

Chapter 1

Antibiotics

The increase in life expectancy seen during the twentieth century in many parts of the world is by now too familiar to require lengthy discussion. Expectancy at birth in the United States, for example, increased by close to two decades from 49.2 years at the beginning of that century to 68.1 years in 1950.¹ This remarkable jump generally has been attributed to improvements in sanitation and the advent of drugs for the treatment of infectious disease. Many bacterial infections that required hospitalization before World War II are now treated with a course of antibiotics. Usage is so well accepted that treatment will involve prescription called into the pharmacy by the physician's office and self-administration at home. The isolation of pure penicillin in 1939 in England is often used to date the beginning of the development that has led to today's armamentarium of antibiotics drugs. However, the story in fact begins back in Germany in the early 1930s.

It was well recognized by then that the structure and enzyme systems that allow bacteria to thrive are very different from their mammalian counterparts. Scientists devoted considerable effort from the late nineteenth century on to the search of chemicals that would exploit those differences so as to specifically eradicate bacterial cells. A tantalizing early clue that such differences might exist lay in the stains that were used by microbiologists to study their prey. Drawing on the wealth of synthetic dyes produced by the burgeoning chemical industry in Germany in the nineteenth century, scientists had identified a series of substances that stained bacteria in preference to mammalian cells.² The key to finding a drug that would preferentially eradicate bacteria seemed to be to find a stain that would kill the cells that accepted that particular dye. The long search seemed to have finally borne fruit in 1932. Gerhard Domagk, working in a laboratory set up by I. G. Farben, discovered that the red dye Prontosil Rubrum (Fig. 1) protected mice that had been injected with otherwise lethal doses of staphylococci.³ Use of the dye to treat successfully a human infection (Domagk's daughter) confirmed that this was indeed a therapeutic breakthrough. There had by then, however, been more than a few reports of seemingly miraculous cures of disease due to bacterial infection.

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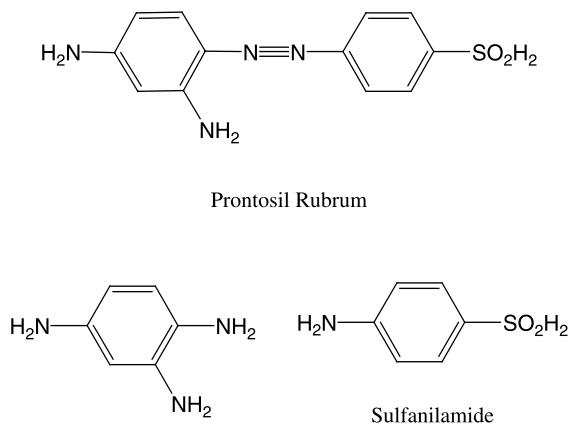


Figure 1 Prontosil Rubrum and its conversion to sulfanilamide.

The resulting skepticism from the inevitable failure of those earlier treatments led to surprisingly slow acceptance of the dye, by now simply called Prontosil. There was also the puzzling fact that the dye was only marginally effective in killing bacteria *in vitro*, that is, in the then standard test tube experiment for antibacterial activity. A group of scientists at the Pasteur Institute in France then showed that the dye molecule is transformed chemically in animals.^{4,5} Liver enzymes, they found, split the molecule in two at the central nitrogen to nitrogen azo linkage (N=N). One of the halves, subsequently named sulfanilamide, turned out to be a fully effective antibacterial compound both in test tubes or when administered to infected mice. The other half showed no antibacterial activity whatsoever. This work incidentally gave birth to the discipline of drug metabolism. Prontosil was to be but the first case of a drug that needed to be modified by the body for activity.

An immediate result of this finding was the abandonment of Prontosil in favor of the chemically much simpler sulfanilamide. This drug can be synthesized from benzene in just a few steps.⁶ In fact, this synthesis was for many years a laboratory exercise at the beginning of organic chemistry courses. It probably enticed more than one student, including the author, into a career in pharmaceutical research.

The discovery of sulfanilamide marked the beginning of the search for agents to treat infectious disease among compounds made from scratch by organic chemists. We come back to that story later. The discovery of the other important source of compounds that selectively kill microbes dates back to 1929 and Alexander Fleming's well-known serendipitous discovery. He noted a microbe-free clear zone around a mold colony that had contaminated a culture in a Petri dish, and correctly ascribed that to an antibiotic substance secreted by the mold. He named this unknown secretion penicillin after the producing mold, which he had identified as *Penicillium notatum*.⁷ The imminence of World War II is said to have spurred the transition of what had been considered as simply an interesting laboratory observation into a useful antibiotic drug. Beginning in about 1938, Howard Florey led the very major effort to isolate penicillin. This was finally accomplished in 1940, largely through the work of his Oxford collaborator Ernst Chain. The team isolated

just enough pure penicillin to ascertain its near miraculous activity in humans.⁸ Production of penicillin was transferred to the United States, because the British chemical industry was at that time fully tied up with war production. In its original form, *Penicillium notatum* grew best as a surface mat. Production in large quantities invoked visions of the use of shallow tanks with enormous surface areas, so the project was assigned to the U.S. Department of Agriculture Northern Laboratory in Peoria, Illinois, which had experience in industrial fermentation. There they devised a method for growing the mold as a submerged culture. By this and other means they greatly increased the yield of penicillin.^{8,9} The method developed by USDA was then transferred to industry, and a large number of companies with expertise and facilities for fermentation were enlisted in the effort.¹⁰ This even at one time included Schenley, better known as a producer of spirits.

Penicillin in the form used then had a number of very serious shortcomings. The drug had to be administered by injection as it was not orally active. The molecule is also very reactive, leading to poor stability. Early research aimed at producing more stable congeners led to several salts with improved stability. Reasons for the sensitivity of penicillins emerged with the determination of their chemical structure. The compounds are in essence comprised of two discrete connected pieces. The essential part consists of a fused ring structure called a beta-lactam. This is the reactive part that in the end kills bacteria; it also contributes to the lack of stability. The rest of the structure, which is also required for activity, consists of an organic acid connected through a chemical bond. Penicillin obtained from fermentation is a mixture of closely related compounds in which the invariant beta-lactam is hooked to slightly different acids. Penicillin G is one of the major components. Scientists had noted that they could increase the proportion of one or another congener by adding a large amount of that acid to the culture medium (Fig. 2).^{11,12} This allowed them selectively to produce one or another of the congeners such as Penicillin V. These still, however, shared many of the same shortcomings. This included poor stability and lack of oral activity; the drugs were also not effective against a significant number of classes of bacteria. It had become apparent by 1960 that further improvements would require manipulation of the chemical structure.

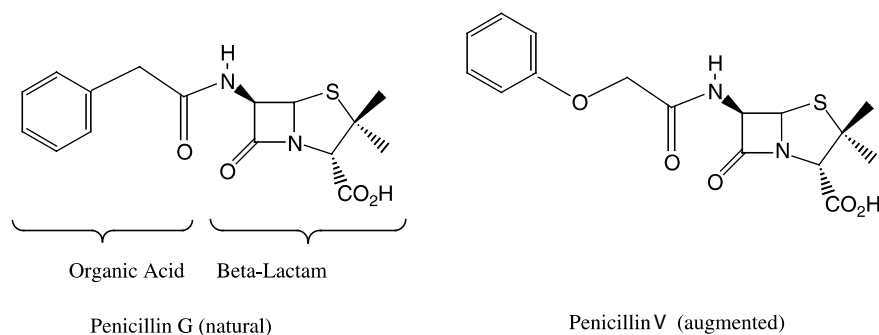


Figure 2 A penicillin from the naturally occurring complex and a typical augmented feeding product.

