Chemotherapeutic Targets in Parasites

Contemporary Strategies

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with the assistance of

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The Search for Antiparasitic Agents

Early Beginnings of Chemotherapy

Historical records from almost every culture have examples of successful treatment of different ailments by potions, mainly from vegetable sources and occasionally from animals. A good example of an antiparasitic agent is cinchona bark (quinine), which was used against malaria by the early Peruvians and introduced to the Western World by the Jesuits. Extracts of male fern and of santonin obtained from *Artemisia maritima* var. *anthelmintica* were held in high regard as anthelmintics by Theophratus (370–285 B.C.) and Galen (A.D. 130–201) and were also recommended as effective anthelmintics until the 1940s and 1950s (Goodman & Gilman, 1955).

The beginning of chemotherapy as a science had to await the establishment of the germ theory of infection by Louis Pasteur more than a century ago. Rather than accepting the concept of spontaneous generation of infection, Pasteur suggested that diseases are caused by microbes that are transmitted among humans. Pasteur's inspiration for this theory came during his early studies on fermentation for the wine industry in France. Pasteur's contributions influenced bacteriology, immunology, the study of antibiotics, sterilization, epidemiology, and public health. This remarkable era was followed by the discovery and identification of many of the infectious microorganisms and methods for their culture in the laboratory and for infecting experimental animals. The study of parasites was initiated at the time of Sir Patrick Manson (1844–1922), who established the first institute for studying and teaching about tropical diseases: The London School of Hygiene and Tropical Medicine. Manson was a prominent researcher who made the seminal identification of the life cycle of the filarial nematode *Wuchereria bancrofti* in humans and the mosquito vector. He emphasized the importance of studies on tropical diseases and parasitology. The early part of the twentieth century saw the identification of many of the main parasites that

afflict humans in the tropics. These included trypanosomiasis (sleeping sickness), leishmaniasis (kala azar), lymphatic filariasis, and malaria.

Paul Ehrlich and the Principle of Selective Toxicity

The father of chemotherapy was Paul Ehrlich (1854–1915), a physician initially interested in immunology. He won the Nobel Prize in 1908 for his work in the field of immunology following Emil Behring (1901), Ronald Ross (1902), and Robert Koch (1905). Ehrlich was fascinated by the selectivity of interaction between antigens and the antibodies that could be produced to them by animals. In the 1870s and 1880s, while using different dyes to stain tissues and parasites, he saw a similar kind of selectivity. Some of these dyes could bind to certain cells and even to certain parts of the cell whereas other cells remained free of dye. He was attracted to the idea that selectivity of binding of chemical agents that are toxic to parasites but not to human cells could be the basis of identifying effective chemotherapeutic agents. These observations in his laboratory inspired his thoughts about the basic principles of chemotherapy. It also proved Pasteur's famous dictum "Chance only favors the prepared mind."

During the first decade of the twentieth century there was a great interest among European countries in exploiting the natural resources from their African colonies. A way had to be found to protect the European colonizers and the native workers they employed from endemic diseases in the tropics. Attempts to use their knowledge of immunology were not successful in protecting people against parasitic infections. In addition, very little was known about the biology of these parasites. There was a great need for chemical agents to treat these infections.

Germany, at that time, had one of the largest and most modern chemical industries in Europe, particularly for the manufacture of dyes. Guided by his early observation that some dyes have selective affinity to certain cells and the availability of thousands of dyes from the neighboring Höchst laboratories Ehrlich embarked on a program to identify dyes that would have selective affinity to microorganisms responsible for different infections. He started with methylene blue, which showed some effects against malaria but also had a specific affinity to nerve cells and was therefore too toxic to humans. Hundreds of dyes were examined against both malaria and trypanosome infections in experimental animals without success.

