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Wrocław University of Technology

Medicinal Chemistry

Paweł Kafarski

PRINCIPLES OF DRUG DESIGN

Wrocław 2011

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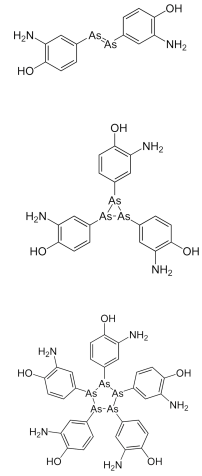
The era of rational drug design started with conclusion of Paul Ehrlich that substances, which are used to dye bacteria for their visualization under the microscope must interfere with bacterial cells. If so, some of them may interfere lethally and therefore Ehrlich started systematic search on the action of various dyes (and further other organic compounds) on bacterial growth. In that manner he had discovered first synthetic antibacterial agent - salvarsan, a cure for syphilis. These studies carried out brought him Nobel Prize in 1908.

Paul Ehrlich was born in Strzelin and studied medicine at the University of Wrocław. This book is dedicated to him and his achievements.



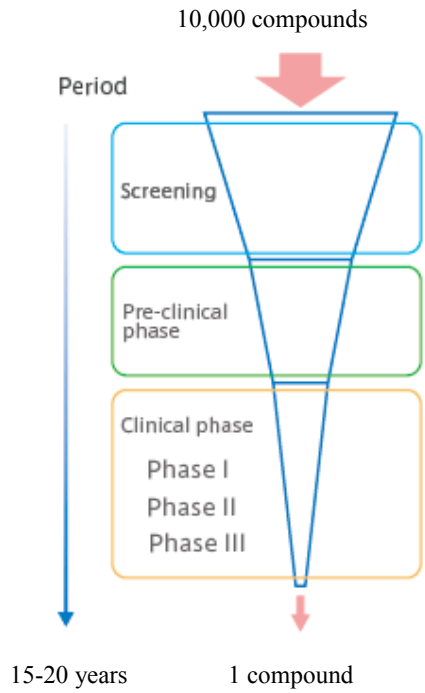
Photograph of Paul Ehlich taken from: http://pl.wikipedia.org/wiki/Paul_Ehrlich
and chemical formulas of salvarsan

Chapter 1. How much costs the development of new drug?

The process starting from new idea and finishing at introduction of drug to market is called drug development. Thus it is an entire process of bringing the new drug to market. The same process considers introduction of new agrochemical.

Drug development process is precarious pharmaceutical business. Before the drug is marketed it has to undergo several stages of the development. The first stage

considers the choice of certain disease with simultaneous identification of target (metabolic pathway, enzyme or receptor ect.) and elaboration of simple assay, which enable to evaluate the biological properties of studied compounds in a most simple way. Then the studies of series of compounds, libraries of compounds (mixtures of structurally similar compounds) or tissue extracts are assayed (screened) to find out the model compound (called lead), which is believed to have potential to treat studied diesease. Leads are sometimes developed as collections (libraries) of individual molecules that posseses properties required to become a new drug. When the lead enters the pre-clinical phase of studies the intensive efforts to modify its structure are undertaken simultaneously.



Phases of drug development process

Lead optimization relies on studies how the small modifications introduced to its structure influence the studied activity. These studies provide information, which enables pharmaceutical company to select the compound to be safe and effective medicine.

Then preclinical studies are undertaken. In that stage an investigational drug must be tested extensively *in vitro* (tests on cells and tissues) and *in vivo* (test using animals) to ensure that it will be safe to administer to humans with toxicological studies being the most important. At the same period studies how to best formulate drug are being carried out.

Finally potential drug reaches phases of clinical studies. Phase 1 is devoted to studies carried out on healthy volunteers, which are small in number – usually 20-100 persons. The purpose of these studies is to evaluate metabolic and pharmacological effects of potential drug in humans and possibly to determine safety and possible side effects associated with increasing doses of the drug. Phase 2 studies carried out on several hundreds of patients are devoted to the evaluation of effectiveness of the drug as particular indication on the patients with the disease or condition. This phase is carefully monitored and helps to determine the common short time effects and risks associated with the drug. The most vital is Phase 3 of the studies. It is given to large groups of people (1,000-3,000) to confirm drug effectiveness, to determine side effects, to compare to commonly used medications and to collect information, which will allow to use drug safely.

After that, drug has to be registered for by governmental agency. For registration of new drug, the results of all preclinical and clinical studies, the quality data and the description of manufacturing process (elaborated independently when drug was in Phase 3 of studies) are compiled and submitted to the regulatory authorities. If the regulators agree that the data prove efficiency, quality and safety of the drug, a marketing authorization is granted. Thus the drug becomes commercially available for patients.

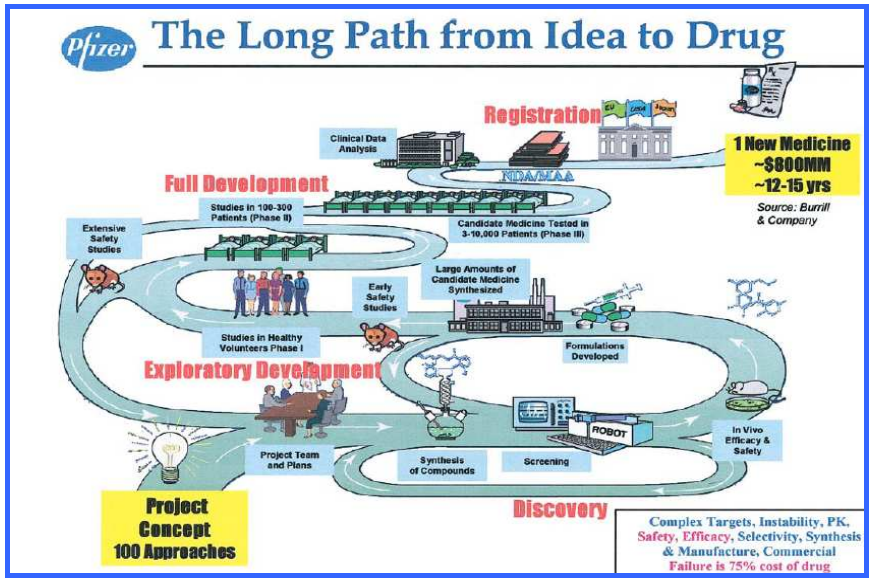
This is not, however, the end of the saga. Since the drug is on a market, adverse effects have to be constantly monitored and reported to the regulatory authorities. In many cases Phase 4 studies are carried out in order to add new indications and to improve drug formulas. Phase 4, known as Post Marketing Surveillance, is mostly carried out to find out safety profile of the drug in large patent pool across the world and to establish its safety profile.

It is estimated that among 10,000 compounds tested in laboratory only one reaches market. Thus, the success rate of this activity is really very small, what causes that cost of drug development process is exceptionally high. Basing on twelve-year panel of research and development expenditure for 183 publicly traded firms in the

pharmaceutical industry with panel of drugs in human clinical trials for each firm over the same period C. P. Adams and V.V. Brantner in their paper published in 2010 claim that the cost of drug development is actually $ 1 billion.

Thus, drug development process is not only very complex but also extremely costly. In order to cut these costs down and to improve economical prospectus, the pharmaceutical industry has plenty of huge mergers during last 15 years. However, these consolidations have not led to an upsurge of new drugs. There is even an opinion that innovation is an area where scale does not work being even counterproductive.

Summing up, the drug discovery process is extremely complex and requires cooperation of scientists representing a wide variety of branches. In order to introduce the drug to the market 15-20 years of research is required. The complexity of this process is well illustrated by the Pfizer's cartoon presented below.



Pfizer's vision of drug development process

Chapter 2. Drugs found "by chance"

On December 7, 1854 during the lecture given in Lille Ludwik Pasteur said "*Dans les champs de l'observation le hasard ne favorise que les esprits préparés*". This means

that chances and accidents play role in scientific discoveries but they are not blind luck.

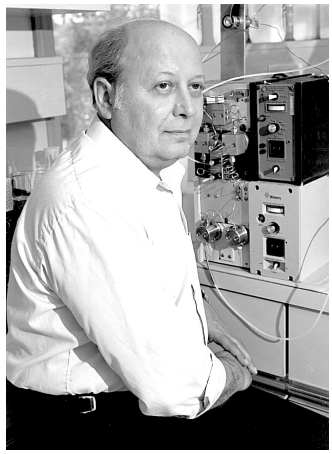
Pasteur was a worldwide renowned chemist and biologist. Among many discoveries he had done the most important was finding that microorganisms are the cause of most infectious diseases (this became known as "germ theory" of diseases). He was also inventor of pasteurization process and developed vaccination.

Photo of Louis Pasteur taken from http://media-2.web.britannica.com/

Today, screening collections of compounds is a major source of drugs. This is achieved by screening corporate compound collections, which usually consists of in house compounds, compounds made under proprietary contracts and purchased from public domain collections. Chemists then need to develop focused libraries of related compounds, seeking structural clues to improved potency and selectivity through iterative screening and analogue design. Thus, one may say that the searching new drug is like looking for a needle in a haystack.

Serendipity is one of the many factors that may contribute to drug discovery. This implies the finding of one thing while looking for something else. The discovery of anticancer *cis*-platin is a good example here. B. Rosenberg and L. van Camp studied the influence of weak electrical current on movement of *Escherichia coli* in aqueous solutions. Surprisingly they found that bacteria had grown 300 times its normal length. This was caused by formation of *cis*-platinum at platinum electrode, which

inhibited bacterial cell division. Stimulated by this finding B. Rosenberg conducted a series of experiments to test the effects of various platinum coordination complexes on human leukemia cells and found that *cis*-platinum was the most effective. It started the medicinal career of this compound.



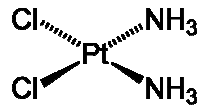


Photo of Barnett Rosenberg taken from http://news.msu.edu/media/
and the structure of *cis*-platinum

The discovery of acetaminophen (*Paracetamol*) a popular analgesic drug, which works, for example, against headaches, muscle pain, knee pain, and toothaches may serve as the second example. J. Axelrod who was working as a technician in a food safety laboratory was asked to determine why his fellow citizens of New York were turning blue. Together with B. Brodie they determined that acetanilide, a popular analgesic, was metabolized to aniline, which elicited methemoglobinemia, an elevated level of methemoglobin that causes cyanosis. They also discovered another metabolite, which was safer and more effective pain reliever – acetaminophen. Thanks to his works on this drug he won share of the Nobel Prize in 1970.

Third story considers sildenafil (*Viagra*). Sildenafil was synthesized by a group of pharmaceutical chemists working at Pfizer's Sandwich (Kent), research facility in England. It was designed as an inhibitor of the PDE5 enzyme, which they hoped would be effective in relaxing coronary arteries and relieving chest pain. It was initially studied in angina pectoris (a symptom of ischaemic heart disease).

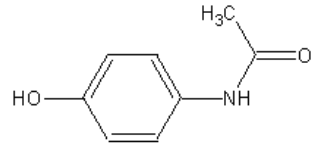
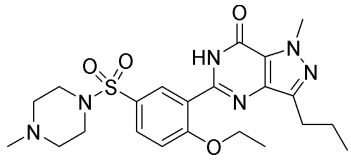


Photo of Julius Axelrod taken from http://www.nature.com/mp/journal/v10/n3/
and the structure of acteaminophen

Phase 1 clinical trials, under the direction of I. Osterloh, suggested that the drug had little effect on angina. Gloomily, the researchers terminated the trial and asked participants to return the unused drug. Many men refused, clutching to the drug as if it was gold. Idiosyncrasies started being present in all clinical tests, researchers gave the objection little thought until they heard rumors about the drug's side effects on sex life. Pfizer therefore decided to market it for erectile dysfunction, rather than for angina. The drug was patented in 1996, approved for by the US Food and Drug Administration on March 27, 1998, becoming the first oral treatment approved to treat erectile dysfunction.