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It was later established that the selectivity of the beta-lactam antibiotics traces back to the fact that bacteria are more closely related to plants than animals. Individual animal cells are surrounded by a membrane, whereas plants and thus bacteria depend on a wall for cell integrity. In bacteria that structure is composed of a dense network of protein filaments that is cross-linked by chemical bonds. A significant number of the amino acids that compose the proteins have chemical structures that are mirror images of those found in animals. The beta-lactams (penicillins and cephalosporins) are mistaken by bacterial enzymes as small pieces that will be used to form the cross-links. Once they get incorporated, they bring the process to a dead halt, causing the cell wall to rupture.¹³ The drugs are thus selective, because mammals do not use cell walls and in addition utilize enzymes that do not recognize the mirror image amino acids used to make bacterial cell walls. The beta-lactam enzymes are consequently known for their very large safety margins. There is, however, a distinct portion of the population that is extremely allergic to these drugs.

By 1940 sulfanilamide had come into widespread use particularly in treating war wounds. The drug was used both as a tablet and sprinkled directly onto open lesions. Although the drug saved numerous lives, many types of bacteria were immune to its action. Chemists in a number of pharmaceutical laboratories then tried to make changes in the molecule in attempts to broaden its activity against other classes of bacteria (Fig. 3). Systematic work showed that there was only a single place on the molecule that could be manipulated and still retain antibacterial activity. Hundreds of analogues of sulfanilamide such as sulfathiazole were probably prepared in a number of laboratories between 1940 and the late 1950s, by which time the work was finally abandoned.¹⁴ No fewer than 27 of these were granted nonproprietary names, which is often an indication that the sponsor intends to test the compound in the clinic. At least eight of these so-called sulfa drugs are currently used in the clinic.

The antibacterial activity and selectivity of this class of drugs again depends on the fact that bacteria uniquely rely on biochemical processes that have no counterpart in more complex organisms (Fig. 4). Folic acid, perhaps better known as one of the B vitamins, is an essential factor in various metabolic processes such as formation of red blood cells and DNA itself. Over the course of evolution many organisms have lost the ability to make this compound and rely on obtaining it in food. Bacteria, on the other hand, synthesize this vitamin from scratch. An important biochemical step involves hooking a small molecule, PABA (para-aminobenzoic acid), onto the growing molecule. The chemical structures of the sulfa drug are similar enough to PABA to cause bacterial enzymes to incorporate these molecules.

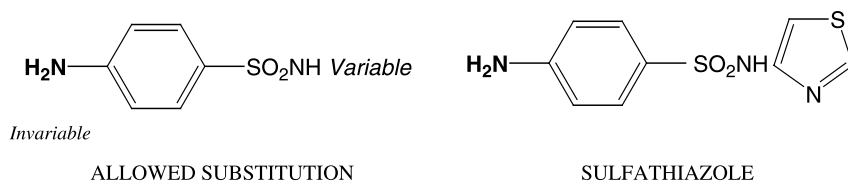


Figure 3 Sites that may be modified on sulfa drugs.

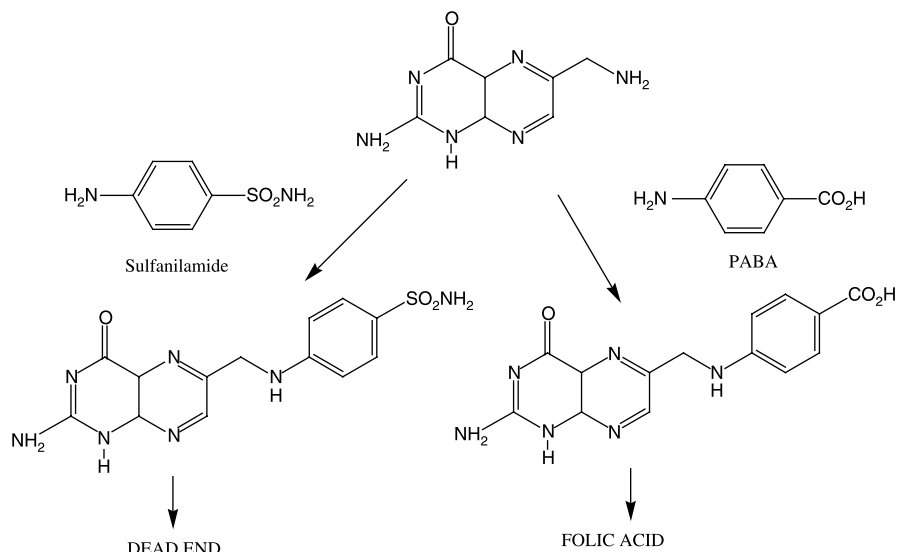


Figure 4 Sulfa drugs' mode of action.

However, the resulting product can go no further, in effect causing the bacterium to die for lack of folic acid.¹⁵

One of the sulfa drugs, sulfamethoxazole, is a constituent of combination tablets, such as Bactrim[®], that still comprise first-line treatment for urinary infections. The other active ingredient, trimethoprim, originates in work carried out by future Nobel Prize winner George Hitchings at Burroughs Wellcome in the late 1950s.¹⁶ Taking their cue from compounds involved in enzyme action, he and his associates prepared a congener called pyrimethamine.¹⁷ This agent proved to inhibit bacterial growth by interfering with an enzyme, dihydrofolate reductase (DHFR), involved further down the line in the synthesis of folic acid. Further work along the same lines led to the synthesis of trimethoprim. The combination tablet exploits the fact that each of the active ingredients inhibits bacterial growth by interfering with different enzymes that bacteria need survive (Fig. 5). This combination has been used for treating HIV/AIDS opportunistic infections.

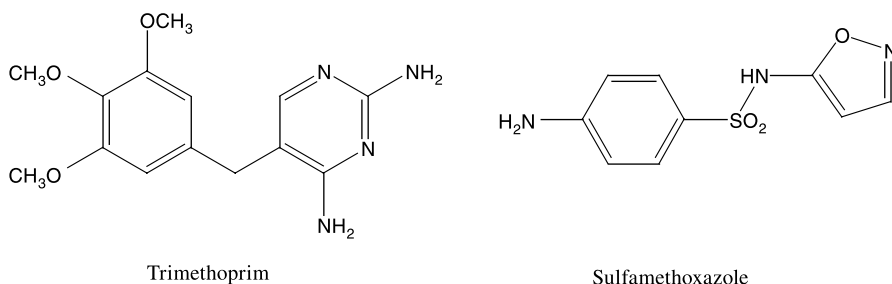


Figure 5 Active ingredients in Bactrim.