Paul Ehrlich's new strategy was based on rational screening for identifying new chemotherapeutic agents. One should try to find chemicals that have been proven to be selectively toxic to the microorganisms but not to the human hosts. Ehrlich's thinking was greatly influenced by the specificity of antibody

formation in response to antigens from infectious microorganisms. He perceived an analogy between the process of antigen/antibody interaction and the selective toxicity of chemical agents against parasites. He advocated the search for a toxic chemical agent that can selectively bind to a receptor in the parasite but not to one in the host. In an address to the 17th International Congress of Medicine in England (Ehrlich, 1913) he presented his ideas about the rational approach to chemotherapy. His first principle was "If the law is true in chemistry that *Corpora non agunt nisi liquida* (substances are effective only in the fluid state), then for chemotherapy the principle is true that *Corpora non agunt nisi fixata* (only attached, anchored substances are effective)." A metaphoric term "the magic bullet" was given to the ideal chemotherapeutic agent, a drug that disables the "germ" but not the host cells. Ehrlich envisioned a "*therapia sterilisans magna* . . . that by means of one or at most two injections the body is freed from the parasites." Chemotherapy is a term that was invented by Paul Ehrlich and was defined as the use of chemical agents to "injure" an invading organism without harming the host.

In 1906, after his disappointing work with dyes, Ehrlich shifted his attention to the spirochetes that cause syphilis (which was widespread in Europe at that time). Mercury was the only known treatment, but this metal did not meet Ehrlich's conception of *therapia sterilisans magna*. Ehrlich studied arsenicals for his first research on identifying new, effective chemotherapeutic agents against syphilis. At that time Atoxyl (sodium arsanilate) was being used as an antiprotozoal agent, but because of toxicity, its therapeutic usage was discontinued. Several hundred arsanilate derivatives were examined against an infection of a spirochete-like organism, *Spirillum morsus muris*, in laboratory animals. Compound 606 (diaminodioxyarsenobenzol) was successful in treating *Spirilla* infections in chickens, rabbits, rats, and mice because *Spirilla* readily absorbed the drug, killing the bacterial cells (Baumler, 1965). Subsequently, Compound 606 was used with a dramatic effect in patients suffering from syphilis. The human cells did not appear to take up the drug. An improved preparation of Compound 606 (neosalvarsan, Na diamino dihydroxyarsenobenzene methanol sulfoxylate), which was more soluble and thus more suitable for injection, was subsequently discovered. Within five years the incidence of syphilis was greatly reduced in many European countries. This was a triumphant success.

Rational Discovery of Antiparasitic Agents

Subsequent to the work of Paul Ehrlich, a strategy was gradually established to search for antiparasitic agents on the basis of identifying drugs that are selectively

toxic to the parasite with either no or minor effect on the host. This is now referred to as rational drug design. Because of its intellectual appeal the rational approach has been espoused predominantly by scientists in academic medicine. With the advances in biochemistry, molecular biology, and the techniques for synthesizing new chemical agents, significant progress has been made toward achieving Paul Ehrlich's goals. The ideal chemotherapeutic targets are those that are unique to the parasite. A few examples, which will be more fully discussed later in this book, can best explain this approach.

In trypanosomes regulation of an intracellular reducing milieu is controlled by an enzyme that is quite different from that in mammals. Glutathione reductase catalyzes the transfer of electrons from NADPH to oxidized glutathione in mammals. In trypanosomes, instead of glutathione, a peptide polyamine conjugate of glutathione and spermidine (named trypanothione) is used. Reduction of trypanothione is catalyzed by trypanothione reductase (Fairlamb & Cerami, 1992). Because of its uniqueness, trypanothione reductase is a favorite target for screening prospective trypanosomicidal agents. Melarsoprol (a trivalent organic arsenical) and nifurtimox (a nitrofuran derivative) are known to be inhibitors of trypanothione metabolism and are used as trypanosomicidal agents. The opportunity was, therefore, open for identifying new and more effective antitrypanosomal agents using the reductase system as a drug target (see Chapter 5).

Another example of a target is dihydropteroate synthase (DHPS). This enzyme catalyzes the first reaction involved in the synthesis of folic acid. Several protozoal parasites including *Plasmodium, Toxoplasma*, and *Eimeria* cannot absorb folic acid from the host and they depend on their own *de novo* synthesis of folic acid. DHPS is considered to be a good target for antiparasitic agents in these organisms (Wang, 1997). The enzyme is inhibited by sulfonamides or pyrimethamine. These compounds have antiparasitic effects and are used therapeutically in patients (see Chapters 4 and 5).