Chemical structure of sildenafil and famous blue *Viagra* pills

We like to think drugs are designed for exact purposes, but as it is seen from these three examples they are often not as targeted as we may hope. The surprising truth is that drug development process owes more to serendipity than to careful rational design, and their potential may only be discovered when we take them.

Chapter 3. Drugs from natural sources

Organic compounds from terrestrial and marine organisms have an extensive past and present in the treatment of many diseases and serve as compounds of interest both in their natural form and as templates for synthetic modification. About 40% of the drugs currently used are derived from natural sources. Most of them are pure substances, which are isolated from various organisms and used directly or after chemical modification. An analysis of the origin of the drugs developed between 1981 and 2002 showed that natural products or natural product-derived drugs comprised 28% of all new chemical entities launched onto the market.

For thousands of years, natural products have played an important role throughout the world in treating and preventing human diseases. Natural product medicines have come from various source of materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates.

Archaeological evidence reveals that drug taking is an extremely old human phenomenon. By necessity, the drugs used in ancient civilizations were extracts of plants or animal products, with a few inorganic salts. In India, the Ayurvedic system of medicine developed an extensive of medicines from plants dating at least 1000 BC. The earliest Chinese records give descriptions of diseases but not medicines: illnesses were thought to be godly punishments. Thus prayers and offerings were being considered as proper cure. The earliest recorded Chinese prescriptions date back to approximately 500 BC and show the beginning of natural products as drugs. The first classic texts in Chinese medicine appeared in AD 25-220, and some of their formulae remain in use up to now. Similarly, the Egyptian Ebers papyrus (around 1550 BC) contains descriptions of several active ingredients (notably purgatives) that are still used today.

The history of morphine is a good example of the discovery and development of the ancient drug being still in use today. Morphine is a potent opiate analgesic psychoactive drug regarded as the gold standard, or benchmark, of analgesics used to

relieve severe or agonizing pain and suffering.

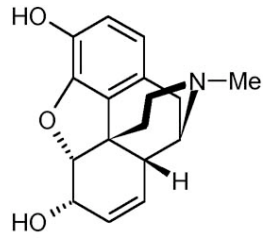
Cultivation of poppy was first documented in lower Mesopotamia as far as 3400 BC. The Sumerians called it joy plant (*Hul Gil*). In 1300 BC the Egyptians were cultivating *opium thebaicum*, and its name was derived from their capital city of Thebes. Opium's effects were considered magical or mystical. Hippocrates (460 BC), dismissed the idea that opium was "magical." Instead, he noted its effectiveness as a painkiller and a styptic (a drug used to staunch bleeding). Alexander the Great (330 BC) highly prized opium, and always gave it to his troops to ease the pains of battle and marching. This is partially why his troops were so successful. He introduced opium to the people of Persia and India. Indian medical treatises *The Shodal Gadanigrah* and *Sharangdhar Samahita* (around 1200) describe the use of opium for diarrhea and sexual disability. At the same time opium become a taboo topic during the Holy Inquisition.

An opium-based elixir has been ascribed to alchemists of Byzantine times, but the specific formula was supposedly lost during the Ottoman conquest of Constantinople. In 1522, Paracelsus introduced *laudanum* - "*Stones of Immortality*", which was made of *opium thebaicum*, citrus juice and quintessence of gold and prescribed as a potent painkiller; but he recommended using it sparingly. The word *laudanum* comes from the Latin word *laudare* meaning "to praise."

Opium and a portrait of Paracelsus
taken from http://www.asmalldoseof.org/historyoftox/renaissance/

In 1606 ships chartered by Elizabeth I are instructed to purchase the finest Indian opium and transport it back to England. In 1680 English apothecary, T. Sydenham,

introduces *Sydenham's Laudanum*, a mixture of opium, sherry wine and herbs. His pills, along with others of the time, become popular remedies for numerous ailments. *Laudanum* was actually cheaper than beer or wine. In 1803 F. Sertürner of Paderborn (Germany) discovers the active ingredient of opium by dissolving it in acid and then neutralizing it with ammonia. As a result he obtained *Principium somniferum* or morphine. The drug was marketed in 1817 to the general public by Sertürner and Company as an analgesic, and also as a treatment for opium and alcohol addiction.



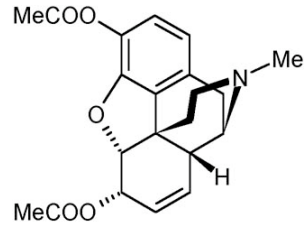
A portrait of Firdrich Sertürner and the chemical structure of morphine
taken from http://www.general-anaesthesia.com/

In 1827 E. Merck & Company of Darmstadt (Germany) begins commercial manufacturing of morphine. At the time, the British dependence on opium for medicinal and recreational usage reaches as high as 22,000 pounds annually. It is worth noticing that in 1839 Lin Tse-Hsu, imperial Chinese commissioner in charge of suppressing the opium traffic, ordered all foreign traders to surrender their opium. In response, the British sent expeditionary warships to the coast of China, beginning The First Opium War (there were two such wars).

H. Dreser working for The Bayer Company of Elberfeld (Germany) finds that diluting morphine with acetyls produces a drug without the common morphine side effects. Bayer begins its production in 1874 and coins the name "*heroin*". Heroin is approximately 1.5–2 times more potent than morphine on a milligram-for-milligram basis. It was as late as in 1920 when J. Kabay of Hungary elaborated the method of

extraction of morphine from dry poppy-straw.

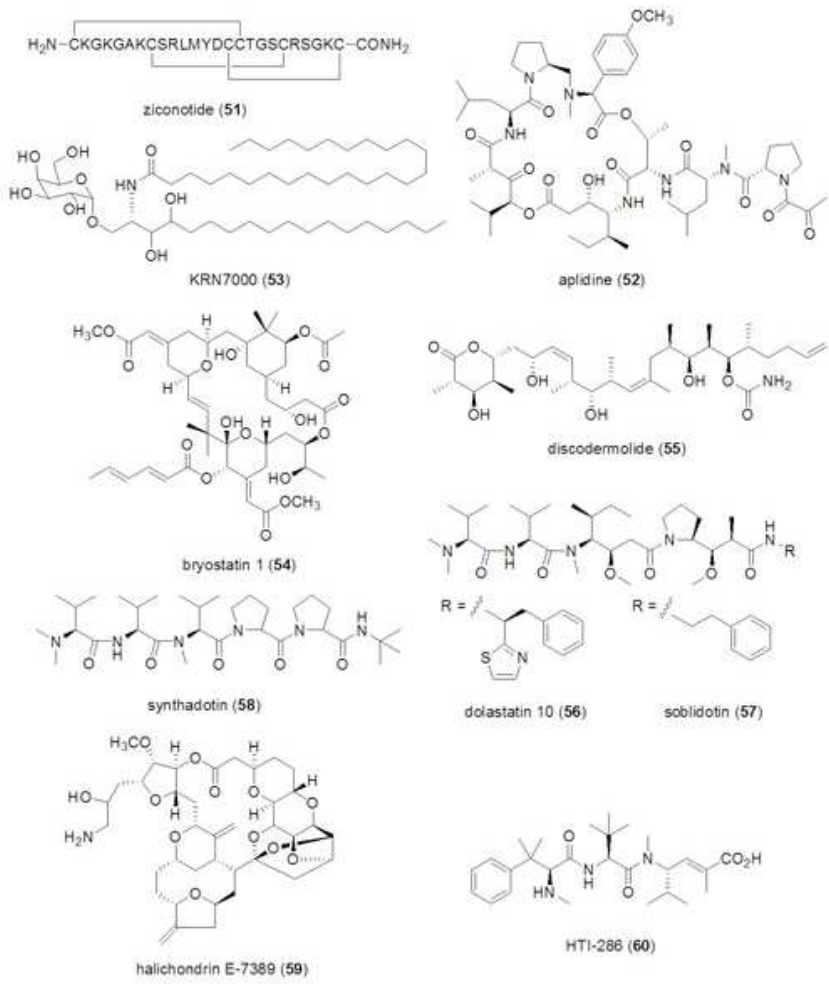
Morphine became a controlled substance in the US under the Harrison Narcotics Tax Act of 1914, and possession without a prescription in the US is a criminal offense. Morphine was the most commonly abused narcotic analgesic in the world until heroin was synthesized and came into use. That is why a Tasmanian company in 2004 published details of its genetically-engineered mutants, which do not produce morphine or codeine.



Bayer Company logo and the structure of heroin

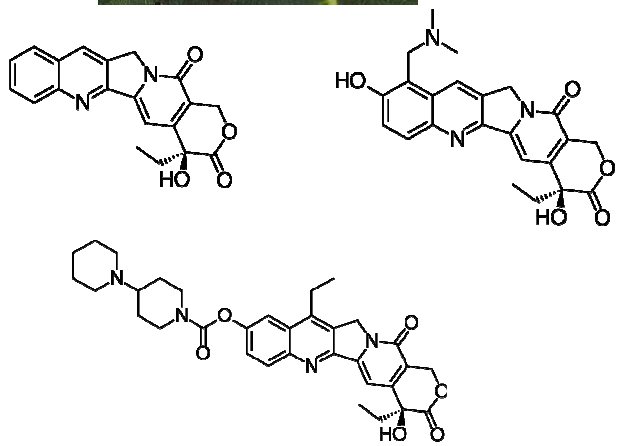
The molecular basis of morphine action was discovered in 1972 when S. Snyder and C. Pert discovered opiate receptor in brain. Opioid receptors are a group of G protein-coupled receptors, which mediate pain by binding low-molecular substances (opioids). In 1975 H. Kosterlitz and his colleagues isolated and purified an endogenous opioid in a brain - a pentapeptide enkephalin. It is of interest that in 2005 researchers at Ernest Gallo Clinic and Research Center in Emeryville, California, inhibit expression of the AGS3 gene, which curbs the desire for heroin in addicted rodents.

As seen from that example the process of natural drug discovery through the ages is complex and sometimes dramatic. The development of both analytical methods and screening techniques causes that today it is much faster. This might be well documented by a small collection of drugs isolated from marine environment in 2000-2005. The diversity and complexity of structures shows the progress done during this time.



Marine organisms derived drug candidates found in 2000-2005

It is also worth noting that natural substances are also treated as lead compounds and undergo chemical modification done in order to improve their action. Camptothecin is an alkaloid found in the barks of the Chinese camptotheca tree (happy tree) and the Asian nothapodytes tree. It is the only known naturally-occurring DNA topoisomerase I inhibitor, thus exhibiting remarkable anticancer activity but also low solubility and high adverse drug reaction. Because of these disadvantages, various derivatives, to increase the benefits of this alkaloid, have been synthesized. Two of them, topotecan and irinotecan, have been approved for anticancer therapy.



