterium smegmatis* and *M. tuberculosis* (Feng and Barletta 2003).

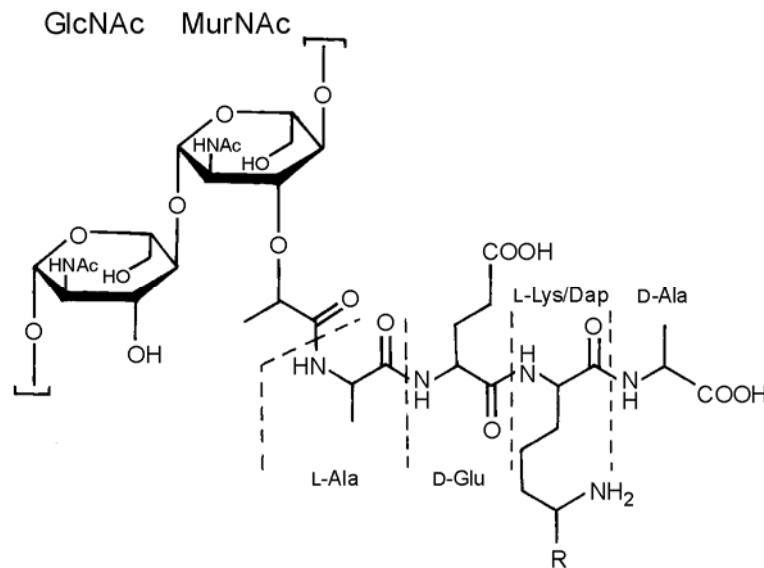


Fig. 1. A building unit of peptidoglycans: R = H (L-Lys), $-\text{COOH}$ (Dap) GlcNAc – N-acetylglucosamine
MurNAc – N-acetylmuramic acid Dap – meso-2,6-diaminopimelate

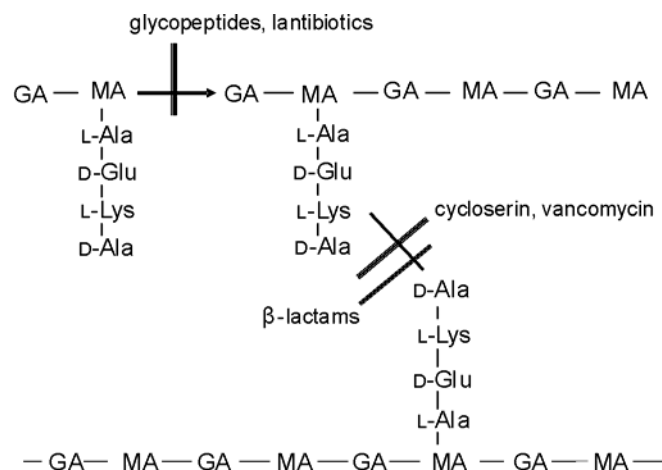


Fig. 2. Inhibition of cell-wall formation by cycloserin, vancomycin and β -lactams.

Peptide antibiotics target cytoplasmic membrane. Cecropins and melittin fold into amphipathic α -helices in membranous environments. A number of peptides have been shown to bind to lipopolysaccharide and to permeabilize the outer membrane to self-promote uptake into G^- bacteria. Because cationic peptides have affinity for lipopolysaccharides that is at least 1000 \times higher than that for the native divalent cations

Ca^{2+} and Mg^{2+} , they competitively displace these ions and, being bulky, disrupt the normal barrier property of the outer membrane (Hancock and Chaple 1999).

3 INHIBITION OF PROTEIN SYNTHESIS

A large group of antibiotics (tetracyclines, macrolides, chloramphenicol, streptomycin, neomycin *etc.*) inhibits the growth of bacteria by inhibiting protein biosynthesis. RNA accounts for two-thirds of ribosomal mass and is responsible for important functions of the ribosome. For this reason the target site of several antibiotics is rRNA. Our knowledge of the tertiary structure of the ribosome has increased in the last few years. Models at resolutions approaching 0.5 nm have been obtained by X-ray crystallographic analysis of the small (30S) and large (50S) subunits as well as of functional 70S ribosome complex of these 2 subunits (Vester and Douthwaite 2001). Large ribosomal subunit is composed of 23S rRNA and of a minimum of 30 proteins.