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Isolated reports of unusual side effects came with widespread use of sulfa drugs.¹⁸ Very high doses caused some patients to excrete water and others to show a drop in blood sugar levels. Chemists in pharmaceutical laboratories seized on these apparent side effects to develop entirely new classes of drugs. By manipulating the chemical structures, scientists at Hoechst came up with a compound that normalized blood sugar in adult onset diabetics. This drug, chlorpropamide, in which the all-essential amino group is replaced by methyl, is virtually devoid of antibacterial activity. Replacement of the sulfonamide (SO_2NH_2) by a sulfonylurea function ($\text{SO}_2\text{NHCONH}_2$) proved crucial for antidiabetic activity and was present in the widely marketed drug tolbutamide.¹⁹ The only drugs available in the 1940s for treating conditions that required loss of body water, the diuretics, included mercury in their chemical composition. Scientists at Merck, led by Sprague, were able to introduce changes on the structure of the sulfa drugs to produce a well-tolerated diuretic drug. These principally involved adding an additional sulfonamide onto the parent molecule. This compound, chloraminophenamide, also devoid of antibacterial activity, is no longer in use. It has been superseded by hydrochlorothiazide, which was first synthesized by chemists at Ciba. This drug, often better known by its acronym, HCTZ (Fig. 6), is still used as first-line treatment of patients with mildly elevated blood pressure.²⁰

The discovery of penicillin led to the recognition of the ability of fungi to protect themselves against microorganisms by secreting compounds that inhibit bacterial replication and in fact often kill off those threatening organisms. Penicillin itself showed that such antibiotics may act specifically on enzymes that do not have counterparts in mammals. This property, shared with the sulfa drugs, led to low toxicity to humans. The search for new molecules in this class turned to the

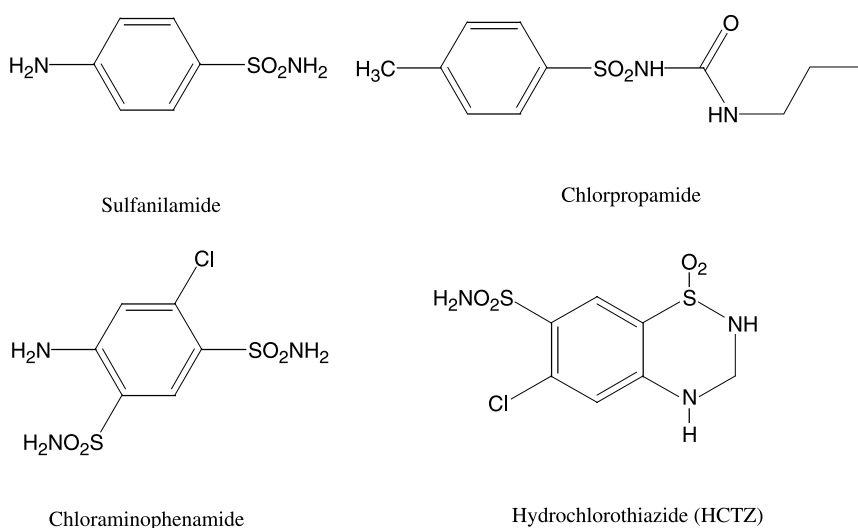


Figure 6 Nonantibiotic drugs related to sulfonamides.

investigation of the large number of fungi that inhabit the world. The competition between these organisms and bacteria in soils pointed to that domain as a potentially rich source of antibiotics. In the early 1940s, Albert Schatz, working at Rutgers University under the supervision of his professor, Selman Waksman, discovered that the mold *Streptomyces Griseus*, produced an antibiotic that had a complex sugar-based chemical structure that was quite different from penicillin.^{21,22} It was more stable than penicillin and was effective against a somewhat different set of pathogens. This therapeutic agent, streptomycin, is today still one of the indispensable drugs used to combat tuberculosis. This was the first in a series of antibiotics composed of linked sugar-like components. The presence of basic nitrogen sets those fragments apart from simple sugars. These antibiotics, known as aminoglycosides, are largely used for treating severe infections (Fig. 7).

The aminoglycosides are part of a group that acts on the machinery within cells, be they bacterial or mammalian, that assemble the proteins involved in reproduction and growth. To accomplish this task, a central component, called the ribosome, slides along the string of RNA that incorporates the sequential three base codes for amino acids that will form part of the protein chain. Smaller nucleic acids, called transfer RNA, which include only the three-base sequence for individual amino acids, convey those fragments to the ribosome. The aminoglycosides in essence cause the messenger RNA, which carries the template for a new protein, to be misread by the ribosome. This causes the ribosome to cause errors in making new proteins, which are then lethal to the cell. Selective toxicity to bacteria and not the host depends on the species difference in the parts used in this process. Aminoglycosides, like many other fermentation products, often occur as mixtures of very closely related compounds. The commercial form of the antibiotic gentamycin, for example, consists of a mixture of C₁, C_{1A}, and C₂, all of which have similar activity.^{2,3} In order to overcome some of the shortcomings of the aminoglycoside

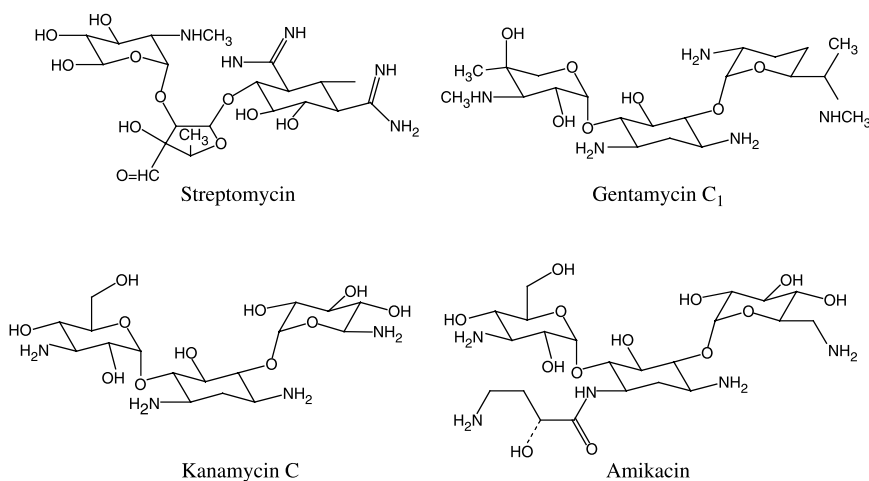


Figure 7 Aminoglycoside antibiotics.

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kanamycin, which had first been isolated in 1957, scientists at Bristol–Myers undertook a program to prepare semi-synthetic derivatives. This led to the marketed aminoglycoside amikacin, which differs largely in the incorporation of a new side chain on the central ring.²⁴ Aminoglycoside antibiotics retain activity against bacteria that have developed resistance to some of the more common drugs because they act on a very different target on the organism.²⁵ An apocryphal tale has it that the approval of a novel and highly potent aminoglycoside was met with high expectations for commercial success on the part of its sponsor. The very properties of the drug led to an agreement on the part of infectious disease specialists to use the drug extremely sparingly. Setting the antibiotic aside in a reserve category, it was felt, would provide last resort treatment for infections with bacteria with multidrug resistance.

This discovery also launched a major effort in the laboratories of many pharmaceutical companies to screen soils from a wide variety of sources. Thus, in 1949, Benjamin Duggar of the University of Wisconsin, who was a consultant to the Lederle Laboratories, discovered a new antibiotic that he called aureomycin.²⁶ Scientists at Lederle used this finding to develop a family of chemically closely related antibiotics. These are called tetracyclines after the chemical structure, which involves four linked rings (Fig. 8). The original drug had a relatively short duration of action due to inactivation in the bloodstream. Chemical manipulation of the natural product led to doxycycline, the first tetracycline that was effective when taken just once per day.²⁷ This class of drugs also inhibits protein synthesis