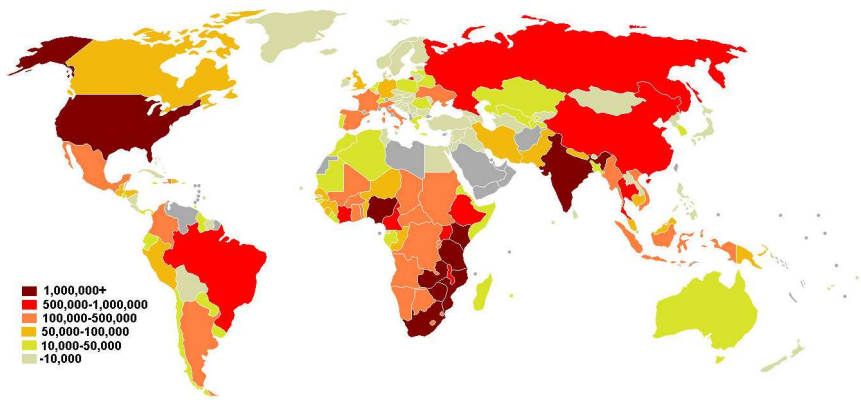
Camptotheca acuminata taken from http://www.exogarden.nl/palmzaden/
and structures of camptothecin, topotecan and irinotecan

Chapter 4. Choice of target using AIDS as an example

Acquired immune deficiency syndrome or acquired immunodeficiency syndrome (AIDS) is a disease of human immune system caused by the human immunodeficiency virus (HIV). This is a serious (often fatal) disease of immune system transmitted through blood products especially by sexual contact or contaminated needles. Individuals who suffer from it, are at risk of severe illnesses that are usually not a threat to anyone whose immune system is working properly. Thus, the disease is manifested by increased susceptibility to opportunistic infections and to certain rare cancers, especially Kaposi's sarcoma.

According to estimates from the UNAIDS 2009 AIDS Epidemic Update, around 31.3 million adults and 2.1 million children were living with HIV at the end of 2008.

During 2008, some 2.7 million people became infected with HIV and this year also faced 2 million deaths because of AIDS. More than 25 million people have died of AIDS since 1981. Globally, around 11% of HIV infections are among babies who acquire the virus from their mothers; 10% result from the use of narcotics injected with syringe; 5-10% are due to sex between men; and 5-10% occur in healthcare settings. Sex between men and women accounts for the remaining proportion, that means around two thirds of new infections. Around half of the people who acquire HIV become infected before they turn 25, and AIDS is the second most common cause of death among 20-24 year olds.

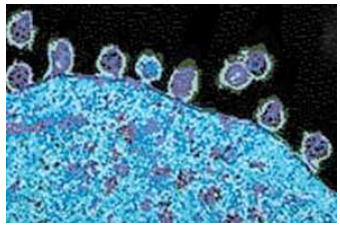


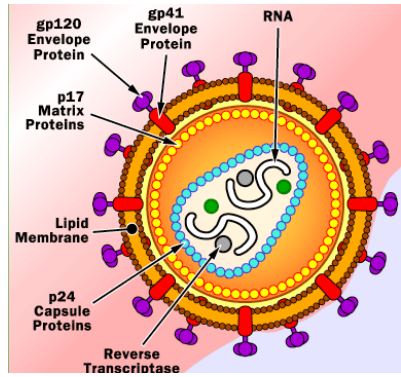
The spread of AIDS all around the world in 2008

The disease is caused by human immunodeficiency virus (HIV). Outside a human cell, HIV exists as roughly spherical particles (sometimes called virions). An HIV particle is around 100-120 nm in diameter. Its cone-shaped core surrounded by lipid matrix containing key surface antigens and glycoproteins. Viral core contains 2 copies of genomic RNA (thus it is retrovirus), and three enzymes: reverse transcriptase, integrase and protease. Its genome is well recognized and is composed of 9 genes encoding 3 structural, 2 envelope, and 6 regulatory proteins in addition to 3 enzymes. It consists of a homodimer of linear, positive-sense, single-stranded RNA of approximately 9.2 kb in size. Both strands of RNA are capped, polyadenylated and non-covalently joined through the dimerization domain.

The knowledge on HIV anatomy is quite impressive but still there is no cure for HIV infection or AIDS nor is there a vaccine to prevent HIV infection. However, there are

new medications available, which not only can slow the progression of the infection, but can also markedly suppress the virus, thereby restoring the body's immune function and permitting many HIV-infected individuals to lead a normal, disease-free life.



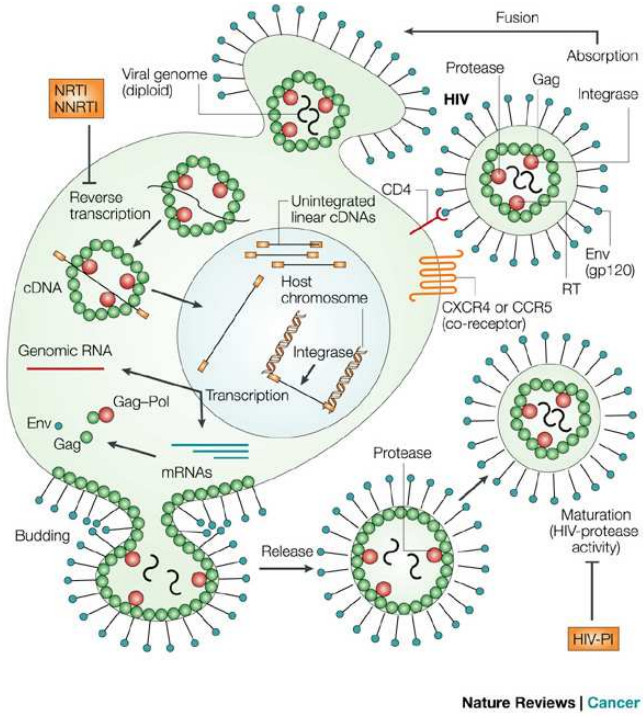


Electron microscope photo showing newly formed HIV particles budding from a human cell and anatomy of HIV cell taken from http://static.howstuffworks.com/

Typically, the infection begins when an HIV particle encounters a cell with a surface molecule called cluster designation 4 (CD4 glycoprotein). Cells carrying this molecule are known as CD4+ T cells and are crucial for immunological response. A healthy, uninfected person usually has 800 to 1,200 CD4+ T cells per cubic millimeter of blood. During untreated HIV infection, the number of these cells in a human's blood progressively declines. When the CD4+ T cell count falls below 200/mm^3, a person becomes particularly vulnerable to the opportunistic infections and non-typical cancers.

One or more of the virus's gp120 molecules binds tightly to CD4 molecule(s) on the cell's surface. The binding of gp120 to CD4 results in a conformational change in the gp120 molecule allowing it to bind to a second molecule on the cell surface known as a co-receptor. The membrane of the virus and the cell membrane then fuse, leading to entry of the virus into the cell. Then the protein envelope is being removed. Its uncoating involves disassembly of the capsid core and liberation of viral RNA with associated virion proteins into the cytoplasm. This is a complex and not fully recognized process in which the cellular protein cyclophilin A, which interacts with the capsid, may promote disassembly of the core. Then the released reverse

transcriptase synthesizes proviral double-stranded cDNA, which is transported to the nucleus and its integration with cell DNA occurs. Transcription of the integrated viral cDNA leads to the production of genomic (unspliced) and messanger (spliced) RNA (mRNA) molecules that are transported to the cell cytoplasm. Translation of HIV mRNAs leads to the production of viral proteins. Immature precursors of capsid (Gag) and fused with thempolymerase (Pol) proteins are transported to the cell membrane, where viral progeny begin assembling and 'bud' from the infected cells. Viral particles released from the cell following budding, however, do not contain the characteristic HIV condensed core and are not infectious. Virus's infectivity is acquired after particle maturation, which is mediated outside the host's cell by the virion-associated HIV protease. This enzyme cleaves the immature Gag and Gag–Pol precursors into functional polypeptides.



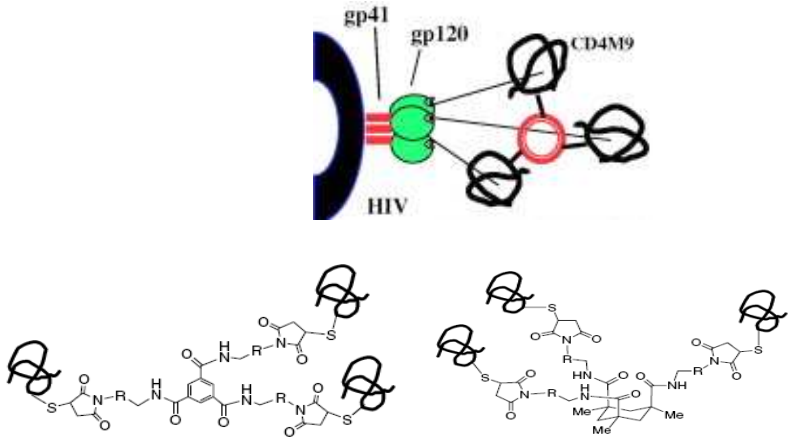
Detailed presentation of HIV cell cycle
taken from http://www.nature.com/nrc/journal/v4/n11/

When designing anti-HIV drug, any of the cell cycle processes might be chosen as a target. Thus, antagonists and inhibitors of virus attachment, fusion and uncoating are

developed to interfere with virus entry into the cell. Inhibitors of three vital virus enzymes: reverse transcriptase, integrase and protease seem to be good targets if considering huge progress in general inhibitor design. Also virus maturation offers some possibilities. Of course, this is not the end of possibilities because a genetic approach can also be used by application of antisense oligonucleotides, Si RNAs or catalytic RNAs.

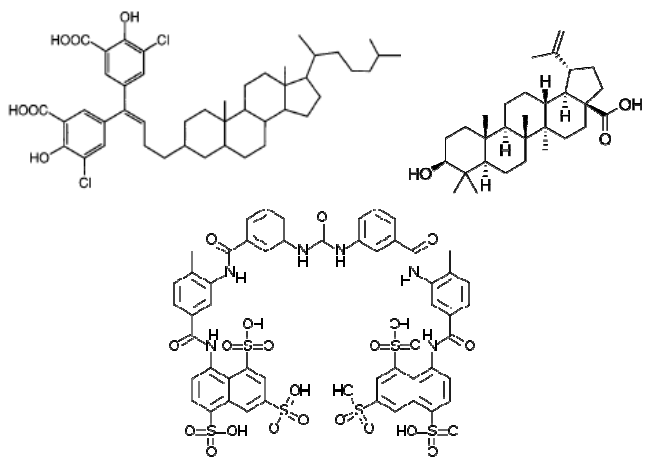
Major research and pharmaceutical companies continue to place precedence on the search for a vaccine, however, it is estimated that it will take another ten to fifteen years to find it. That is why the major stream of research is directed toward finding a cure against AIDS. Lets consider two targets here: inhibitors of attachement of HIV virus to CD+ T cell and inhibitors of viral protease.

The entry of virus to host cell is mediated by interaction of CD4 receptor on human cell and viral gp120 protein, which lies on the outer envelope of the HIV. The exact mechanism by means of which the virus enters the cell is unknown; however, it is known that gp120 plays a critical role. The protein's role is threefold: to seek receptors suitable for viral entry, to fix the viral particle to the cell. Since gp120 is trimeric, trivalent synthetic miniproteins CD4M9, mimicking DC4 receptor, were designed to target the CD4-binding sites displayed in the trimeric gp120 complex of HIV-1. These miniproteins were bound via thiol moieties to symmetrical synthetic scaffolds and demonstrated significantly enhanced anti-HIV activities over the monomeric miniproteins.