Biology of Parasites

In the past decade there have been great advances in our understanding of the biology of parasites. Recent discoveries regarding the physiology and biochemistry of protozoal and helminthic parasites have elucidated many of the prospective targets that are unique to each parasite. There is currently more basic biological information available than has been sufficiently exploited for chemotherapy of parasites. The rational approach to the discovery of new antiparasitic agents is to identify as many of these prospective targets as possible and to aim the new chemical agents selectively against these targets.

Although parasites may be quite biologically diverse, there are examples of chemotherapeutic agents that are effective against more than one type of parasite. A compound that is effective against one species may very well be effective against other unrelated organisms. Sulfonamides, originally discovered for their effect against streptococci, were later found to be active against chicken malaria (an experimental infection) as well as chicken coccidiosis. Therefore, it is important to study the mechanism of action of these agents in species other than those where they are presently used. Scientists directed their attention to the physiology and biochemistry of these organisms to explain the mechanism of action of certain effective chemotherapeutic agents. Some of the best antiparasitic agents were recognized long before a modern understanding of the biology of these parasites was established. Elucidation of their mechanism of action usually uncovered targets that are amenable to further biological studies. Biologists who are interested in the discovery of antiparasitic agents should study what parasites need from their hosts.

Biochemical Adaptation of Parasites to the Host

If it is assumed that parasites were derived from free-living ancestors then they must have been subjected to the process of natural selection within the mammalian host. This evolutionary process must have favored their ability to metabolize food from the host for energy and for reproduction. They must also have developed ingenious biochemical systems for self-maintenance within the unfriendly environment of the host. These evolutionary processes result in the selection of metabolic pathways and enzymes that are adapted for the survival of the parasites in their hosts. Evolution of the parasite had to keep pace with evolution of the host. Since internal parasites were not subjected to the same environmental factors that influence their hosts, the parasites have resorted, in many cases, to strategies that are different from those of the host. A rational approach to selection of antiparasitic agents must include identification of biochemical and molecular differences between the parasite and the host. Such differences could be manifested in the form of a biochemical pathway or an enzyme that is unique to the parasite. This would represent the ideal target against which antiparasitic agents should be designed. Evolutionary adaptation of the parasite to the host may not have involved drastic changes. The parasite may use metabolic pathways that are similar to those of the host, but it may have adapted its own enzymes to have different structures or different kinetic properties. Differences between the parasite and host enzymes may include differential sensitivity to inhibitors. Knowledge of the biochemical

and molecular differences between parasite and host and of rate-limiting points in the parasite metabolism are both necessary for successful selection of targets.

Parasites are well adapted for survival in the host's environment. Here they satisfy all their needs, while usually providing no benefit to the host. The result of this one-sided dependency is that it is not in the parasites' best interests to kill the host and in many cases parasites are able to survive for a long time. Such habituation may deprive the host of essential nutrients and usually exposes the host's tissues to the parasites' metabolic and degradative products, causing pathological changes in and damage to the host. Obviously, remedies against parasites should subsequently include treatment of the host for clinical and pathological changes induced by the parasites.

Drug Receptor Selectivity

The term "drug receptor" has been loosely used to designate the primary site that selectively binds a drug. The more restrictive use of the term is: a macromolecule that has as one of its main functions the binding of a hormone or a neurotransmitter that is naturally present in the body (e.g., acetylcholine for nicotinic receptors). Throughout this book I shall be using the restrictive usage of the term "drug target" to designate the macromolecule that binds a drug selectively. Through the use of modern biological techniques our knowledge of antiparasitic agents has been sufficiently advanced to identify some targets that are critical for the survival of parasitic organisms. In most of these cases several chemical agents that interact with these targets have been screened by different mechanisms. There is still, however, a need to establish the stereospecificity of chemical agents for obtaining optimal biological effect against the parasite. It is necessary to establish an assay procedure that is accurate and simple. This could be achieved by isolating the target and measuring the binding of the candidate chemicals to the target or by examining the biological effect of the chemical on enzyme activity. Mathematical calculations of structure–effect data for chemical analogs are important for deducing the relationship between functional chemical constituents of the drug and the biological effect on the target. Information regarding the optimal size and shape of the drug, the hydrophilic/lipophylic ratio, and the reactive functional groups that determine the selectivity of the binding to the parasite target are all crucial in selecting for potent antiparasitic agents. The nature of the target should be investigated. A requirement for certain reactive groups in the prospective drug that favor binding may suggest the presence of complementary groups in the target molecule.