Most of clinically important antibiotics interact with various segments of the central loop domain in the 50S subunit (Belova *et al.* 2001). Spectinomycin reversibly interferes with mRNA interaction with 30S subunits (Fig. 3). Aminoglycosides (streptomycin, kanamycin, gentamicin, *etc.*) irreversibly bind to the 16S

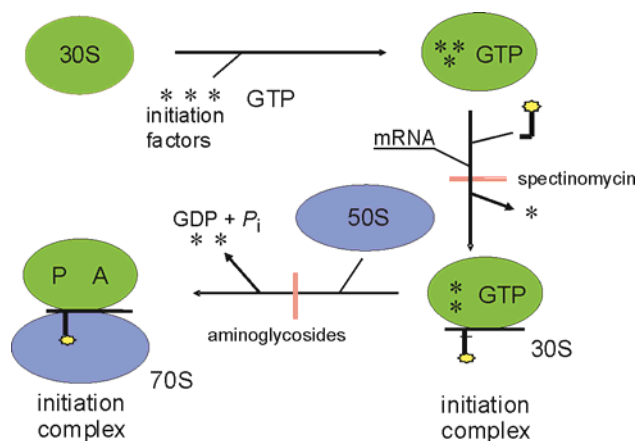


Fig. 3. Inhibition of protein synthesis by spectinomycin and aminoglycosides.

rRNA and freeze the 30S initiation complex (30S–mRNA–tRNA) so that no further initiation can occur (Fig. 4). Different classes of aminoglycoside antibiotics bind to different sites on the rRNA. Neomycin, paromomycin, gentamicin and kanamycin are believed to bind to the A-site on the 16S rRNA. The binding of the above aminoglycosides to the A-site in the decoding region interferes with the accurate recognition of cognate tRNA by rRNA during translation (Kotra *et al.* 2000). The binding specificity of aminoglycosides to prokaryotic rRNA is only $\approx 10\times$ higher than to that of eukaryotes and it can explain the toxic effect of these antibiotics in mammalian system (Noller 1991). Hygromycin B selectively inhibits the ATPase activity of ribosomal ATPase by selectively releasing ribosomal ATPase from 70S ribosome (Ganoza and Kiel 2001).

The tetracyclines block bacterial translation by binding reversibly to the 30S subunit and distorting it in such a way that the anticodon of the charged tRNAs cannot align properly with the codons of the mRNA (Fig. 5) (Chopra and Roberts 2001). Chloramphenicol binds to the 50S subunit and inhibits peptidyl transferase activity (Hopps *et al.* 1956). The macrolides also bind to the 50S ribosomal subunit, inhibiting thereby the synthesis of peptides.

The site of evernimicin (an oligosaccharide antibiotic) binding and its mode of action are distinct from other ribosome targeted antibiotics. Evernimicin inhibits the activity of initiation factor 2 *in vitro*, suggesting that the drug interferes with the formation of the 70S initiation complex (Belova *et al.* 2001).

4 INHIBITION OF MULTIDRUG RESISTANCE

Resistance to antitumor agents is a major problem in the treatment of cancer. One type of resistance, *viz.* MDR, is a mechanism whereby the intracellular concentration of the drugs is reduced by the

action of proteins which are ATP- or proton-gradient dependent drug pumps that can export a broad range of commonly used chemotherapeutic drugs from cells. Most of the genes encoding MDR pumps are normal constituents of bacterial chromosomes. ATP-dependent transmembrane drug transporters such as *P*-glyco-