Idea of false attachement of HIV to synthetic CD4 mimics
(H. Li et al., Bioorg. Med. Chem., 2007)

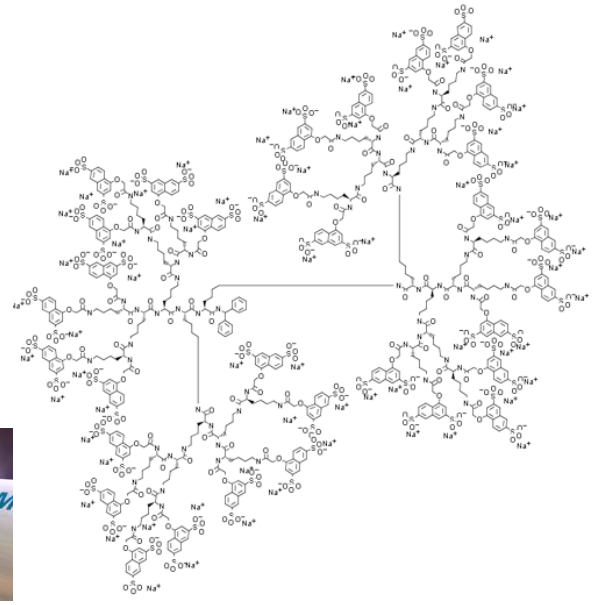
Binding of gp120 to CD4 is mainly electrostatic in nature, although there are van der Waals' interactions and hydrogen bonds. There is a site at CD4 protein, which strongly binds polyanions and is located in close proximity to gp120 binding site. Thus, blocking this site cause disruption of CD4-g120 interactions and consequently inhibits viral infection. It is also well known that gp120 V3 loop, which plays an essential role in chemokine , bounds polyanions. Therefore polyanions (polysulfates, polysulfonates, polycarboxylates, polyphosphonates ect.) may function as double inhibitors by interacting with the two proteins. One of he first drugs of this type was cosalane developed by researchers at Purdue University in 1993. Similar action exerts structurally related betulinic acid, a component of white birch's bark and suramin disclosed in 1916 as antiprotozoan drug. Since then, it was found that antiviral effect increases, together with molecular weight and degree, saturation of the molecule with polyanions. However, clinical trials of polyanionic compound did not result in remarkable effects, most probably due to the low bioavailability of these compounds.



Structures of cosalane, betulinic acid and suramin

In order to become infectious, HIV must enter the blood stream of a person for trasmision. It enters through blood, semen, vaginal fluid, and breast milk. Prevention is usually focused around sexual activities, because HIV transmission is more likely to occur during sex. Currently, the primary form of HIV prevention during sexual activity is the condom. Microbicides are a broad range of products that prevent viral

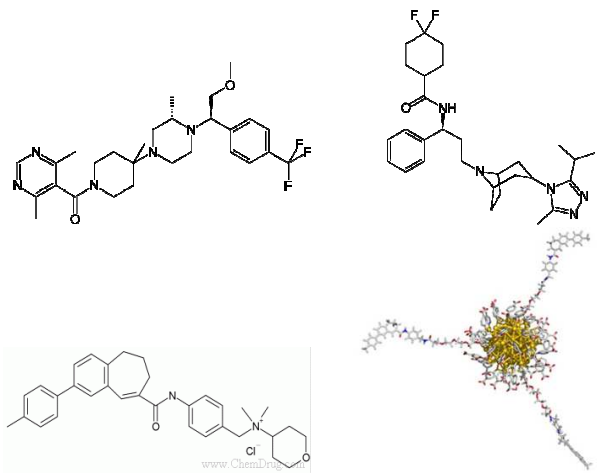
infections, especially sexually transmitted diseases. In this case they are used as HIV pathogen infection prevention when applied in the vagina or rectum. First of them was a sexual lubricant VivaGel® introduced by Australian firm Starpharma. It prevents HIV and herpes as well as other infections caused through sexual intercourse. It is a water-based vaginal product of 3% weight/weight (w/w) SPL7013 mixed in Carbopol® gel buffered to a pH physiologically compatible with the normal human vagina. SPL7013 is a lysine-based dendrimer with naphthalene disulfonic acid surface groups. Thus, its surface bears negative chargeand dendrimer acts as polyanionic species. Newer drug of this type is PRO2000 manufactured by Indevus Pharmaceuticals. It is also anti-HIV intravaginal gel with synthetic, long-chain, naphthalene sulfonic acid polymer being its active ingredient. It was proved to bind to HIV-1 gp120 and to interfere with virus binding to $CD4^+$ T cells. However, studies on over 9,000 women in Uganda showed only limited usefulness of this preparation.



VivaGel® and structure of SPL7013

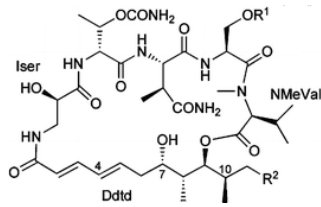
The process of HIV attachment infusion entails CD4glycoprotein 120 (gp120) binding followed by co-receptor binding, utilizing either the C-C chemokine receptor-5 (CCR5) or CX chemokine receptor-4 (CXCR4) co-receptor followed by viral/cell fusion. Chemokines mediate the immune response by attracting and, in some cases, activating specific populations of leukocytes. These small (8-10 kDa) proteins bind specifically to G protein-coupled receptors of the transmembrane, including CCR5

and CXCR4. Thus, the ß-chemokines MIP-1, MIP-1ß, and RANTES inhibit HIV-1 infection of CD4+ T cells by inhibiting interactions between the virus and CCR5 receptors. Thus the search of their antagonists might be a useful approach to HIV treatment. At least two of such drugs appeared to be successful – Vicriviroc developed by Schering-Plough and Maraviroc developed by Pfizer. Vicriviroc is a noncompetitive allosteric antagonist of CCR5. It binds to a small hydrophobic pocket between the transmembrane helices near the extracellular surface of the CCR5 receptor, which cause conformational change of the receptor and prevents gp120 binding. Vicriviroc is orally administered and, because it is effective at nanomolar concentrations, it can be administered once daily (30 mg). Maraviroc acts by means of virtually the same mechanism and has to be taken twice a day in a high (300 mg) dose. Third drug, TAK-779, was designed in the 1990s in Japan. However, it caused harmful side effects, which were assigned to ammonium salt fragment of the molecule. The conjunction of a modified version of the TAK-779 (minus the ammonium salt) with gold nanoparticles creates a drug that prevents the virus from gaining a cellular foothold.



Structures of Vicriviroc, Maraviroc, TAK-779 and its conjugate with gold nanoparticle

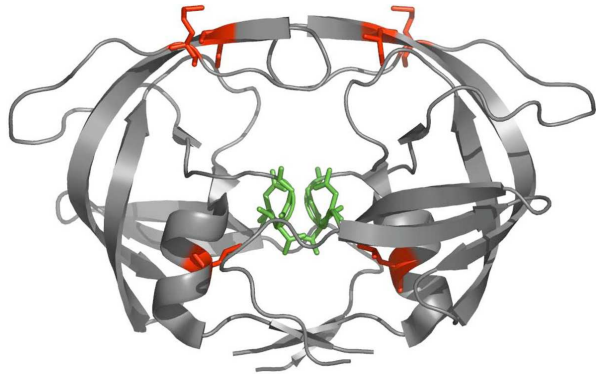
As seen from the above examples, the choice of the only one target offers many possibilities of designing new drugs. Of course, the methods discussed in Chapter 2 and 3 may also be successfully applied here as demonstrated by the finding of activity of celebesides isolated from Indonesian marine sponge *Siliquariaspongia mirabilis*. They act as entry inhibitors by unidentified mechanism. They do not act, however, on entry proteins.



Structure of celebesides

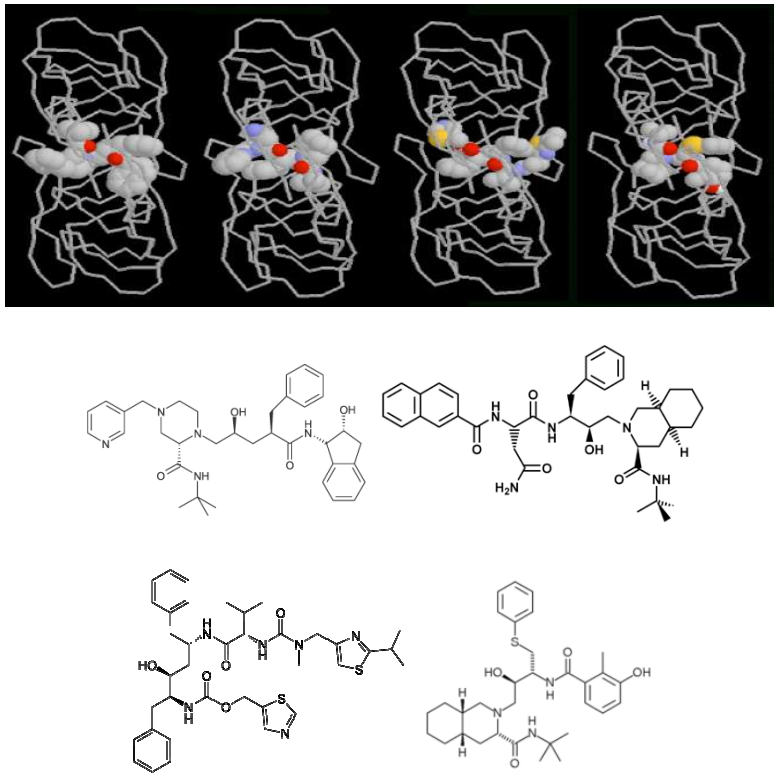
The second target is HIV protease. It is an aspartic protease that is essential for the life-cycle of the virus. Like many viruses, HIV makes many of its proteins in one long piece, with several proteins strung together. This protease cleaves newly synthesized polyproteins to create the mature protein components of an infectious HIV. Since there is a huge amount of data accumulated on the design of inhibitors of various proteases, HIV protease appeared, right from the beginning an attractive target for drugs to cure AIDS.

Its three dimensional structure has been investigated using X-ray crystallography. It exists as a C2-symmetric homodimer, with each subunit made up of 99 amino acids. The active site lies between the identical subunits and has the characteristic Asp-Thr-Gly sequence common to aspartic proteases. The two Asp25 residues (one from each chain) act as the catalytic residues, with water molecule acting as nucleophile. Drugs that attack HIV protease, are one of the triumphs of modern medicine. They were obtained by using all the methods elaborated for other proteases taking into account specific spatial arrangement of the active and binding sites of the enzyme reflecting its non-typical C2-symmetry. This symmetry might be best visualized by two right hands attached together via wrists.



C-2 symmetrical structure of HIV protease
taken from http://www.biomedcentral.com/

HIV protease inhibitors were first invented between 1989 and 1994 and were considered the first breakthrough in over a decade of AIDS research. Currently, there are several HIV protease inhibitors approved for the treatment for HIV infection. These medications work at the final stage of viral replication and attempt to prevent HIV from making new copies of the virus. Despite mild toxicity and adverse effects these inhibitors, used in combination with reverse transcriptase nucleoside inhibitors, have turned AIDS into a chronic, manageable disease.



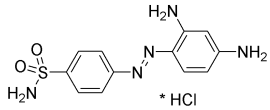
Inhibitors of HIV protease, from left to right: Indinavir, Saquinavir, Ritonavir and Nelfinavir

Chapter 5. Structural analogy

Gerhard Domagk at the Bayer Laboratories of the IG Farben conglomerate in Germany studied the effect of new synthetic dyes on streptococci. He tested each chemical on both agar plates inoculated with microbes and mice and rabbits injected

with pathogens. In 1932, colleagues brought Domagk a new dye, sulfamidochrysoidine, called Prontosil Rubrum, for its red color. It seemed to have no effect on the bacteria *in vitro*, but he went ahead and tested it on 26 mice injected with streptococci. Fourteen were kept as controls, and 12 were treated after the injection with a large dose of the dye, administered by stomach tube. All of the controls died within a few days, while all of the treated mice survived. Prontosil had not been tested on humans when Domagk's only daughter developed a severe streptococcal infection in her arm. Both arm and life were at risk and Domagk injected her with prontosil. She responded well and the diseased arm was restored to full health. Domagk did not reveal this discovery until other human tests were conducted over the next few years. Impressive successes of Prontosil started to be reported from all over Europe, and especially after the widely published treatment of F. D. Roosvelt Jr. (a son of U.S. president F. D. Roosevelt) who was suffering from sinus trouble (*Streptococcus haemolyticus*).