Metabolic Pathways as Targets

Human as well as parasite cells have hundreds of metabolic pathways that are vital for their normal function. There is always a need to study mammalian pathways and compare them with those of the parasite. Each pathway has a number of enzyme reactions that catalyze different steps in the pathway. Enzyme activity at every step is regulated to ensure that the final product of the pathway provides for the needs of the cell. Metabolic pathways are interconnected to others. Each enzyme reaction in a pathway is governed by the law of mass action. In each pathway one or more enzymes are considered to be rate limiting or pacemaker enzymes. These enzymes usually are endowed with allosteric control mechanisms. Activity of these regulatory enzymes is subject to feedback inhibition or activation by one or more of the substrates in the pathway. Regulation of these key enzymes is critical for coupling the activity of the pathway to the metabolic needs of the cell. The Embden–Meyerhof glycolytic pathway is a good example of regulation of a multienzyme system. This pathway has eleven enzymes to convert glucose to lactic acid with the net production of two molecules of ATP. Three of these enzymes are considered to be rate limiting: hexokinase, phosphofructokinase, and pyruvic kinase. If cellular levels of ATP generated by glycolysis are high, ATP inhibits the three rate-limiting enzymes, particularly phosphofructokinase. Higher levels of AMP and ADP favor the "deinhibition" of these enzymes and thus favor increasing glycolysis and ATP production. Rate-limiting enzymes such as phosphofructokinase are usually good enzyme targets to inhibit the vitally important glycolytic pathway (Mansour, 1979). In a multienzyme pathway inhibition of two enzymes by two different drugs can have a more inhibitory effect on the metabolic pathway than the use of either drug alone. An example of such synergistic effects can be seen in the synthesis of tetrahydrofolic acid. In the treatment of malaria the use of a sulfonamide that inhibits dihydropteroate synthetase together with trimethoprim that inhibits dihydrofolic acid reductase has a greater effect than using either drug alone (see Chapter 4).

In the past, studies on the nature of enzymes have focused mainly on their properties in the purified form. Although kinetics and other biophysical information about isolated purified enzymes are essential in deducing their ultimate physiological role, very often regulation of these enzymes within the constraints of activity of other enzymes in the metabolic pathway cannot be predicted. It is now understood that in a multienzyme system each enzyme is influenced by the activity of preceding and following enzymes in the pathway as well as extrinsic signaling systems outside the pathway. The regulation of these enzymes is made more complicated by the fact that some components of the multienzyme

system are regulated by collateral signaling systems such as protein kinases or transmembrane transport of substrates for the metabolic pathway. We now have a better understanding of such regulation in many mammalian pathways, such as the glycolytic pathway in muscle or liver cells.

Once an enzyme is found to catalyze a reaction that limits the rate of an essential metabolic pathway, more information will be needed about its properties and chemistry. The next essential step for these studies is isolation and purification of the enzyme. Studies of the properties of an enzyme in crude extracts very frequently give misleading information. Enzyme specificity, regulation, kinetics, and other physical parameters cannot be studied with certainty using impure enzyme preparations. Detailed information regarding the chemistry of the enzyme, particularly the active site, can be obtained by cloning the enzyme and determining the amino acid sequence (see Chapter 2). In addition, the cloned enzyme can be expressed in appropriate bacterial or eukaryotic cells to provide ample amounts of material for further studies as well as for screening inhibitors.