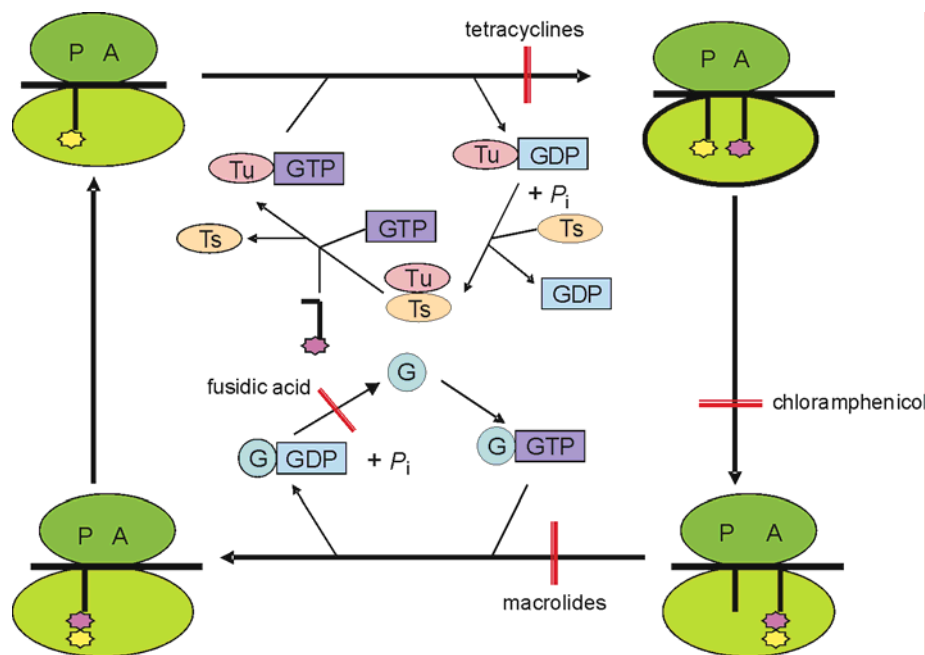


Fig. 4. Inhibition of protein synthesis by tetracyclines, chloramphenicol and macrolides; Tu, Ts, G – elongation factors.

protein and MRP1 can render cell multidrug resistant. *P*-Glycoprotein is a 1280-amino acid integral membrane phosphoglycoprotein with 2 homologous halves connected by a short stretch of a linker region. Both of the ATP-binding sites are catalytically active and are required for the protein to function as a transporter (Dey *et al.* 1997). *P*-Glycoprotein interacts with a large scale of chemical compounds and defends cells in normal tissues against drugs and other xenotoxins. MRP1 is involved in reducing the passage of drugs across some specialized epithelia (Wijnholds *et al.* 2000). Several other MRP family members may play a role in MDR. Cancer cells overproducing drug-transporting proteins become resistant to a wide spectrum of drugs. One possibility how to eliminate the overproduction of transmembrane drug transporters is an inhibition of transcriptional activation of drug resistance genes.

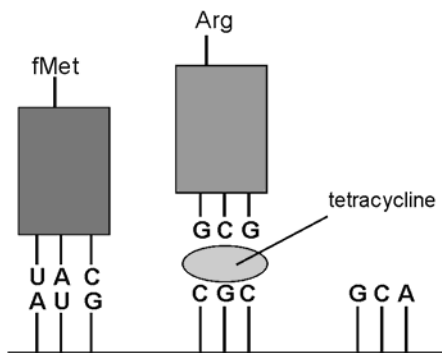


Fig. 5. Inhibition of bacterial translation by tetracyclines.

Ecteinascidin 743 (isolated from the marine tunicate *Ecteinascida turbinata*) has been shown to interfere with the binding of minor-groove-interacting transcription factor, particularly NF-Y, with their cognate promoter elements *in vitro* (Jin *et al.* 2000).

In *Staphylococcus aureus*, export of substances out of cells is effected by the protein NorA, which appears to straddle the cell membrane and act as an efflux pump and can decrease susceptibility to fluoroquinolones (Aeschlimann *et al.* 1999; Piddock *et al.* 2001). Overproduction of NorA evokes resistance to fluoroquinolones and other structurally unrelated compounds.

NorA inhibitors dramatically improved the activity of the hydrophilic fluoroquinolones. These compounds presumably affect the activity of NorA by affecting the cell proton gradient similarly as the protonophore mesoxalonyl 3-chlorophenylhydrazide ('carbonyl cyanide *m*-chlorophenylhydrazide', CCCP) and the competitive pump blocker reserpine (Aeschlimann *et al.* 1999). Staurosporin, which enhances accumulation of vincristine in multidrug-resistant cells, inhibits protein kinases (EC 2.7.1.37), including serine/threonine and serine/tyrosine kinases which mediate phosphorylation of transmembrane glycoprotein (Sato *et al.* 1990; Omura *et al.* 1995).

5 INHIBITION OF NUCLEIC ACID SYNTHESIS

Inhibition of DNA synthesis. DNA topoisomerases are enzymes responsible for the alteration of the topological state of DNA. The topological state of DNA is regulated by topoisomerases through the action of breaking and resealing DNA strand. The topoisomerization reaction is achieved by cleavage of DNA and the formation of transient DNA gate through which a part of the same or another DNA molecule is passed. Topoisomerases work by temporarily attaching to the DNA like a clamp, and making a cut that partially unravels this molecule. These enzymes have been classified into two major classes, based on their mode of cleaving DNA. Type I enzymes introduce a single-stranded break in DNA and facilitate the passage of a second strand through this gate. Type II topoisomerases (DNA gyrases) create a double-stranded break and allow the passage of a second double-stranded DNA segment. Topoisomerases have a pivotal role in the process of DNA replication, transcription, and recombination (Hengstler *et al.* 2002).