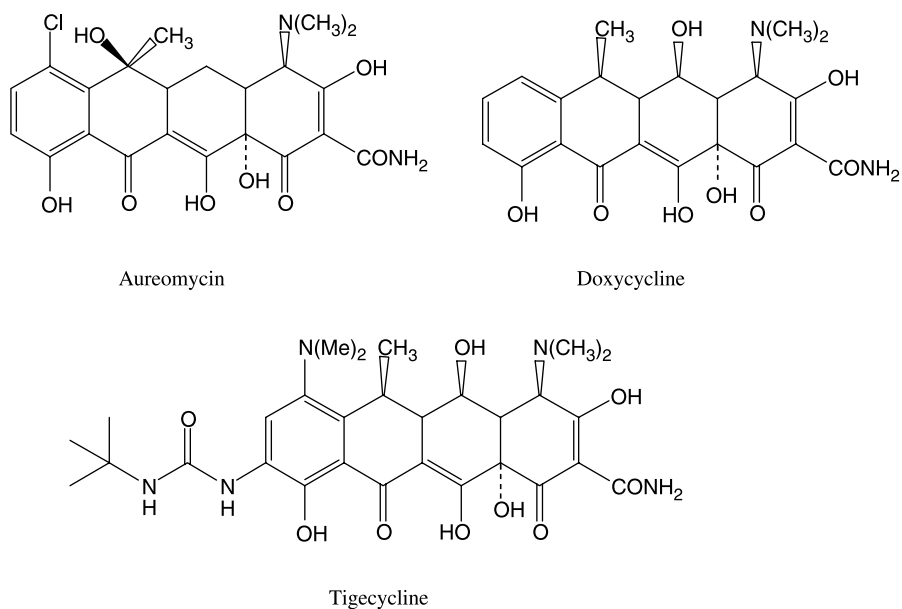


Figure 8 Tetracycline antibiotics.

in bacteria, although at a different biochemical site than do the aminoglycosides. Although the causative agent of malaria is a plasmodium parasite, it is susceptible to doxycycline at one of the stages of its very involved life cycle. The drug thus finds extensive use as a prophylactic for travelers in malaria-infested areas, and is used to treat the disease in combination with other drugs. The tetracyclines exhibited activity against a wide variety of microbes when they were first introduced. Resistant strains, however, developed over time, as has happened with most other classes of drugs. Very recent work in the original laboratories, by the now Wyeth–Ayerst, resulted in a chemically highly modified derivative that is effective even against tetracycline-resistant strains. This compound, tigecycline, was approved for sale in the United States in 2005.²⁸

A soil sample from the Phillipines was sent back to the Indianapolis labs of Eli Lilly by one of their local employees at about the same time. Screening of that sample led to the isolation of a new antibiotic with yet another novel chemical structure. The organism, at that time called *Streptomyces erythraeus*, gave the compound its name. (Many *Streptomyces* species have since been reclassified as *Actinomyces* for reasons of taxonomic accuracy.) This antibiotic was developed into the still widely used drug erythromycin by a team at Lilly led by J. M. McGuire. The structure is a good bit more complex than preceding antibiotics, and consists of a very large fourteen-membered ring with attached sugars, one of which has a basic amino group (omitted in Fig. 9 for reasons of clarity).²⁹ This compound too inhibits bacterial protein synthesis, again by a somewhat different mechanism. The relatively short duration of blood levels of the drug encouraged chemists to try to modify the basic structure so as to overcome that property. In the late 1980s a group of chemists at the Croatian (then Yugoslav) pharmaceutical company Sour Pliva succeeded in preparing a derivative in which a basic nitrogen atom had been introduced into the fourteen-membered ring.³⁰ The compound, which was subsequently developed

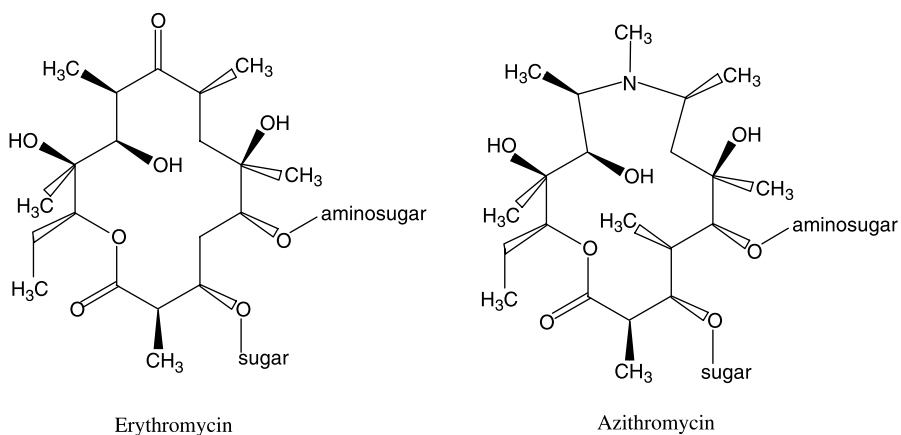


Figure 9 Erythronolides.

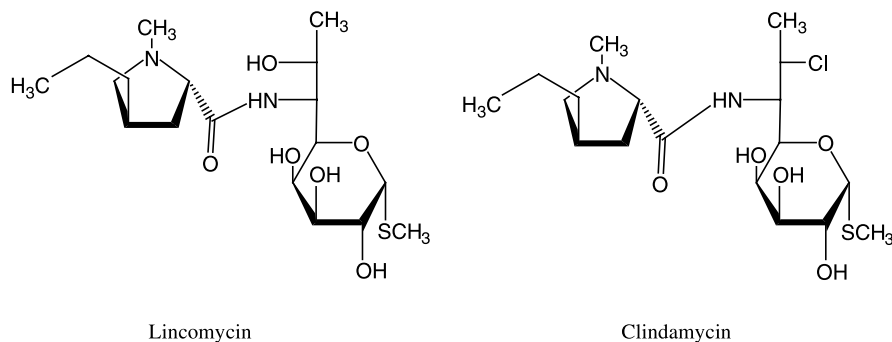


Figure 10 Lincomycin and clindamycin.

under license by Pfizer, proved significantly more stable than the parent. It provided long-lasting blood levels that made once per day treatment feasible.

A soil sample from a much less exotic source led to another new class of drugs. A detail man in the American Midwest sent a sample from Lincoln, Nebraska, back to his employer, the Upjohn Company, in Kalamazoo. There, scientists isolated an antibiotic whose chemical structure and range of activity was markedly different from the other hitherto known agents. It was named lincomycin (Fig. 10) after the producing organism, *Streptomyces Lincolnensis*, which in turn had been named after the capital of Nebraska.³¹ The drug was eventually largely superseded by the derivative clindamycin, which was produced by modifying the structure chemically.

The forgoing touches on only the highlights of what was a major program in many laboratories from the late 1940s on. The screening process became highly efficient with time. In brief, this involved culturing soil samples so as to encourage formation of colonies of native fungi. Samples of the fungi would then be streaked onto plates inoculated with some test bacterium. The finding of clear kill zones around the colony would start an involved process for characterizing the substance that had killed the test bacteria. An unexpected major stumbling block in this search was the frequent rediscovery of previously known antibiotics. Many laboratories thus maintained extensive dictionaries of the properties of all antibacterial substances produced by soil organisms. These tomes, maintained in order to avoid wasting time on known substances, were considered highly confidential and were jealously guarded, particularly on occasions where an employee left the company.