Empirical Screening of Antiparasitic Agents

Most of our currently used antiparasitic drugs have been identified as a result of random screening of a series of chemicals that are related to compounds with recognized therapeutic value. This is referred to as the empirical approach to drug discovery. A successful example of this approach was the vast research program of the United States Army at Walter Reed Institute to screen for chemicals with potential antimalarial effects, focusing particularly on malaria strains resistant to chloroquine or pyrimethamine or both (Schmidt *et al.*, 1978). A special group of chemicals considered were the 4-quinolinemethanols. This group was chosen because quinine, the first naturally occurring antimalarial agent discovered, belongs to the 4-quinolinemethanol group. Moreover, quinine was an effective antimalarial agent against multidrug-resistant strains of *P. falciparum*. Of the thousands of 4-quinolinemethanol derivatives tested in mice infected with *Plasmodium berghei* (mouse malaria), 300 quinolinemethanol derivatives were chosen. In considering chemotherapeutic data based on the use of mice, the question always arises whether these compounds would be equally effective against the human malarial species *P. falciparum*. In a pilot experiment the most potent 4-quinolinemethanol derivatives were tested against resistant *P. falciparum* in owl monkeys and the best eight compounds were selected. These were tested for their antimalarial properties and tolerability in human volunteers. Six of the

eight compounds tested were found to be less active than quinine. Compound WE-142,490 (subsequently given the name mefloquine) was among the most effective agents against resistant *P. falciparum*. It was effective when given as a single dose and it was also effective against *Plasmodium vivax*.

Use of Animal Models in Screening

The advantages and disadvantages of the empirical screening procedure described above depend in part on the number of animals needed. In the case of *P. falciparum,* the need for testing in owl monkeys was a disadvantage because of the limitation on the number of available animals and the high cost of maintenance. The human malarias are very specific about the host in which they can live and thus cannot be transmitted to small laboratory animals. Furthermore, antimalarial agents that are effective against mouse or bird malaria do not necessarily have chemotherapeutic value in humans. In the case of many other parasites, rodents or birds have been of use only for a first screening before human subject trials. In addition to high cost, using experimental animals for empirical screening of a large number of compounds entails adherence to extensive government regulations. However, examining the effects of drugs against the parasites in their natural animal habitat has several advantages. One advantage in using laboratory animals for drug screening is that information on the pharmacokinetic and toxic properties of the drug can be obtained earlier (Hudson, 1994).

Use of *in vitro* Parasite Cultures for Screening

In vitro cultures have been extensively used for screening chemotherapeutic agents against bacteria. This technique is less costly than animal screening but depends on the ease of establishing laboratory cultures. An additional advantage is the opportunity to genetically manipulate most of these organisms. Bacterial cultures can also be used for vaccine development. In the case of most of the protozoal and helminth parasites it has not always been possible to establish continuous *in vitro* cultures. The relationship between the parasite and the host is very intricate and disruptions of the host–parasite relationship *in vitro* leads to gradual death of the parasite. Studies on the metabolism or physiology of movement were carried out for only a short period of time after removing the parasite from the host. Attempts are always made to provide the parasite with everything the investigator can think of, but it is often difficult to keep a continuous culture of the parasite. One obvious disadvantage of *in vitro* tests is that

some compounds being tested (pro-drugs) may have to be metabolized by the host to an active form and therefore would not be recognized by *in vitro* screens.

In many cases maintaining the complete life cycle of a human parasite in the laboratory has not yet been possible. For many years numerous scientists tried to establish the erythrocytic stage of *P. falciparum*, in the laboratory. Trager achieved a breakthrough in maintaining this stage of the human malaria parasite in continuous *in vitro* culture (Trager & Jensen, 1976). The procedure involved using an appropriate medium over a layer of human red cells maintained in a gas phase of 3–5% CO_2 and less than 21% oxygen. Media preparation and the correct gas content are now so simple that the practice of culturing human malaria has been established all over the world even in relatively primitive laboratory conditions. This procedure has enabled scientists in many countries to learn more about the biochemistry and cell biology of the parasite and about chemotherapy and drug resistance. The *in vitro* culture techniques have been used to study the mechanism of action of antimalarial agents and to screen for antimalarial agents.

In the case of *Toxoplasma gondii*, one of the opportunistic infections that afflict some AIDS patients, the tachyzoite stage of the parasites can be harvested from the peritoneal cavities of infected mice and cultured *in vitro* (Remington, Krahenbuhl, & Mendenhall, 1972). Replication of *T. gondii* can be measured by determining the incorporation of [^{3}H] uracil by the tachyzoites, while the mammalian host cells show no significant incorporation of uracil (Pfefferkorn & Pfefferkorn, 1977). Chemical agents being tested are added to the medium after the *T. gondii* have infected the host cells.