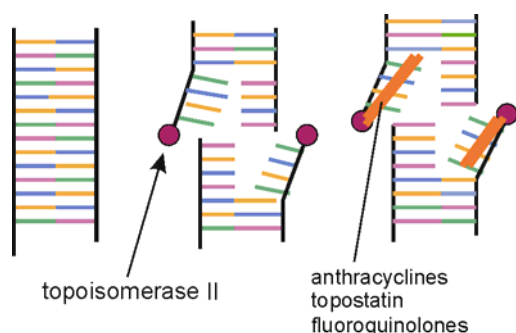


Fig. 6. Inhibition of nucleic acid synthesis by anthracyclines, topostatin and fluoroquinolones.

topoisomerases I and II in a competitive manner with respect to DNA and also some restriction endonucleases (Suzuki *et al.* 1999).

Inhibition of RNA synthesis. The antibiotics rifampicin, rifampin, rifamycin *etc.* inhibit the synthesis of RNA by binding to DNA-dependent RNA polymerase, inhibiting the initiation of RNA synthesis (Murray *et al.* 2005).

6 INHIBITION OF BACTERIAL TWO-COMPONENT SIGNALING SYSTEM

Many bacteria utilize a two-component system consisting of a histidine protein kinase and a response regulator for signal transduction. Some substances inhibit this enzyme (Fig. 7). Other antibacterial histidine protein kinase inhibitors decrease incorporation of DNA, RNA, and protein precursors, possibly as a result of concomitant disruption of cytoplasmic membrane (Hillard *et al.* 1999).

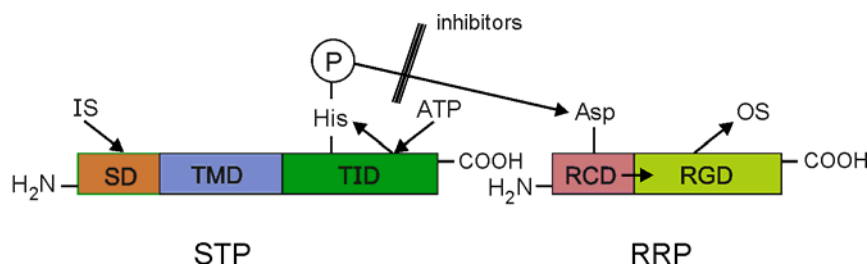
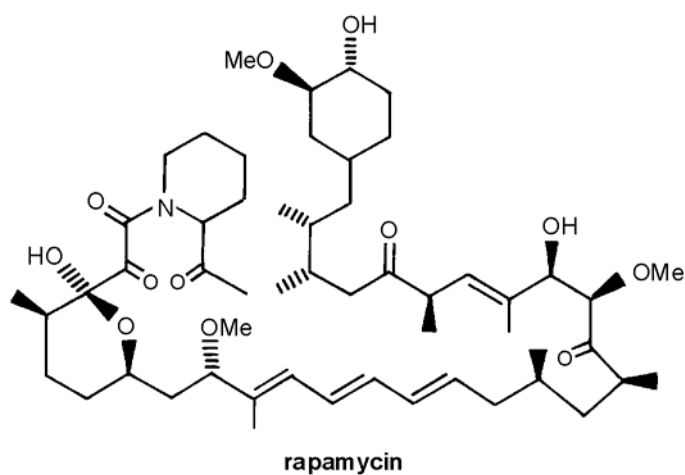
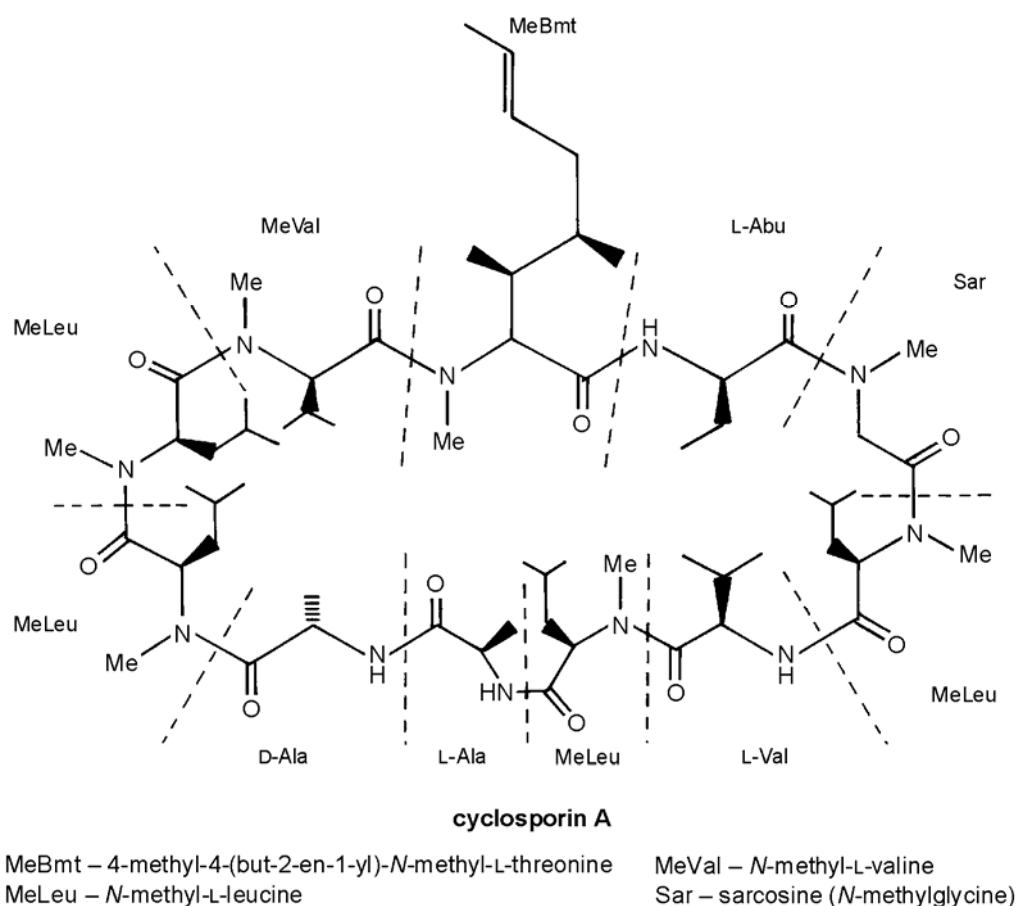