Penicillin had in the meantime not been forgotten. Limited work on attaching unusual acids to the beta-lactam part of the molecule by feeding those to the fermentation tanks had not been particularly successful. In 1959, however, scientists at Beecham Laboratories in the UK managed to devise conditions that allowed them to isolate the beta-lactam itself without the attached acid part from fermentation broths.³² The availability of this substance, called 6-aminopenicillanic acid, or more familiarly 6-APA, offered organic chemists the chance to hook acids (also called side chains) that are never found in nature onto the active part of the molecule. The first drug from this research, pheneticillin, was developed by Beecham in

collaboration with Bristol Myers in Syracuse, New York. This development was eagerly seized upon by a number of competing laboratories. Many of these now launched their own programs aimed at synthesizing and testing analogs of penicillin with novel side chains (Fig. 11). The output of these penicillin analogs was limited only by the high skill needed to carry out manipulations on these sensitive and reactive molecules. The work was subsequently facilitated by new methods for converting the much more easily available penicillin-V into 6-APA by either chemical or enzymatic means.³³ These new so-called semi-synthetic penicillins included much more stable drugs as well as a number with broader activity and some that were active when taken by mouth. This massive research effort resulted in at least 35 discrete substances that showed enough promise that their sponsors went through the process of acquiring nonproprietary names. Not all, needless to say, made it to the market. The orally active semi-synthetic drug amoxicillin is one of the more widely prescribed antibiotics to date. The beta-lactam pirlbencillin is typical of some of the more highly modified penicillin-based drugs.³⁴

The development of resistance to antibiotics on the part of bacteria can be considered a simple expression of evolution, fitness in this case being expressed as sheer survival. This phenomenon was probably first observed with penicillins because of their early widespread and indiscriminate use. The strained four-membered beta-lactam ring in these compounds is at one and the same time the

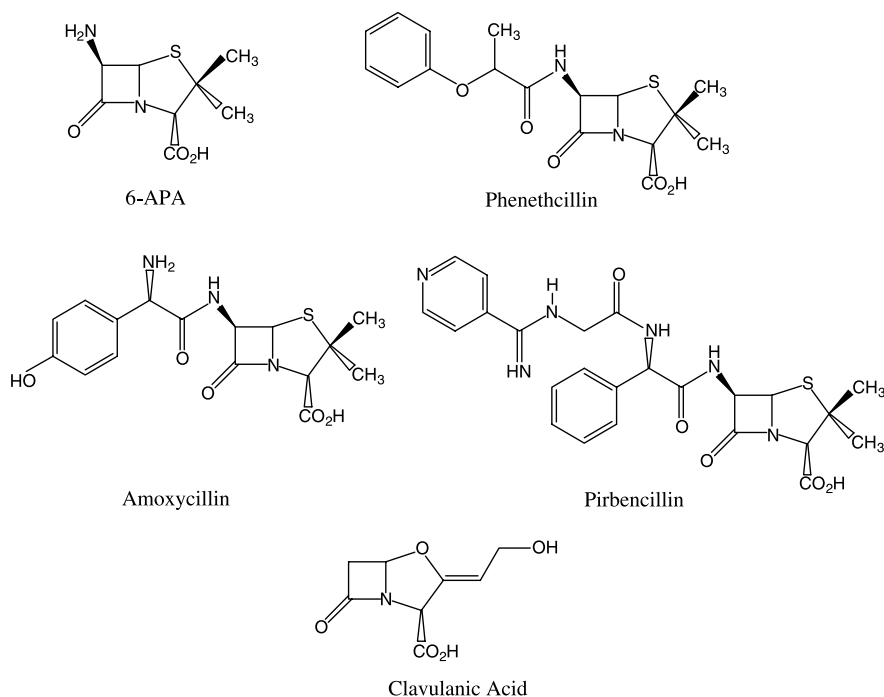


Figure 11 Penicillins.

mode of action and Achilles heel. Resistant organisms elaborate an enzyme, beta-lactamase, that specifically inactivates that function. A fermentation product closely related structurally to the penicillins was found to compete for beta-lactamase with penicillins. This compound, clavulanic acid, which has little if any antibacterial activity in its own right, will thus retard inactivation of co-administered antibiotic.³⁵ Augmentin[®], a fixed combination with amoxicillin, is widely prescribed for many infections.

Other environments rich in bacteria and fungi were examined for potential antibiotics as well. The lead for a new series of drugs based on a beta-lactam came from the isolation, in 1945, of a mixture of antibiotics from Sardinian sewage sludge by the Italian scientist Brotzu.³⁶ The active principle, named Cephalosporin C after the producing species, *Cephalosporium acremonium*, was too weakly active to be considered as a drug candidate. However, some of its properties, such as resistance to beta-lactamase, a bacterial enzyme that destroyed the four-membered ring in penicillin, made it an attractive starting point for further research. Although the chemical structure differed from penicillin, it shared enough similar features, such as the beta-lactam part, to lead scientists to apply the same methodology to try to prepare more active compounds. Attempts to introduce different side-chain acids by adding those to the fermentation were not very successful, nor were experiments aimed at producing the bare beta-lactam part of the molecule, equivalent to 6-APA, by fermentation. A procedure for obtaining the bare nucleus, 7-ACA (7-aminocephalosporanic acid), from cephalosporin C either chemically or by treatment with enzymes was finally published in 1962 by a team of chemists at Ciba in Basel (Fig. 12).³⁷

The availability of this intermediate now made it possible to launch research programs analogous to those that had led to the collection of semi-synthetic penicillins. The group at Eli Lilly was particularly active in this field. Its first drug from this program was the injectable antibiotic cephalothin (Keflin). The starting material for this and later products, Cephalosporin C, was more difficult to obtain than the penicillins. Extensive research on production methods combined with demand had drastically lowered the price of bulk penicillins. This had in fact become a bulk commodity chemical, which, by the late 1990s, could be bought for less than one dollar per gram. The fact that both molecules shared a beta-lactam led to

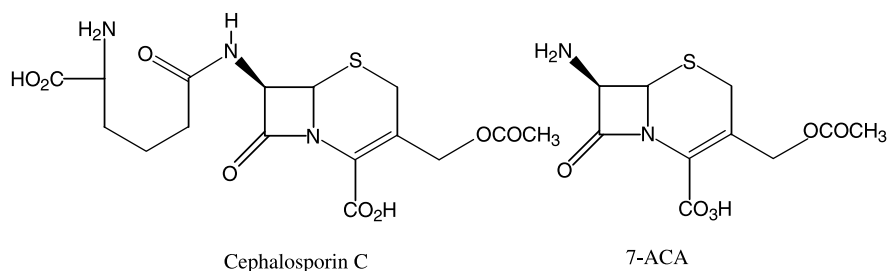


Figure 12 Cephalosporin C and 7-ACA.

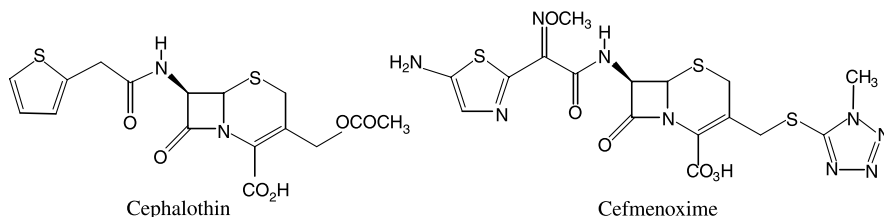


Figure 13 Cephalothin and Cefmenoxime.

considerable research on the part of chemists to find a way to transform penicillins into cephalosporins. These efforts were rewarded when Robert Morin devised just such a procedure at the Lilly labs. It is likely that the great preponderance of today's cephalosporin drugs start life as penicillin V or possibly its cousin penicillin G. The now ready availability of 7-ACA led to intensified research throughout pharmaceutical company laboratories. This resulted in a virtual flood of antibiotics, of which no fewer than 44 of these were assigned nonproprietary names. The group of drugs that were made available to physicians included many that could be administered orally. Most were quite resistant to the bacterial enzyme that destroys the beta-lactam. One of the most significant advances lay in the fact that selected semi-synthetic cephalosporins were active against a great many types of bacteria that were not sensitive to the earlier drugs. The beta-lactam nucleus is barely perceptible in the structure of some of the so-called third-generation antibiotics derived from 7-ACA such as cefmenoxime (Fig. 13).