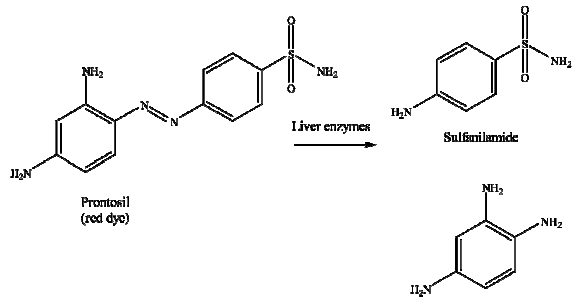
G. Domagk received Nobel Prize in 1939 but the Nazi regime in Germany would not allow him to travel to Stockholm to receive the honor. He did receive the medal in 1947, but because of the time that elapsed, he was not given the monetary award.



Portrait of Gerhard Domagk by Otto Dix and structure of prontosil

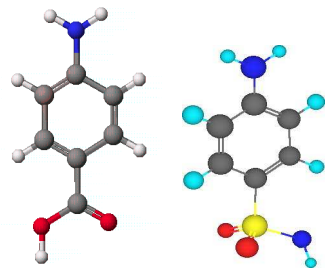
Researchers at the French Pasteur Institute found, at the end of 1935, that prontosil is metabolized to sulfanilamide, which acts as real antibiotic. Thus, Prontosil acts as a prodrug. Sulfanilamide became the first oral version of an antibiotic by Bayer, which had actually obtained a German patent on this compound as early as in 1909, without realizing its medical potential at this time. It inhibits multiplication of bacteria by

acting as antimetabolite of *p*-aminobenzoic acid in the folic acid metabolism cycle. This cycle is vital to bacterial growth and development and does not exist in mammals.



Activation of prontosil with release of sulfanilamide

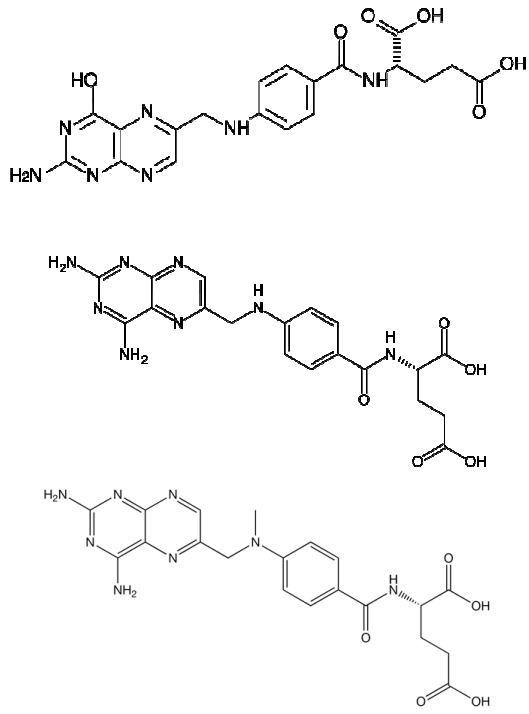
Sulfanilamide is considered as a first drug, which action bases on structural analogy to natural compound. Thus it is formally obtained by replacement of p-aminobenzoic acid (metabolite) carboxylic group with sulfonamide moiety. The obtained antimetabolite blocks biosynthesis of dihydrofolic acid by inhibiting enzyme dehydropteroate synthase. Thus, structural analogue is a compound, structure of which resembles the structure of metabolite and thus fits the same enzyme or receptor exerting different action than parent compound. Sulfanilamide is considered as isosteric and isoelectronic analogue of *p*-aminobenzoic acid. Thus, its three dimensional and electronic structure resembles closely that of the metabolite.



Structures of *p*-aminobenzoic acid (left) and sufanilanide (right)

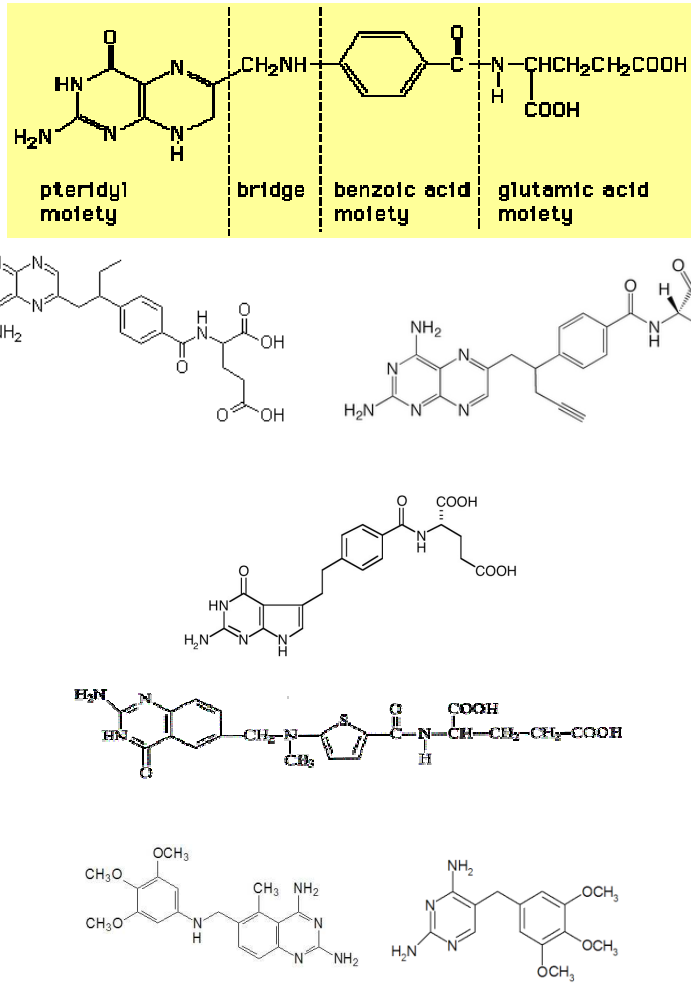
Concept of structural analogy gave an impetus to general search for antimetabolites of therapeutic utility. The principal approach involves introduction of minor changes to

the chemical structure of chosen metabolite by replacement its specific functional groups by related ones. The invention of methotrexate is one of the oldest examples of successful implementation of this methodology. Methotrexate is N-methylated aminopterine, a formal antimetabolite of folic acid, in which hydroxyl group of pteridynyl fragment of folic acid is replaced by amino moiety.



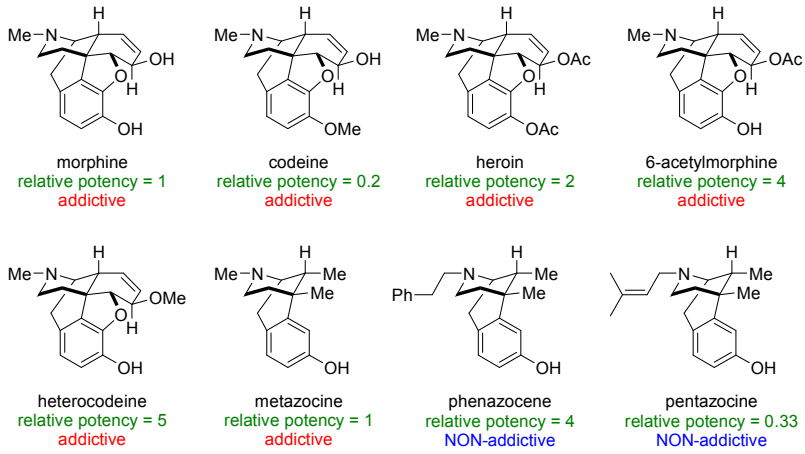
Structures of folic acid, aminopterine and methotrexate

Aminopterine and methotrexate were developed to inhibit mammalian folate metabolism and thus act as anticancer drugs. Its discovery is considered as one of the milestones in modern chemotherapy. It is used to treat certain cancers of breast, skin, head and neck and lung but also severe psoriasis and rheumatoid arthritis. Interestingly, first developed to treat malignancies it is now used to treat gynecological problems. Further modification of its structure led to the drugs less and less resembling folic acid, as it is well demonstrated by the structure of antiparasitic Trimethoprim.



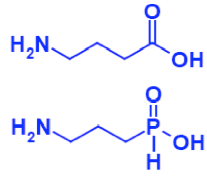
Sites of the modification of folic acid and some of its anlogues,
the last structure represents Trimethoprim

A good example how small modifications introduced to the molecule of the drug represents comparison of the activity of morphine analogues given below. As it is seen from that example, the application of the theory of structural analogy is quite cumbersome because it requires synthesis of many new chemical entities in order to evaluate how small structural changes introduced to parent molecule affect its biological activity.



Structure-activity relations in morphine derivatives

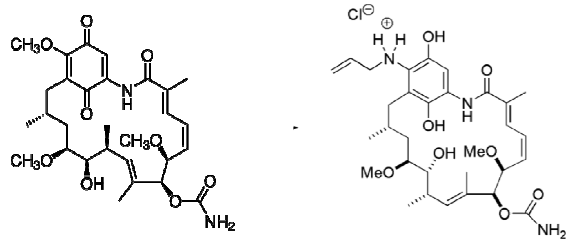
Sometimes the use of structural analogy approach yields in quite surprising results. It is well illustrated by the activity of phosphinic acid analogue of γ–aminobutyric acid (GABA). GABA is a chief inhibitory neurotransmitter in mammalian central nervous system. There are two classes of GBA receptors: $GABA_A$ and $GABA_B$. $GABA_A$ receptors are ligand-gated channels, whereas $GABA_B$ ones are G protein-coupled receptors. In order to understand their physiological functions a molecular tools are able to switch off one of the receptors when not influencing the other are required. The activating affinity of GABA to the two receptors is equal and values 20 nM. Phosphinic acid analogue is 4,500 times more selective towards $GABA_B$ receptor, with affinity of 1 nM.



GABA and its phosphinic acid analogue

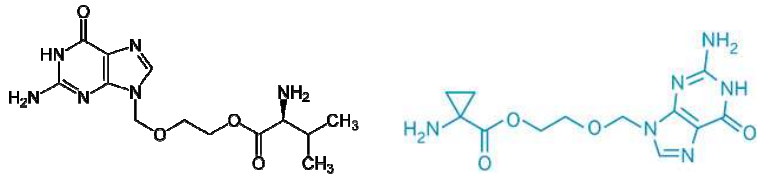
Theory of structural analogy is most commonly used to modify structures of the known drug molecules. This process is called drug optimization and is done in order to enhance drug secondary properties such as: absorption, stability, distribution, metabolism and toxicity. This is also cumbersome and time-consuming process.

However there are some indications that help to achieve the goal. A useful example is modification of geldanamycin, an antimelanotic compound isolated from *Streptomyces hygroscopicus*. It binds to Heat Shock Protein 90 and alters its function inducing degradation of proteins that are mutated in tumor cells. Despite its potent antitumor potential, geldanamycin presents several major drawbacks as a drug candidate, with hepatotoxicity being the most dangerous. That is why Kosan Biosciences introduced improved geldanamycins obtained by replacement of methoxyl at the17 position by amines.



Geladanamycin and its most acive analogue IPI 504

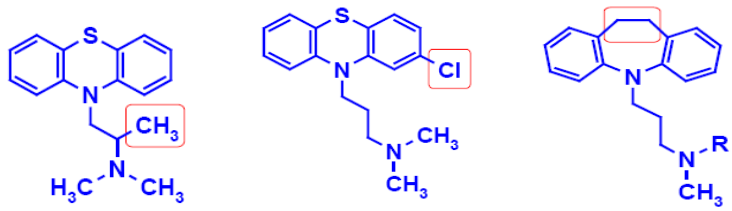
Another example is modification of the valacyclovir structure. It is an antiviral agent produced by GlaxoSmithKline, active against herpex simplex and herpex zoster (shingles). It is a prodrug since the hydrolysis of *L*-valine releases popular antiviral agent – acyclovir. Replacement of valine by aminocyclopropanecarboxylic acid improves the stability of the prodrug and results in the increase of its oral availability.