Fig. 7. Inhibition of bacterial two-composition signaling system;

IS	input signal	OS	output signal	RCD	receiver domain
RGD	regulatory domain	RRP	response regulator protein	SD	sensor domain
STP	sensor-transmitter protein	TID	transmitter domain	TMD	transmembrane domain

7 IMMUNOSUPPRESSANTS

Immunosuppressants are compounds that reduce the effects of an activated immune system. They are clinically effective at suppressing organ graft rejection and in the treatment of autoimmune diseases (*e.g.*, rheumatoid arthritis, gastritis, multiple sclerosis, primary biliary cirrhosis). The most widely used immunosuppressants are cyclosporin A, antibiotic FK506, and rapamycin, isolated from microbial cultures (Russell *et al.* 1992). Cyclosporin A is a neutral cyclic undecapeptide, FK506 and rapamycin are macrolides, but at



present we know several tens of immunosuppressants isolated from microorganisms. Cyclosporin A affects various components of an immune system, including T-helper and T-effector cell function, antigen presentation and B cell function. *In vitro* studies have suggested that cyclosporin A exerts its immunosuppressive effect on T cells by inhibiting interleukin-2 production (Fukuzava *et al.* 1989). Cyclosporin A inhibits selec-

tively transcription of interleukin-2 gene. Cyclosporin A and FK506 bind to membrane receptors and after pervading the cytoplasm they bind to cyclosporin- and FK506-binding proteins (immunophilins). Both of these proteins exhibit the peptidyl-prolyl *cis*-*trans* isomerase activity that accelerates the isomerization of the peptidyl-prolyl bond, a rate-limiting step in protein folding (Farutani *et al.* 1998). They regulate T cell activation and other metabolic processes, perhaps by the recognition of proline-containing epitopes in target proteins. A very active immunosuppressant tautomycin blocks tyrosine phosphorylation of T cell-specific signaling mediators in T-cell receptor proximal signal-transduction pathway, leading to induction of apoptosis (Shim *et al.* 2002). Elaiophylin (Čikošová *et al.* 2004) was found to have a cytotoxic effect on human tumor cell lines, antimicrobial effect against G^+ bacteria, a strong inhibitory effect on B-cells stimulated by lipopolysaccharides, and an inhibitory effect on the proliferation of mouse spleen lymphocytes stimulated by mitogens (Lee *et al.* 1997a,b).