Activity of the cephalosporins suggested that the nature of the ring fused to the beta-lactam was not crucial. As chemical methods and expertise accrued, it became possible to make modifications to that fused ring. A number of drugs that included such modifications have been approved for clinical use. Probably the most complex is moxalactam, a cephalosporin-like compound in which oxygen replaces sulfur (Fig. 14).³⁸ The involved lengthy synthesis of this compound at one stage involves degrading penicillin V to a bare four-membered ring. Chemists at Shionigi then built the oxygen-containing ring onto that. Imipenem represents another drastically modified compounds.³⁹ One approach to creating this compound involves an intermediate obtained by degrading the fused five-membered ring in 6-APA; it is

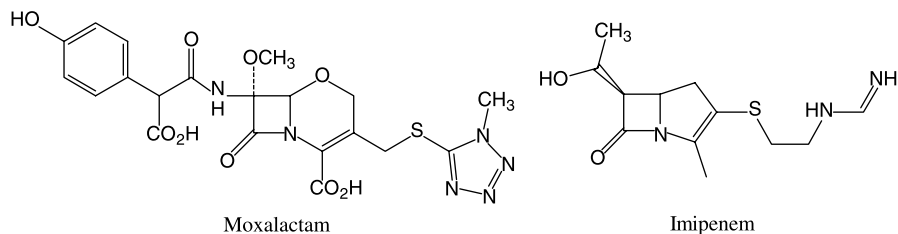


Figure 14 Beta-lactams fused to carbo- and oxacyclic rings.

then built it up again with a ring in which sulfur has been replaced by a carbon atom.⁴⁰ The resulting antibiotic is active against a particularly wide assortment of bacteria. It is usually administered with cilastatin, a compound that slows metabolic destruction of the antibiotic.

The beta-lactam ring in all antibiotics of this class discussed thus far has been invariably fused onto another ring. The diversity of structures generated by that appendage would seem to indicate that this is not a critical element for biological activity. Nature soon demonstrated that this extra ring could be omitted entirely. The basic premise for screening soils as a source for antibiotics relies on the assumption that fungi and actinomycetes carve out a niche in the environment by secreting substances that will eliminate encroaching bacteria. This approach was soon plagued, as noted earlier, by rediscovery of known substances and lack of new findings. Attention then turned to bacteria themselves as a potential source for new antibiotics, because it was logical to assume that different microbial species would compete for the same ecological niches. In 1982, scientist at Squibb announced the discovery of a novel antibiotic produced by the bacterium *Chromobacterium violaceum*; the active compound was dubbed a monobactam as it lacked a second ring fused onto the beta-lactam (Fig. 15).⁴¹ The structure of this compound differs from the previously known antibiotics in this class not only by the lack of the fused ring but also by the lack of a complex organic acid side chain. An unusual sulfonic acid amide (NSO₃H) takes the place of the carboxylic acid (CO₂H) that is attached to the fused ring of the two-ring compounds. As is more often the case than not, the naturally produced antibiotic was not suitable for use as obtained. Its strong resistance to beta-lactamase prompted chemists to undertake programs aimed at producing synthetic monobactams. The first compound that resulted from this program, aztreonam, proved, as predicted from its natural forerunner, to be particularly resistant to beta-lactamases. The synthesis of aztreonam starts with

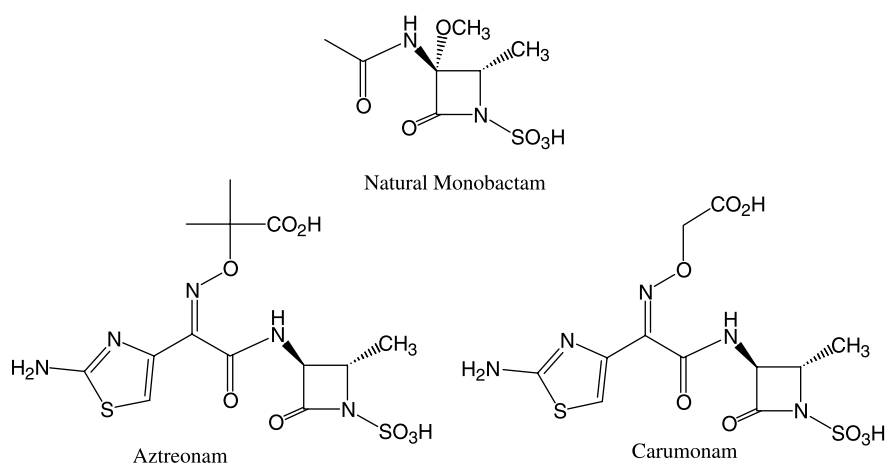


Figure 15 Single-ring beta-lactams.

simple organic chemicals.⁴² This is, of course, in contrast to most modified beta-lactams, whose production involves modification of fermentation-derived starting materials. A parallel program at Takeda led to the commercial monobactam carumonam.⁴³

Pharmaceutical research in the early 1960s relied heavily on the output of its organic chemists. These individuals as a rule synthesized compounds that were aimed at some particular therapeutic target and were tested in a model for that disease. Surplus amounts of the samples were then tested in a standard screen. This usually comprised an array of tests that were designed to identify chemicals that showed some degree of biological activity in other disease models. The rationale for this procedure lay in the observation of the number of compounds that had been found over the years to have more activity on some unintended endpoint than their purported target. Some of the compounds discovered by the screening procedure had provided leads for agents that had gone on to become successful drugs. In that period, the Sterling Drug laboratories had a long-standing program on tropical disease. A compound, submitted by one of their chemists, George Leshner, showed unexpected activity in the ongoing antibacterial screen. According to one account this was a byproduct from the synthesis of an antimalarial drug.^{44,45} The chemical showed enough activity for further development. This drug, nalidixic acid (Fig. 16), was developed further and approved for use in treating urinary tract infections. The chemical structure of this little heralded antibiotic, it should be noted, incorporated all the necessary features that would be found in the later quinolones. The activity of this forerunner was quite modest and was apparently not enough to stimulate research in competing laboratories. Only a few additional

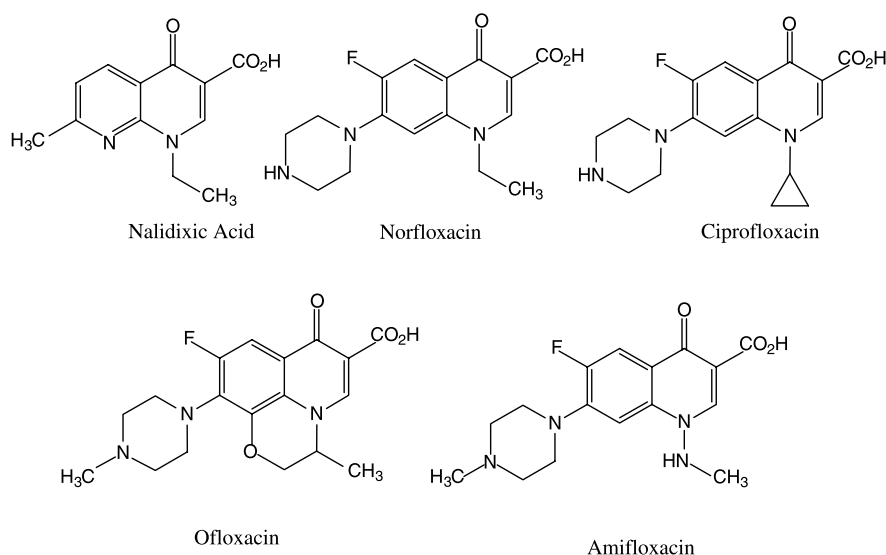


Figure 16 Nalidixic acid and current quinolone antibiotics.

entities reached the clinic from an assortment of other firms over the next decade. These analogs were only modestly more effective than the forerunner. Research did apparently continue at a low level in several laboratories. Replacement of hydrogen by fluorine was known to markedly increase activity in several classes of drugs such as the corticosteroids. Two specific modifications of the chemical structure of the quinolones, one of which interestingly comprised introduction of a fluorine atom, dramatically changed the picture. This compound, norfloxacin, was first synthesized in the late 1970s in the laboratories of the Kyorin Pharmaceutical Company in Japan.⁴⁶ Its activity in test models and subsequently in humans was clearly superior to any of its predecessors. It was then introduced into the United States by Merck. The excellent broad spectrum activity of this novel drug stimulated a virtual race to produce new chemical entities and it has been estimated that over a thousand quinolones have been synthesized in various laboratories, 22 of which have been assigned nonproprietary names. Although the structures of some of these later entities, such as ofloxacin and amifloxacin, have become quite elaborate, they still incorporate the basic elements present in the lead compound. Another of the second-generation congeners, ciprofloxacin, known to the press as simply cipro, gained considerable fame in late 2001. This drug gained wide exposure as a remedy and preventative at the height of the anthrax scare.