Valacyclovir and its analogue

In some cases small modification of the drug structure led unexpectedly to change of the mode of the drug action. This is well illustrated by modifications of promethazine, which is a firs generation of H1 receptor antagonist thus being used medically as antihistamine antiemetic. It prevents motion sickness, nausea or vomiting, itching associated with allergies. Small modification of its structure led to chloropromazine,

which works on a variety of receptors in the central nervous system, producing anticholinergic, antidopaminergic, antihistaminic and weak antiadrenergic effects. Thus, it is used to treat psychotic disorders such as schizophrenia and bipolar disorder. Another minute modification of promethazine led to imipramine, which is mainly used in the treatment of major depression, panic disorder and eunuresis (inability to control urination).



Structures of promethazine, chloropromazine and imipramine

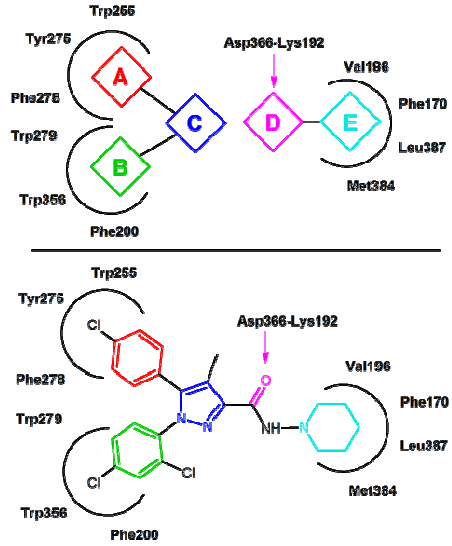
Chapter 6. Identification of the receptor structure

Receptor is a cellular structure that receives information from the environment carried out by a molecule (ligand), which is transferred into a physiologic effect. In most cases the action of the drug results from its direct action on certain receptor. Therefore, the knowledge of drug-receptor interaction is of key importance for drug design process.

One of the techniques to build up possible receptor structure is pharmacophore approach. A pharmacophore is a specific, three-dimensional (3D) map of biological properties common to all active conformations of a set of drugs (ligands), which exhibit a particular activity. Conceptually, a pharmacophore is a distillation of the functional attributes of ligands, which accomplish a specific task. Thus, it is a conceptual template for drug design generated from structural data describing ligands and their interaction with the receptor.

Pharmacophore models are hypotheses on the 3D arrangement of structural properties, such as: charge centers, hydrogen bond donors and acceptors, hydrophobic groups and aromatic rings of compounds that bind to a biological target. In the presence of the 3D structure of this target by comparison with inactive analogs, further geometric and/or steric constraints can be defined.

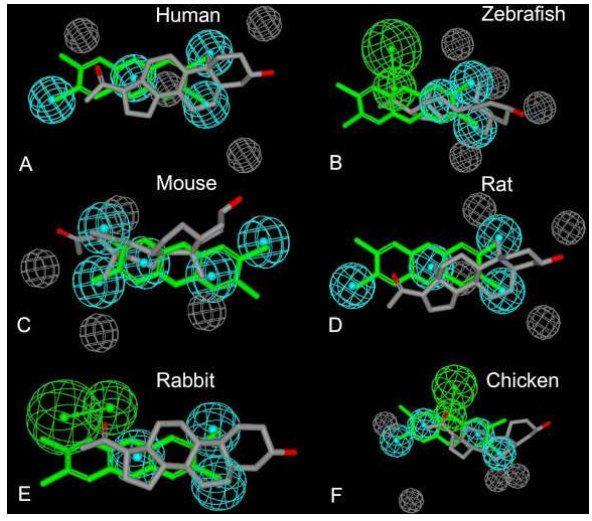
The pharmacophore for cannabinoid receptor might serve as an example here. The discovery of the endogenous cannabinoid system led to the development of CB1 receptor antagonists. The first cannabinoid receptor antagonist, rimonabant, was described in 1994. Rimonabant blocks CB1 receptor selectively and it has been shown to decrease food intake and regulate body-weight gain. The prevalence of obesity worldwide is increasing dramatically and has a great impact on public health. Most CB1 antagonists reported so far are close analogs or isosteric compounds to rimonabant. They were used to extract the common structural features and to build up the receptor model. This pharmacophore contains a cyclic core C (small heterocyclic ring, most likely pyrazole), which is substituted by two aromatic moieties, A and B. A core ring C is additionally bound, via small hydrogen bond forming unit D, with a cyclic lipophilic moiety E.



A general CB1 receptor inverse agonist pharmacophore model. Putative CB1 receptor amino acid side chain residues in receptor-ligand interaction are shown. The binding of the starting drug rimonabant is also presented.
(J. H. M. Lange et al. *Drug Discovery Today* 2005)

Another example comes from construction of pharmacophore for pregnane X receptor (PXR). This is a nuclear receptor whose primary function is to sense the presence of foreign toxic substances and in response regulate the expression of proteins involved in the detoxification and clearance of these substances from the body (for example induction of cytochromes P450).

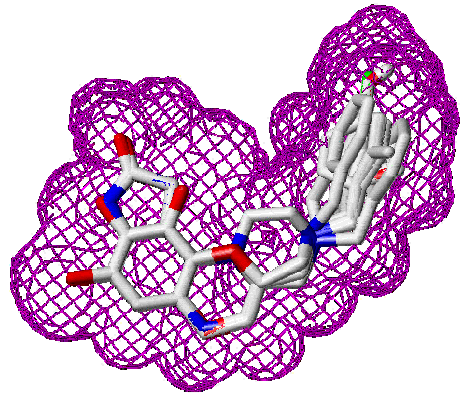
In a comparative study, concentration-response curves for a common set of 16 compounds (steroids, bile salts, xenobiotics) in a set of PXRs from six species (human, zebrafish, mouse, rat, rabbit, and chicken) were determined. These data were use to construct pharmacophore for each individual receptor. The pharmacophore maps show the hydrophobic features as cyan spheres, hydrogen bond acceptor regions as green ones, and volumes where the lack of any structural motif is desirable as grey spheres. The pharmacophores generated are shown mapped to two of the generally more active ligands, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, green) and 5β-pregnane-3,20-dione (gray). It should be noted that these ligands are differently placed in the pharmacophore space. For, example the TCDD is inactive in rabbit PXR and only maps to the hydrophobic features thus indicating different specificities of the two ligands towards this receptor.



Pharmacophore models of PXR activators
(S. Ekins et al., *BMC Evolutionary Biology* 2008)

Another approach relays on construction of quantitative models derived in the absence of protein structures. This technique is used most often when three-dimensional structure of the receptor is not known. They are referred to as Quantitative Structure Activity Relationships (QSAR) and are based on relatively easy to calculate descriptors of chemical structure and on the optimization of these molecular structures for particular biological effects. One of the useful techniques is a

Comparative Molecular Field Analysis (3D-QSAR CoMFA). The aim of CoMFA is to derive a correlation between the biological activity of a set of molecules and their 3D shape. It relies on superimposition of chosen set of biologically active compounds in such a manner, which maximizes electronic and steric overlap of the studied compounds. Then the calculation of steric and electrostatic fields for each molecule by interaction with a probe atom (for example sp^3-hybridized carbon with +1 charge) placed at a series of grid points (usually 2-Å spacing) surrounding the aligned assembly in three-dimensional space is carried out. Thus, CoMFA builds statistical and graphical models that relate to the properties of molecules (including biological activity) to their structures. These models are then used to predict the chosen activities of novel compounds.

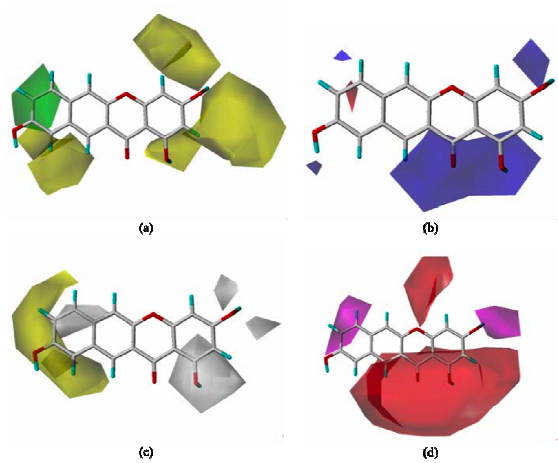


Dopamine D2 receptor partial antagonists placed in a grid
taken from http://www.netsci.org/Science/Compchem/

More advanced version of CoMFA is Comparative Molecular Shape Indices Analysis (CoMSIA). These methods differ from each other in that CoMSIA uses a Gaussian function instead of Lennard-Jones potentials to asses steric, elactostatic, hydrophobic and hydrogen bond donor/acceptor fields.

As a result of studies carried out by these two technique countour maps, which permit the understanding steric, electrostatic, lipophilic and hydrogen bonding requirements of ligand binding. Such an alalysis done on a set of 42 xanthone derivatives (called training set) in order to understand teir glucosidic activities is a good example here. These compounds, which serve as α–glucosidases inhibitors, were studied by two CoMFa and CoMSIA techniques resulting in models of good predive abilities. Results of CoMSIA studies are shown below. The steric fields are represented by green and

yellow contours (green, bulky substitution favoured; yellow, bulky susbtitution disfavoured. The electrostatic fields are indicated by red- and blue-coloured contours (blue, electropositive groups favoured, red, electronegative groups favoured). The hydrophobic fields are denoted by yellow (favoured) an white-coloured (disfavoured) contours, while the hydrogen donor fields are indicatd by cyan- and purple-coloured contours (cyan, favoured; purple, disfavoured). Finally, hydrogen bond acceptor firlds are shown as magneta and red contous (magneta, favoured; red, disfavoured). This set of templates is ready to be used to design new anti-diabetic drugs.



The most active xanthone mapped on CoMSIA (a) steric (b) electrostatic (c) hydrophobic and (d) hydrogen acceptor contour maps
(U. Saqib, M. I. Siddiqi, *Int. J. Intergrative Biol.* 2009)

The third methodology is to use protein structures found by X-ray crystallography or NMR as a template to design potential drugs by computer-aided procedures. Since the amount of information concerning 3D structures of biomolecular targets has increased dramatically and the structural dynamics and electronic properties of the ligands are easily calculated this has encouraged the rapid development of the structure-based drug design.

The first step of the process is to find either active site or other receptor (even of no physiological significance). Armed with this information powerful computer programs are being used to search through databases containing the structures of

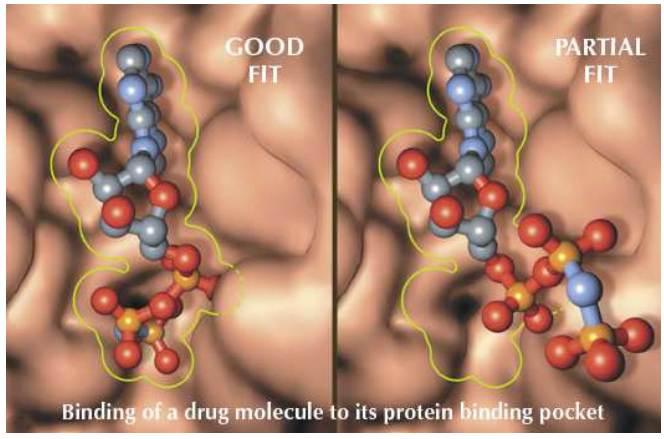
many different chemical compounds (Cambridge Structural Database is the one most frequently used). Such a procedure is called virtual screening, or *in silico* screening. The computer "docks" each molecule from the chosen library into target's binding site and scores its geometric and electrostatic fit. There are quite a big number of docking programs available and all of them predict the possible binding of a ligand by calculating the contribution of certain types of interactions to overall affinity, which is given as a set of specific scores. Scoring system relates the activity to:

– number and stability of hydrogen bonds formed between receptor (usually it is the enzyme) and inhibitor;

–electrostatic interactions between metal ion(s) and negatively charged portions of inhibitor;

–strength of hydrophobic interactions, which depends on receptor-ligand hydrophobic contact area.