With *Trypanosoma cruzi* (the causative agent of Chagas' disease) HeLa cells are used as the host. They are grown for two days before being infected with the trypomastigotes. This stage is flagellated and nondividing. The trypomastigotes are transformed to the amastigote stage after invading the mammalian cells. The percentage of HeLa cells that have one or more amastigotes (intracellular stage) is recorded every day for 5–7 days (Nakajima-Shimada, Hirota, & Aoki, 1996). A similar procedure for *in vitro* drug screening against *T. brucei rhodesiense* was established using human HL-60 feed-layer cells. The number of trypanosomes and of HL-60 cells are counted using a hemocytometer and concentrations of drugs causing 50% growth inhibition are determined.

Leishmania mexicana, a dermatological protozoal infection, has, like trypanosomes, two forms: the flagellated promastigotes, which live in the invertebrate vector, and the nonflagellated amastigotes, which multiply in the vertebrate host. A medium developed by Pan was found to be optimal for *in vitro* culture and rapid transformation of the *Leishmania* promastigotes to amastigotes (Pan, 1984). These cultures can be used for drug screening.

Similar *in vitro* culture experiments were carried out on the protozoan parasites *Giardia duodenalis, Trichomonas vaginalis*, and *Entamoeba histolytica*. All cause intestinal infections in humans. A specific culture medium has been developed for each parasite. The trophozoites are harvested for the drug test experiments when they are in the mid-logarithmic growth phase. Parasite viability is evaluated every day by observing motile parasites. Minimum lethal concentration of the drugs being tested are determined (Upcroft *et al.*, 1999).

It has been difficult to establish continuous cultures of parasitic helminths because of their complex life cycle. In addition to some of them having an invertebrate as intermediate host, many of these parasites have one or more free-living stages. Some of these helminths live in the gut of the host, which creates a serious problem of contamination of the cultures with a variety of gut microorganisms. Because of these considerations *in vitro* drug screening tests are usually limited to short time incubations immediately after the parasites are detached from the host. Most of the parasitic helminths can be kept in the presence of antibiotics under physiological conditions for at least 24 hours. *Ascaris suum*, because of a thick cuticle and large reserves of glycogen, can be kept for several days in a simple saline medium (Bueding, Saz, & Farrow, 1959). *Fasciola hepatica* can also be maintained in good physiological condition for at least 24 hours in a special saline medium containing glucose and antibiotics.

In the case of *Schistosoma mansoni* there has been some success in culturing adult schistosomes from cercariae that were transformed to schistosomula. However, only a few of the mature adults produce eggs, which in most cases are abnormal, indicating that the *in vitro* development was not normal (Basch & Rhine, 1983). For routine *in vitro* testing of drugs adult schistosomes can be removed from infected mice and maintained in culture media (Bueding, 1950). The effects of tested drugs can be determined by survival of the parasites versus controls for a limited time.

In vitro culture of parasitic cestodes has enjoyed greater success. Many of their intermediate stages live in fish or vertebrate muscle and can be isolated under sterile conditions. The entire life cycle of *Hymenolepis diminuta* has been cultured *in vitro* (Smyth, 1990). This has been especially valuable for studies on the developmental biology of the parasites as well as for screening chemical agents against cestodes.

Among nematodes that live in human tissues, filarial worms are of great medical importance. Recently, cultivation of the third-stage larvae of *Brugia malayi* and their transformation to the young adult stage has been reported (Falcone *et al.*, 1995). Of the larvae cultured 17% reached young adulthood after 37 days. This is an important contribution to studies on development of this filarial parasite. These cultures could also be used for *in vitro* screening of

chemical agents. *Brugia* could be used as a representative of other human filarial parasites such as *Wuchereria bancrofti* (which causes elephantiasis) and *Onchocerca volvulus* (which causes onchocerciasis or river blindness).