The quinolones, it has been determined, act by a unique mechanism. Recall that DNA is a very large string-like molecule. The length of this compound must be reckoned in feet rather than millimeters. This entity must obviously be tightly coiled for one to be packed into each and every cell nucleus. It would take far too long to carefully uncoil what has been compared to a snarl of fishing monofilament in order to read a stretch of DNA to exert its controlling role. Biochemistry then does what any impatient fisherman would do: cut sections in order to pass strands through another. A very special enzyme called topoisomerase then temporarily holds the cut ends together until the section has passed through and then subsequently reconnects the cut pieces. Bacterial DNA floats loose within the cell as microbes have no cell nucleus. Their topoisomerase, possibly because of this difference, is quite different from that in organisms that have a cell nucleus. The quinolones act as quite specific inhibitors of bacterial topoisomerase and in effect degrade the DNA as the cut ends are not identified for reconnection. The lack of an intact DNA template prevents the affected organism from replicating.^{47,48}

The very large selection of very effective antibiotics has led to a significant diminution of research on new antibiotics. The increasing incidence of antibiotic-resistant organisms has led to renewed attention in the field. A drug introduced within the past five years may be an early harbinger of this. The development of this agent begins with the discovery of a series of compounds at the Du Pont Laboratories, which showed good activity against a wide range of bacteria.⁴⁹ The most promising candidate, DuP 721, was particularly active against microbes resistant to other classes of drugs. It was not taken further because of safety concerns raised from results in preliminary animal toxicity tests. Following these reports, scientists at Upjohn launched a program to prepare related compounds. All the

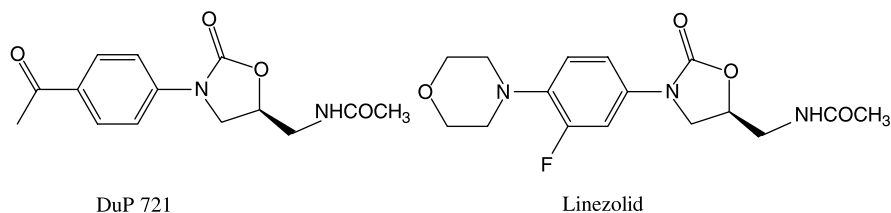


Figure 17 Linezolid.

analogs contained the nitrogen and oxygen five-membered ring, called an oxazolidone, also present in the Du Pont series. Two of the compounds from the collection of new analogs synthesized at Upjohn showed very promising antibacterial activity.⁵⁰ One of these, linezolid, which has been approved for human use, is quite effective against classes of bacteria resistant to known antibiotics (Fig. 17). This drug also acts by inhibiting bacterial protein synthesis. It does so by a rather unique mechanism, by blocking the binding of the transfer RNA intermediate to the unit that adds amino acids to the protein chain. This in essence stops the process before it even starts. It was felt that this differs sufficiently from the way other antibiotics act, by inhibiting bacterial protein, to avoid the development of cross-resistance.⁵¹

The various cell-killing substances uncovered by the soil-screening programs led a number of laboratories to examine some of those extracts for their potential for uncovering antineoplastic agents.⁵² These programs were based more on the availability of large numbers of extracts than on any imagined similarity between antibiotic and anticancer activity. The former of course relies on the fact that bacteria and humans belong to different kingdoms. The highly selective toxicity to bacteria is due to interference with biological mechanisms simply not present in humans. No such marked differences unfortunately obtain between normal and cancer cells. Most current antineoplastic agents thus rely largely on inhibiting processes that occur at a higher rate in cancer cells. The majority of these drugs act to inhibit cell replication at the level of DNA by a variety of mechanisms. This might be called the kinetic approach to selective toxicity. The antitumor antibiotics have not, with the exception of the anthracyclines, led to classes of agents with related structures. Although considerable work may have been devoted to developing more effective and better tolerated representatives, they are, as a rule, represented by a single licensed representative.

The simplest of these agents is the modified sugar streptozotocin (Fig. 18) isolated from a *Streptomyces* fermentation broth. The structure consists of glucose in which one of the hydroxyl groups has been replaced by a very reactive nitrosourea group.⁵³ It is of interest that the very same grouping occurs in the synthetic anticancer drugs BCNU and CCNU. These are some of the very first anticancer drugs; they act as alkylating agents on DNA and thus prevent cell replication. Streptozotocin is somewhat selectively taken up by the pancreas. Its main indication is thus for treatment of pancreatic cancer. This selective uptake and consequent

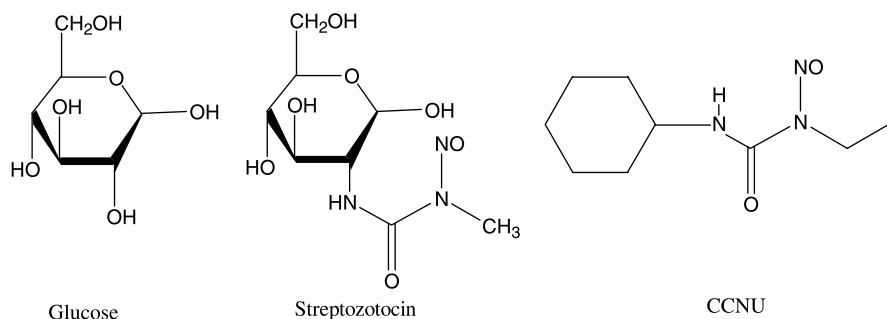
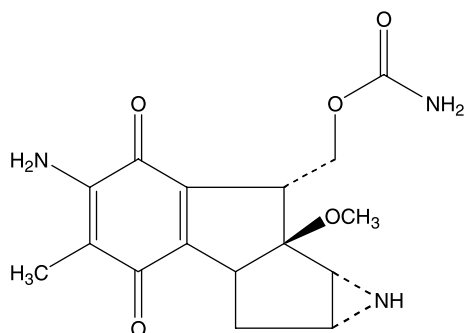


Figure 18 Streptozotocin and CCNU.

toxicity has provided pharmacologists with an important tool. Administration of the drug to laboratory animals causes them to develop diabetes. So-called streptozocin rodents are consequently often used in diabetes research.⁵⁴

Mitomycin C (Fig. 19) is a somewhat more complex alkylating agent isolated from another *Streptomyces* strain.⁵⁵ As is often the case, the compound is accompanied by the closely related mitomycins A and B. This agent is among the drugs that must first undergo a transformation in order to exert its action. The drug seems to be most often used in combinations with other antineoplastic agents in the various combinations designated by acronyms. It is the M in MIP (mitomycin C, ifosfamide, and cyclophosphamide). Although considerable work has been devoted to producing related compounds in the search for more selective and less toxic analogs, it remains the only representative of its class approved for use in the United States.