8 ANTIPARASITICS

Microorganisms produce potent antiparasitic compounds. The most important are avermectins, especially their derivative ivermectin. They are active against a broad spectrum of nematode and arthropod parasites. They bind to a specific, high-affinity site present in nematodes but not in vertebrates (Burg *et al.* 1979). Avermectins bind to a protein (a receptor) in the nerve fiber called Gab chloride channel (Fig. 8) (MacNeil 1995). The cell walls become increasingly permeable to Cl^- ions, hyperpolarizing the neuronal membrane and decreasing nerve transmission, causing paralysis or death of the parasite. In mammals, Gab receptors and neurons are found only in the central nervous system while in arthropods and nematodes Gab is found in the peripheral nervous system.

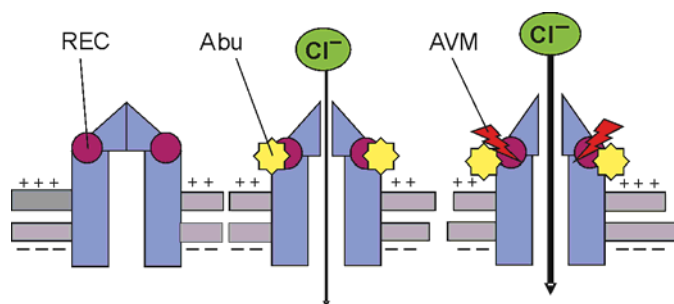


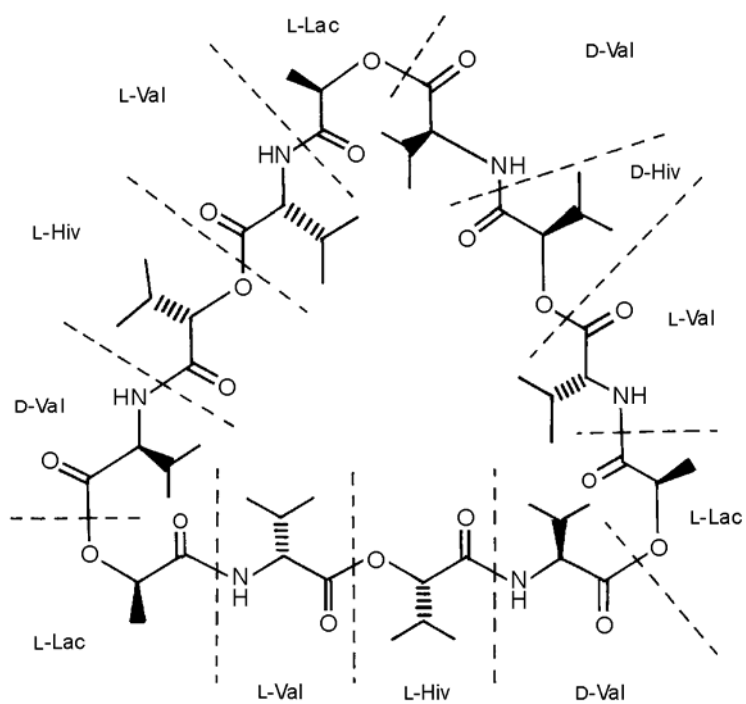
Fig. 8. Mode of action of avermectins; AVM – avermectins, REC – receptor; Abu – 4-aminobutyrate.

9 IONOPHORES

Ionophores are small amphipathic molecules which dissolve in phospholipid bilayers and enormously increase their ionic permeability. Ionophores can be divided into channel formers (gramicidin) which form a tiny pores through the membrane, and mobile carriers which diffuse back and forth across the membrane. We can subdivide carrier ionophores into 2 groups distinguished by whether the ionophore is uncharged or anionic. Typical neutral ionophore is valinomycin (Bookchin *et al.* 2000), anionic ionophores are nigericin (Fig. 9) and monensin which are monovalent antiporters with selective binding affinity for Na^+ and K^+ , respectively. Tetronosin transfers Ca^{2+} and Mg^{2+} .