Designing Chemicals for Selective Targets

The grand revolution in biochemistry during the last half of the twentieth century opened new fields of research for the development of selective antiparasitic agents. Much can be learned from drug development strategies for anticancer and antimicrobial drugs. A classical example is penicillin, the champion of antibacterial agents. The target of penicillin is the cell wall of *Streptococci*, a structure that is not present in host cells. Enzymes that are present in both parasite and host can also be amenable to inhibition by proper design of chemical agents to selectively inhibit the parasite enzyme. The ideal inhibitor would be a chemical that reacts with the target enzyme better than the natural substrate of that enzyme.

Based on the chemical structure of enzyme substrate and possible modifiers, different tentative inhibitors can be selected and tested on enzyme activity. Once a promising inhibitor is chosen, analogs of the molecule should be synthesized with the aim of improving the inhibitory potency of the compound. A measure of the inhibitory effect of the compound on mammalian as well as parasite cells is essential in determining the selectivity of the inhibitors. Chemical changes to improve its selectivity may include addition of new hydrophobic groups to make the molecule more able to penetrate the cells or making the inhibitor more robust by modifying the molecule by cyclization. For reviews of the general principles of design of inhibitors see Stark & Bartlett (1983) or Smith (1988).

Reversible inhibitors bind to the target enzyme physicochemically, either competitively or noncompetitively depending on their relationship to the enzyme–substrate reaction. An example is the use of sulfonamides, the therapeutic efficacy of which can be overcome by *p*-aminobenzoic acid (see Chapter 4).

An example of an irreversible inhibitor is the effect of metronidazole on *Entamoeba histolytica* (see Chapter 6). Transition state analogs or intermediate analogs that have high affinity to the enzyme's substrate site can also be drugs. In many cases the structure of these transition state analogs can be predicted from the mechanism of the enzyme reactions and/or by NMR analysis. Subversive (suicidal) inhibitors (mechanism-based inhibitors) are false substrates for an enzyme, which the enzyme itself converts to an inhibitor. These inhibitors interact preferentially with the target enzyme at the active site, but the enzyme

reaction is not completed and the enzyme is inactivated. Allopurinol is a hypoxanthine analog that acts as a false substrate of the purine phosphoribosyl transferase of some protozoal parasites that cannot synthesize purine *de novo*. Allopurinol is not toxic to humans because they can synthesize purines *de novo* (see Chapter 5).

In the case of a target enzyme that has two substrates, inhibitors could be designed as a single molecule that can recognize both sites. Such an inhibitor would have a higher order of specificity and is usually referred to as a multisubstrate analog inhibitor (MAI) (Broom, 1989). A classical example of MAI is N-(phosphonacetyl)-L-aspartate (PALA), an inhibitor of aspartate transcarbamoylase. PALA binds to the enzyme three orders of magnitude more tightly than the natural substrate, carbamoyl phosphate. PALA has been shown to have antiproliferative effects in mice (Yoshida, Stark, & Hoogenraad, 1974) and against B16 melanoma and Lewis lung carcinoma (Johnson *et al.*, 1976) but has not been used for chemotherapy in humans.

Relationship between Chemical Structure and Biological Activity of Inhibitors

The accepted procedure for studying the relationship between chemical structure and biological activity is to choose a compound that severely inhibits the target enzyme. However, in many cases the drug target has not been isolated and one can only measure the biological effect of the drug. Frequently, there are several chemical relations between the target and the final biological effect. Because the biological effect is always triggered by the initial interaction between the drug and the target, the biological response to the compound is accepted as a measure of drug–enzyme interaction. It is customary to test a series of chemicals that are related to the original drug in an attempt to improve its antiparasitic potency and to reduce the toxic effects against host cells. Chemical modification of the drug molecule could include changes in size, shape, or side chains. After trying many compounds, one can deduce a rough idea of the three-dimensional structure of the drug target. When the enzyme is isolated and the chemical structures of inhibitors are known, more accurate mapping of the target molecule using computer graphics can be undertaken.

Quantitative Structure Activity Relationship

A methodology for drug design, known as Quantitative Structure Activity Relationship (QSAR), has been developed to quantitate the relationship between chemical structure of a compound and its biological activity. A description and