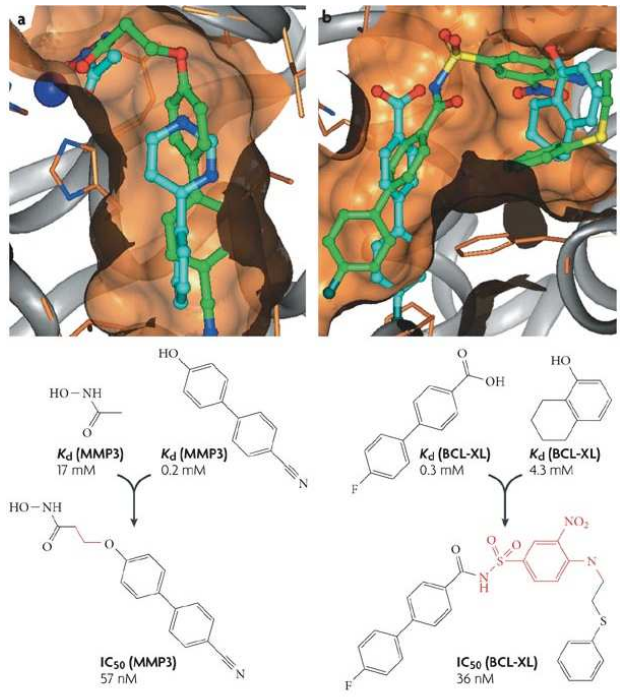
Thus, the computer can select those compounds that are most likely to interact with receptor, and these can be tested in the laboratory.



Docking of the inhibitors to the active site of the enzyme
taken from http://people.rit.edu/japfaa/molecules.html

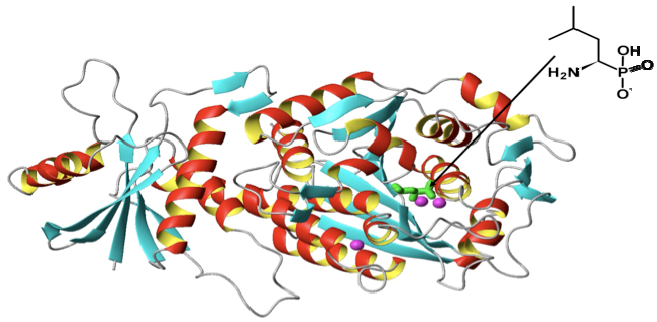
Another approach uses a library of fragments (or molecules). The idea is to position molecular fragments into the active site in such a way that hydrogen bonds can be formed with the enzyme and hydrophobic pockets filled with hydrophobic groups. These fragments are then connected with each other by using suitable spacer fragments to form a single molecule. Such a procedure is called fragment based drug

design and reminds the concept of “Lego”. There are several advantages to this approach. It is very fast and due to the large number of possible fragment combinations the variety of molecules that can be generated is enormous.



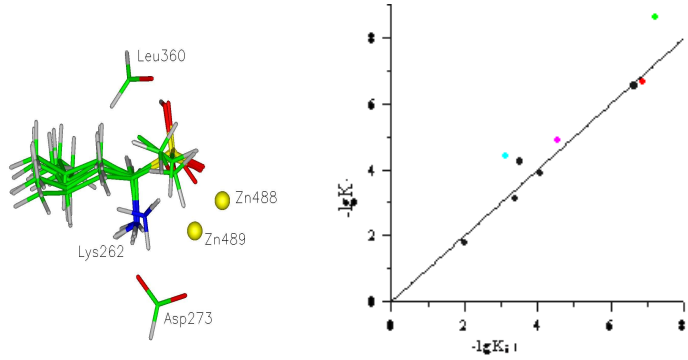
Idea of fragment based drug design as shown by P. J. Hajduk and J. Greer in *Nature Reviews: Drug Discovery,* 2007

The concept of computer aided drug design is well described by structural modifications of phosphonic acid analogue of leucine. This compound is an inhibitor of leucine aminopeptidase (LAP), a protease involved in cell maintenance with critical role in turnover of peptides. Based on the crystal structure of this compound bound in the active site of the enzyme from bovine lens, the influence of the modification of its structure on the inhibitory activity towards porcine enzyme was studied.

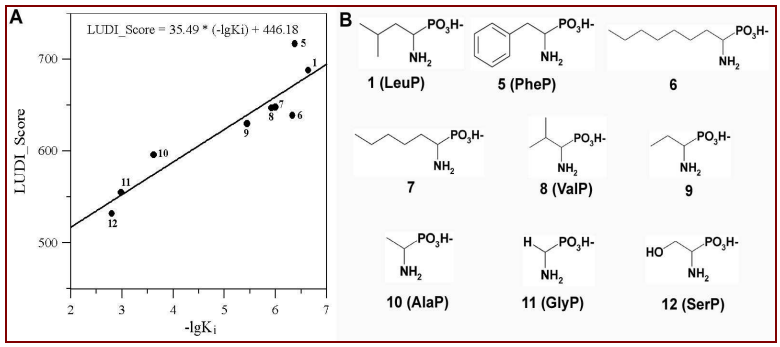


Phosphonic analogue of leucine bound with bovine lens aminopeptidase
(N. Sträter et al. *Biochemistry*, 1995)

First, the influence of the replacement of one of the phosphonate hydroxyl groups by related functions was studied. This resulted in series of analogues of leucine of varying affinity towards LAP. Significant correlation between used computational program (LUDI) and experimental inhibition constant was achieved. Then a precise analysis of enzyme-inhibitor interactions for series of structurally diverse aminophosphonates was performed. Also in this case, the correlation between predicted and determined inhibition constant was found. In this case the differences in binding were mainly attributed to hydrophobic interactions. Further studies resulted in generation by the program more structurally complex phosphinates, which appear to be the most potent low-molecular inhibitors of the enzyme.



Structural modifications of the inhibitory phosphonic acid analogue of leucine by replacement of one of hydroxyl phosphonate groups by -H, $-CH_3$, $-OCH_3$, $-CH_2Cl$. Measured values are plotted on x-axis, whereas calculated ones on y-axis.



Structural modifications of the inhibitory phosphonic acid analogue of leucine by replacement of *iso*-butyl fragment of the molecule

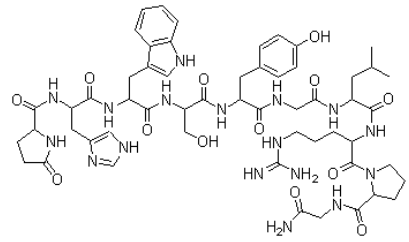
Chapter 7. Topographical complementarities

Throughout the body, peptides are active regulators and information brokers with skill sets that make them interesting for drug discovery. The most commonly search on peptide-like drugs is concentrated on discovery of agonists and antagonists of certain hormones and neuroregulators. In order to do that, low stability in body fluids and the fast clearance of peptides must be overcome.

The simplest solution is a replacement of terminal amino acids of lead compound by their enantiomers. This usually improves peptide hydrolytic stability, since enzymes do not hydrolyze *D*-amino acids.

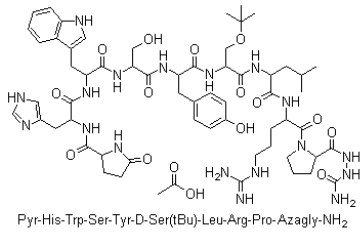
Replacement of one or few amino acids of chosen hormone by their analogues is perhaps the oldest and the most exploited technique for designing new drugs. Such analogues of gonadotropin releasing hormone are a good example here. Gonadotropin releasing hormone (GnRH) is the hypothalamic factor that mediates reproductive competence. This peptide composed of 10 amino acids triggers sexual development and it is essential for normal sexual physiology of both males and females. In both sexes, its secretion occurs in periodic pulses usually occurring every 1–2 hours. GnRH secretion from the hypothalamus acts upon its receptor in the anterior pituitary to regulate the production and release of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH then stimulate sex steroid hormone synthesis and gametogenesis in the gonads. Therefore, its analogues are considered as drugs against sexual disorders.

Zoladex obtained by modifications introduced at C-terminal glycine and serine-4 stops the production of sex hormones (testosterone and estrogen) and is used to treat hormone-sensitive cancers of the prostate and breast (in pre-/perimenopausal women). Cetrorelix obtained by modifications of GnRH chain in positions 1, 2, 3, 6 and 10 is a synthetic decapeptide with gonadotropin-releasing hormone antagonistic activity. It is used in assisted reproduction techniques to prevent premature LH surge in women undergoing controlled ovarian stimulation allowing the follicles to mature for planned oocyte collection.



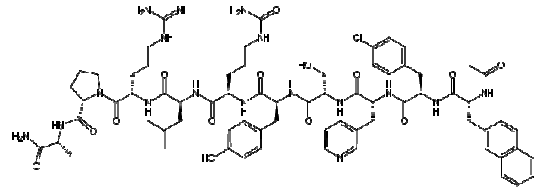
Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

Gonadotropin releasing hormone

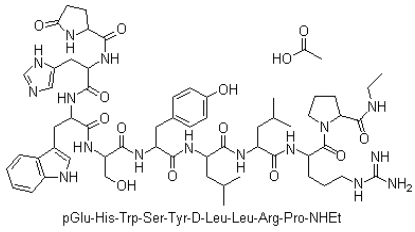


Pyr-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-Azagly-NH_2

Zoladex

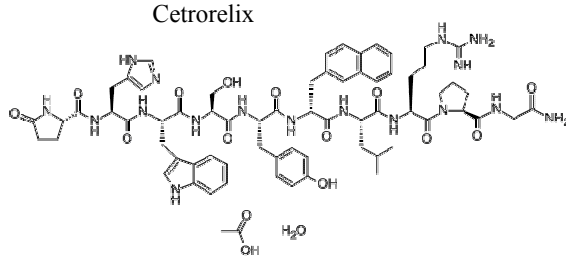


Cetrorelix



pGlu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt

Lupron



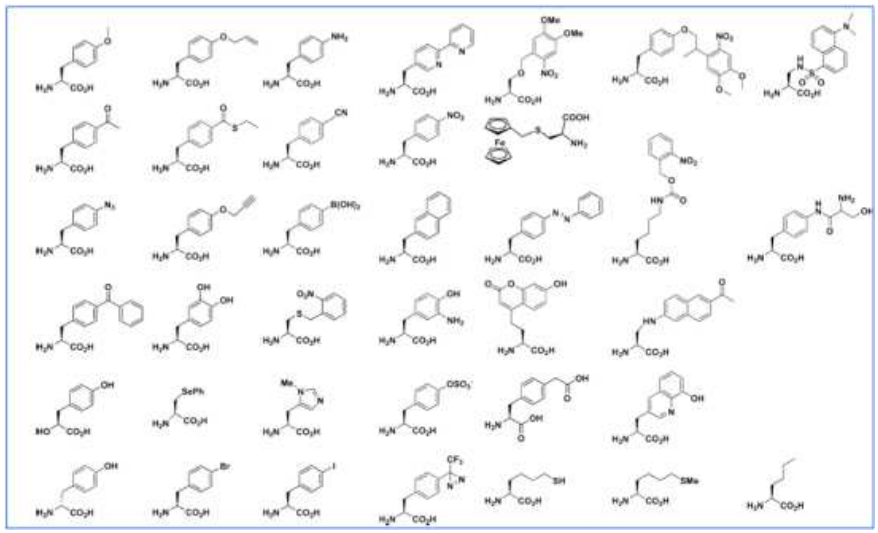
Synarel

Gonadotropin releasing hormone and its analogues

Third analogue, Lupron, which differs from parent hormone by modification of both

glycines (positions 6 and 10), is used for the palliative treatment of advanced prostate cancer. In many cases, Lupron may slow or stop the growth of cancerous cells and relieve some of the associated symptoms. Finally, Synarel, which was obtained by replecement of glycine-6 by bulky aromatic non-proteinous amino acid, is used to relieve the symptoms of endometriosis, including menstrual cramps or low back pain during menstruation. Synarel (Nafarelin) is also indicated for use in controlled ovarian stimulation programs prior to *in vitro* fertilization.