10 RESPIRATION INHIBITORS

Respiration inhibitors block the respiration chain. Antimycin blocks chain at complex 3 between cytochrome *b* and cytochrome *c*₁ (Fig. 10) (Kim *et al.* 1998). It therefore prevents the oxidation of both NADPH and succinate, but has no effect on ascorbate and *N,N,N',N'*-tetramethyl-1,4-benzenediamine (TMBD). Rotenone blocks NADH dehydrogenase in respiration chain but also has no effect on the oxidation of either succinate or ascorbate and TMBD (Nagata *et al.* 2001).



Hiv – 2-hydroxyisovalerate, Lac – lactate

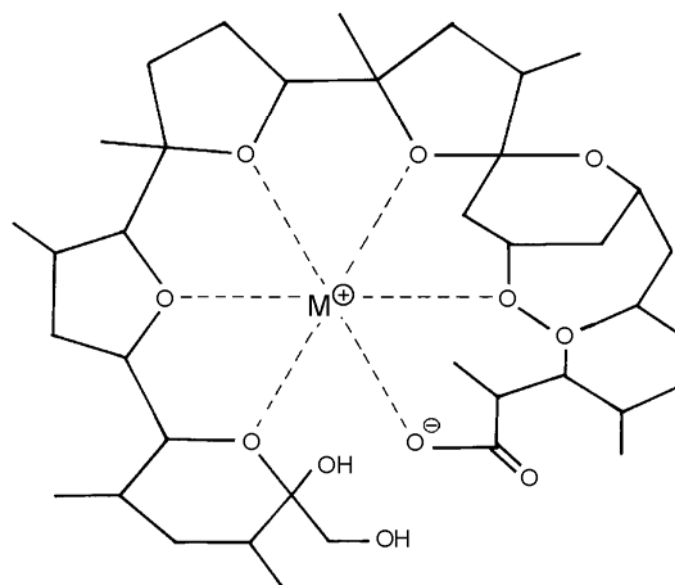
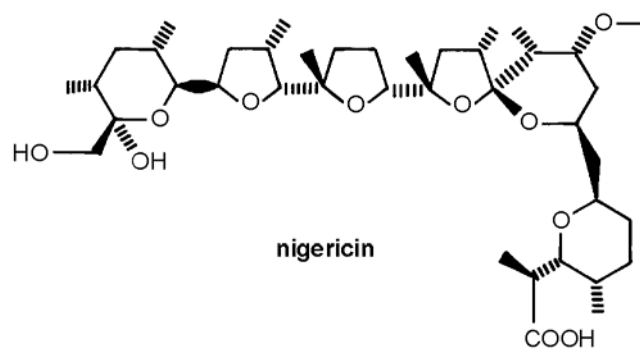


Fig. 9. Nigericin as an ionophore; $M^+ = Na^+$ or K^+ .

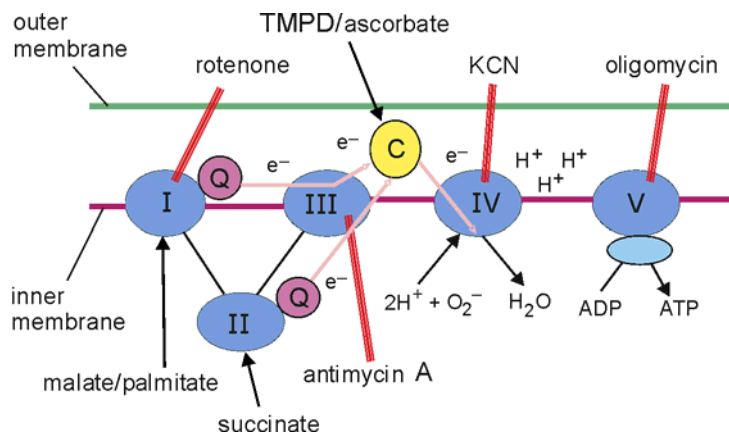


Fig. 10. Inhibition of a respiration chain; I – NAD–ubiquinol, II – succinate–ubiquinol dehydrogenase, III – ubiquinol–cytochrome-*c* oxidoreductase, IV – cytochrome-*c* oxidase, V – F_0F_1 -ATP synthase, C – cytochrome *c*, Q – ubiquinone; TMPD – *N,N,N',N'*-tetramethylbenzene-1,4-diamine

11 ANTIMETABOLITE ANTIMICROBIALS

The mode of action of several antimetabolite antimicrobials consists in inhibition of folic acid synthesis (Fig. 11). Sulfonamides and 4-aminosalicylic acid competitively inhibit pteridine synthetase. Trimethoprim and its derivatives inhibit dihydrofolate reductase (Murray *et al.* 2005).