Three structurally very complex fermentation products, plicamycin,⁵⁶ dactinomycin, and bleomycin have also found their place in oncology (Fig. 20). Each, it should be added, represents a single drug class. The structure of the first of



Mitomycin C

Figure 19 Mitomycin C.

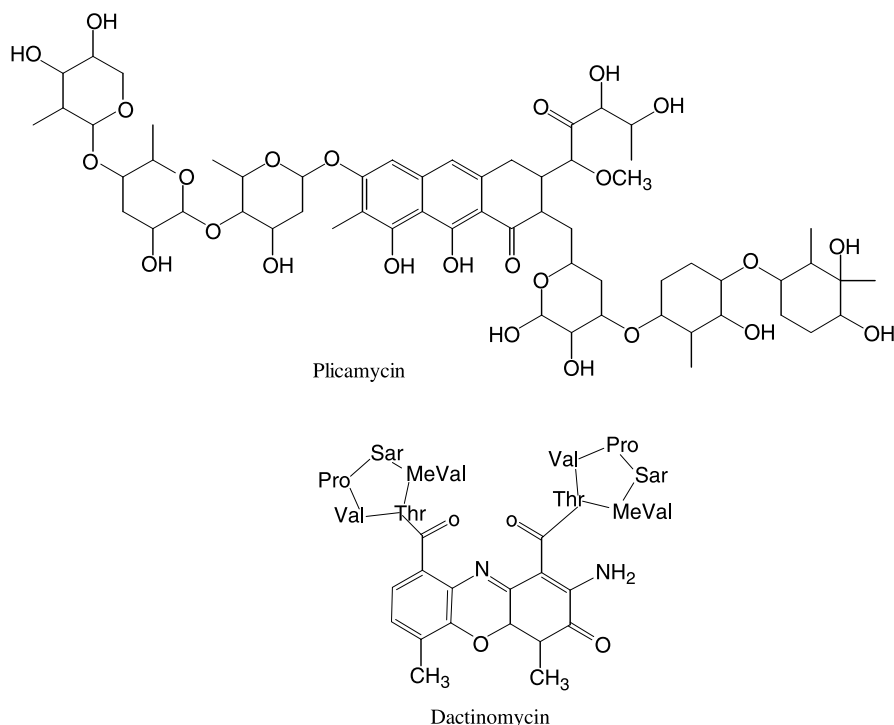


Figure 20 Plicamycin and dactinomycin.

these compounds has several structural features, such as the linear array of rings bearing hydroxyl groups and the attached sugars, which foreshadows the currently widely used anthracyclines. Plicamycin, initially known as aureolic acid, is apparently used mainly for treating testicular cancer. Dactinomycin, also known as actinomycin D, is one of yet another group of closely structurally related compounds isolated from *Streptomyces* fermentation broths.⁵⁷ This compound too has been the subject of a large amount of research aimed at producing a better congener. It is of interest that its mechanism of action is similar to the anthracyclines that will be considered next. The structure of bleomycin is not shown.

FDA approval of adriamycin in the early 1980s marked a significant change in cancer chemotherapy. The wide spectrum of activity of this drug against a variety of tumors led to increased recourse to chemotherapy for treating this disease. The development actually started at the Farmitalia laboratories in the 1950s with the isolation of a red pigment from cultures of *Streptomyces peucitius*. (The name is said to derive from the Peucitia region in Italy.) Over the years, Arcamone and his colleagues at the company isolated a number of pure substances from the mixture that made up the pigment. The first of these was a compound with antibiotic activity that they named daunomycin (Fig. 21). The same compound was isolated in parallel work at Rhône-Poulenc in France, where it was dubbed rubidomycin.

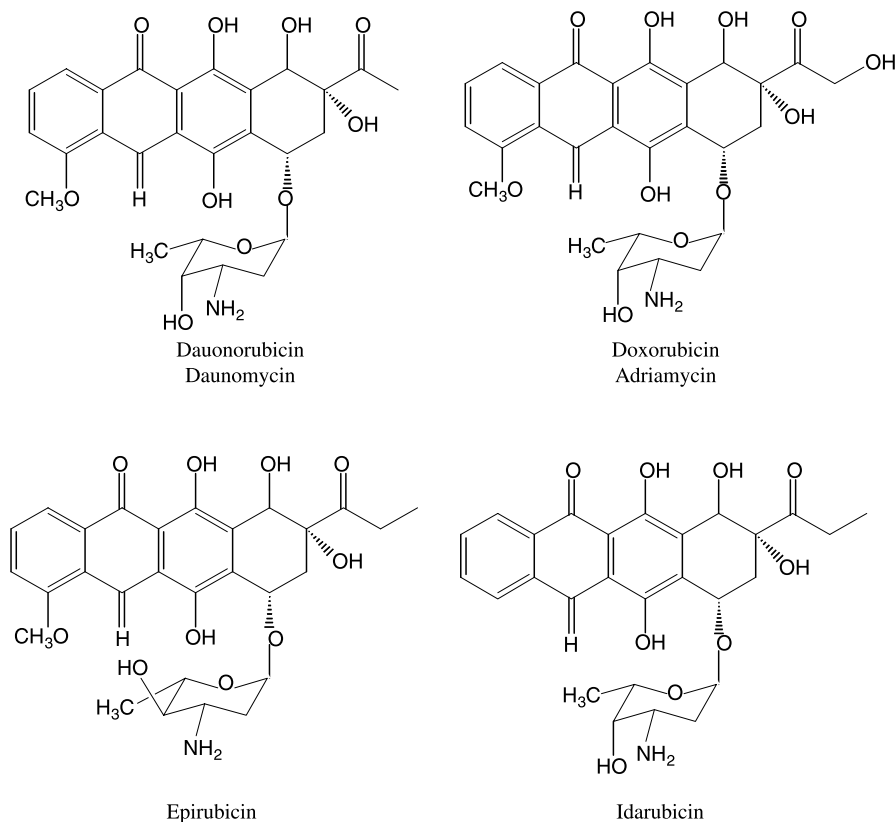


Figure 21 Anthracycline antitumor antibiotics.

This compound was found to have antibiotic activity and, more importantly, to be active against leukemias. The nonproprietary name daunorubicin, which included syllables from each source, was assigned to the drug. The “rubicin” suffix was to be used for all subsequent members of the series. Daunomycin was eventually introduced clinically, where it found its place as part of combinations used to treat leukemias. Further work in Milan then led to the identification of the analog doxorubicin, which has an additional hydroxyl group on the side chain.⁵⁸ This compound is more familiarly known as adriamycin, the name deriving from the Adriatic Sea; it seemed to have much wider activity and found widespread use in oncology. As a side note the Farmitalia’s American affiliate, Adria Laboratories, was actually named after the drug. Clinical use revealed a serious shortcoming of this drug. In addition to the usual toxicities, adriamycin was associated with dose-limiting cardiac toxicity. Chemists at Farmitalia started significant programs to modify various parts of the molecule to try to overcome that effect. Epirubicin, in which one of the hydroxyl groups on the sugar is inverted, is said to be less cardiotoxic, permitting a higher cumulative dose. Idarubicin, the analog missing a methyl ether on the first

ring is not in fact a fermentation product; the compound was first produced by total synthesis.⁵⁹ The anthracyclines were at first thought to exert their cytotoxic effect by intercalation in DNA. The large flat molecule, it was thought, would slide between two hydrogen-bonded base pairs and thus disrupt DNA's organization. The disrupted molecule would then no longer be able to take part in cell replication. More recent results suggest that the compound also disrupts topoisomerase 2, one of the human enzymes involved in cell replication whose action is analogous to the bacterial topoisomerase disrupted by the quinolone antibiotics.

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