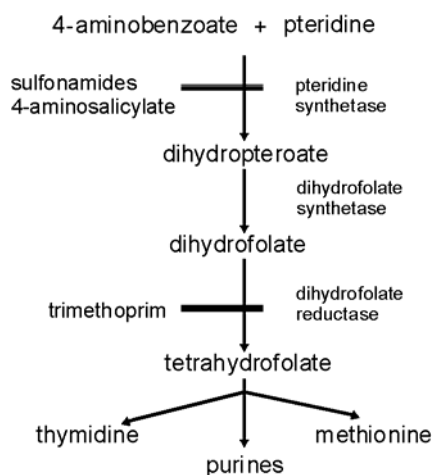
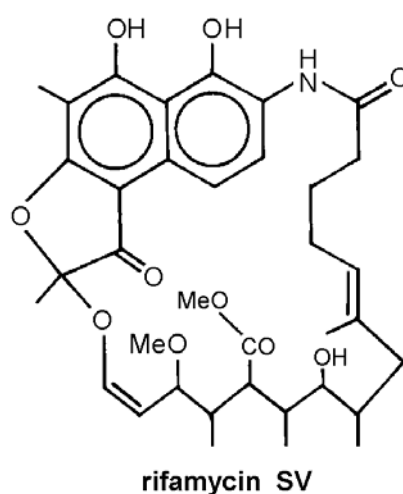


Fig. 11. Mode of action of antimetabolites.

The antibiotic binds to the β subunit of the polymerase and apparently blocks the entry of the first nucleotide which is necessary to activate the polymerase, thereby blocking mRNA synthesis. Rifampicin is an extremely efficient inhibitor of the bacterial enzyme, but fortunately eukaryotic RNA polymerase is not affected. RNA polymerase consists of a core enzyme made up of 4 polypeptide subunits, and rifampicin specifically binds to the β subunit. However, since isolated β subunit does not bind rifampicin, the precise configuration in which it is locked into the core enzyme is important.

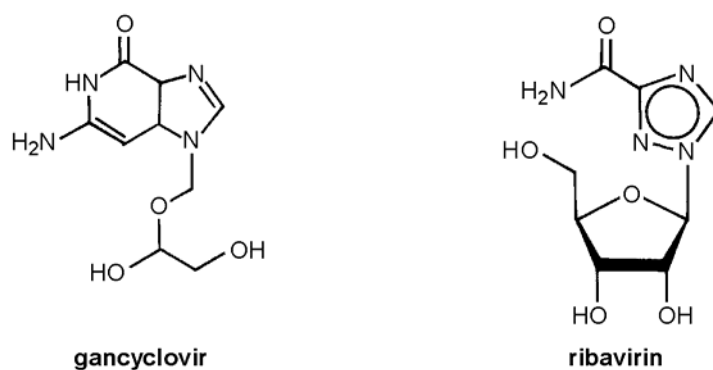
12 RIFAMYCINS

Rifamycins and ansamycins are compounds produced by *Streptomyces* spp. in which an aliphatic bridge links 2 non-adjacent positions on an aromatic component (Chiao *et al.* 1995). Rifampicin is a semisynthetic derivative of rifamycin that is active against G^+ bacteria (including *Mycobacterium tuberculosis*) and some G^- negative bacteria. Rifampicin acts quite specifically on the bacterial RNA polymerase and is inactive towards DNA polymerase or RNA polymerase from animal cells (Heep *et al.* 1999).



13 ANTIVIRAL AGENTS

The anti-HIV compounds are thought to inhibit reverse transcriptase activity by causing premature chain termination during the transcription of DNA from the ssRNA template. Similarly, gancyclovir acts as a chain terminator and DNA polymerase inhibitor during the transcription of cytomegalovirus DNA. Since these compounds lack a hydroxy group on the 2-deoxyribose ring, they are unable to form phosphodiester linkages in the DNA chain. Ribavirin, in contrast, allows DNA synthesis to occur, but prevents the formation of viral proteins, probably by interfering with capping of viral mRNA (Brown 1979). *In vitro*, ribavirin antagonizes the action of zidovudine, probably by feed back inhibition of thymidine kinase so that



the zidovudine is not phosphorylated. Several substances, such as fattiviracin A₁, are active against herpes simplex viruses. The mechanism of its antiviral activity may be due to inactivation of the viral particles and inhibition of viral entry into host cells (Uyeda *et al.* 1998).

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