Of course, it is not possible to predict how the introduced change will reflect in certain activity. Therefore, cumbersome trials are needed to find out proper drug amongst thousands of synthesized analogues. It is worth to note that the replacement of each of ten amino acids in GnRH by 20 proteineous amino acids gives 10^{20} combinations. If considering that each natural amino acid could be replaced by many structurally different analogues not systematic approach but only luck may help to find interesting new drug.

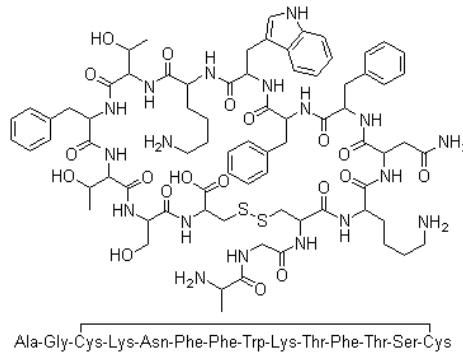


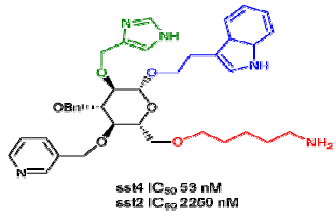
Chosen analogues of phenylalanine

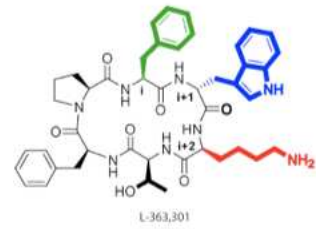
It is well established that only several exposed amino acids of the hormone are responsible for physiologic effect. Therefore it is of interest to place their side chains in such a way that they ensure interaction with the appropriate receptor. Ocreotide is a drug elaborated basing on that concept. Somatostatin has two active forms produced

by alternative cleavage of a single preproprotein: one of 14 amino acids, the other of 28 amino acids. Ocreotide is an octapeptide that mimics natural somatostatin pharmacologically, though is a more potent inhibitor of growth hormone, glucagon, and insulin than the natural hormone. It is approved for the treatment of acromegaly, the treatment of diarrhea and flushing episodes associated with carcinoid syndrome, and treatment of diarrhea in patients with vasoactive intestinal peptide - secreting tumors. Also a L-363,301 hexapeptide with properly exposed side chains of phenylalanine, tryptophan, lysine and threonine displays high biological activity in inhibiting the release of growth hormone, insulin, and glucagon. When the same moieties were place on sugar backbone hey were found to mimic β-turn of L-363,301 and thus display similar activity.

Acromegaly is a hormonal disorder that results when the pituitary gland produces excess growth hormone (GH). It most commonly affects middle-aged adults and can result in serious illness and premature death.



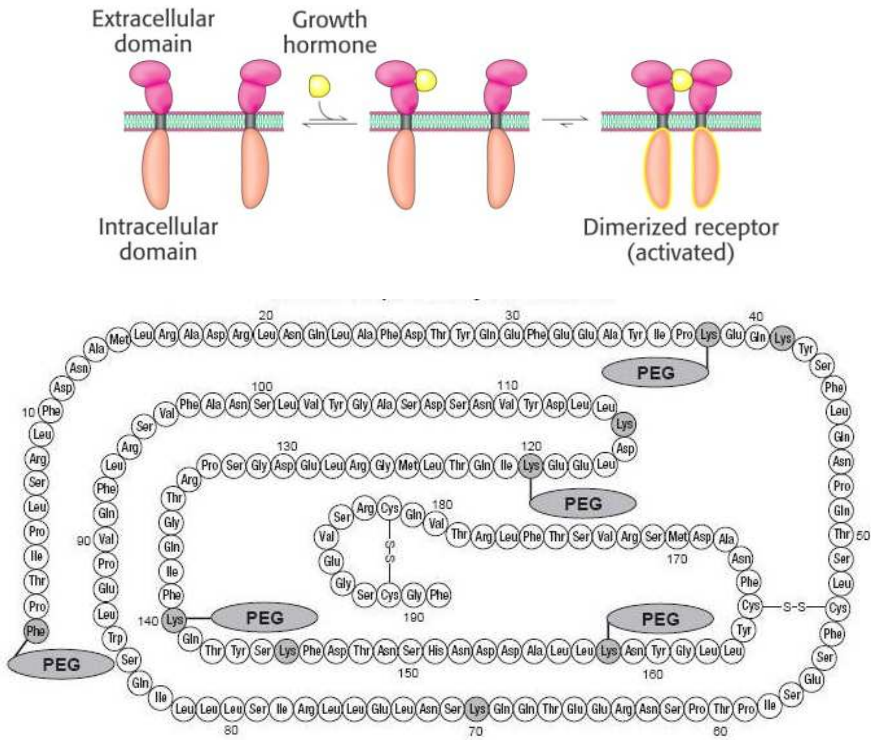




Somatostatin and its analogues

When GH-producing tumors occur in childhood, the disease that results is called

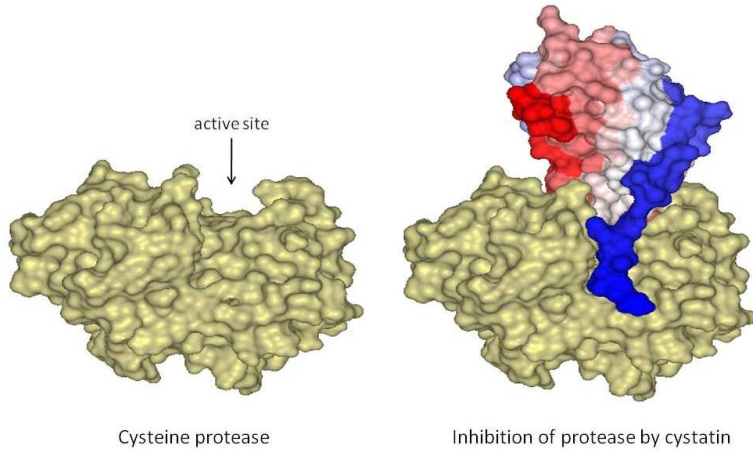
gigantism rather than acromegaly. Somavert is the first in a new class of drugs called growth hormone receptor antagonists. Human growth hormone has two binding sites, each of which binds identical cell receptor. When both sites bind, dimerazing the receptors, signal transduction occurs. Blockage of the dimerization protects the receptor and hormone does not act. Somavert is a human protein of recombinant DNA origin that contains 191 amino acids to which several polyethylene glycol polymers are bound covalently (usually four to six PEG/protein molecule). It is synthesized by a specific strain of *Escherichia coli* that has been genetically modified. Although the hormone binds to the receptor and then bulky polyethylene glycol prevents dimerization.



Functioning of the growth hormone receptor and structure of Somavert taken from http://dailymed.nlm.nih.gov/dailymed. PEG denotes polyethylene glycols attached to the side chains of hormone lysines.

There are many regulatory small proteins, which disclose activity by binding to the

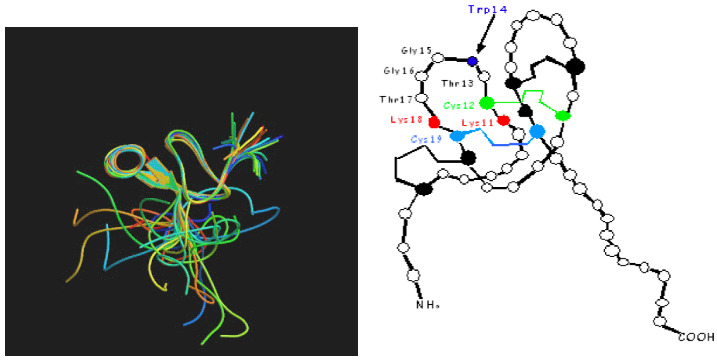
receptor or enzyme by small part of the molecule. Cystatins are a good example here. Cystatins are able to inhibit the tumor-associated activity of intracellular cysteine proteases cathepsins. Cathepsins are regulated proteases with limited tissue expression, which implies specific roles in cellular physiology. These roles appear to include apoptosis, MHC class II immune responses, prohormone processing, and extracellular matrix remodeling important to bone development. Inhibition of cathepsins is considered as a mean of combating cancers and they are even used in experimental treatments. Cystatins interact with the active sites of cathepsins via their inhibitory reactive site, made up of the juxtaposition of three regions of the molecule and which forms a wedge-shaped edge that is highly complementary to cathepsins these sites. Therefore the separation of that entity from the whole protein and stabilization of its structure seems to be an interesting approach to new inhibitors. Unfortunately, such an approach has not been successfully applied to cystatins yet.



Mode of binding of cystatin to cathepsins taken from
http://www.fabinet.up.ac.za/people/juan

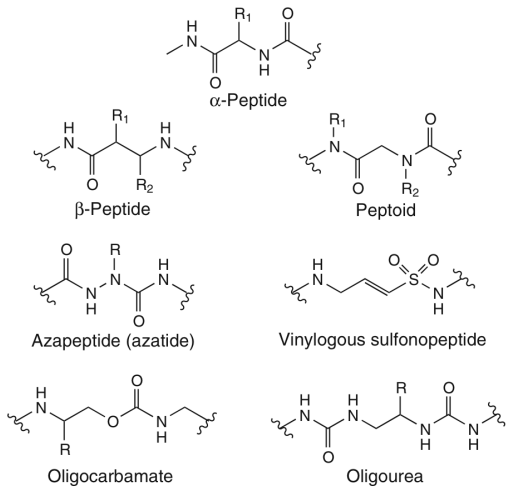
This idea was applied to synthesis of agonists and antagonists of 48 amino acid protein, γ-agatoxin IVB. It is one of the toxins extracted from American funnel web spider *Agelenopsis aperta.* This toxin is a very selective antagonist of the P-type calcium channels. Because γ-agatoxin IV docks to the channel protein via eight amino acids, which are located between the 11 and 18 amino acid this fragment was chosen to find the minimal sequence, which possesses the activity of calcium channel modulators. Therefore, analogues possessing the three-dimensional arrangement

corresponding to the native structure of the loop, which resulted from constrain by application of cyclic peptides, were designed. The neurophysiological experiments confirmed the choice of the mimetics and the necessity of the presence of properly directed tryptophan residue for toxin-channel interactions.



Structure of γ-agatoxin IV, its exposed loop (in the middle on the top of protein structure) and examples of two effectors of calcium channel, taken from PhD Thesis of Dr. Ewelina Minta (Montpellier 2006).

The last approach to obtain drugs basing on the structures of peptides and proteins is construction of peptidomimetics. A peptidomimetic is a compound containing non-peptidic structural elements that is capable of mimicking or antagonizing the biological action(s) of a natural parent peptide. It does not have to have classical peptide characteristics such as enzymatically scissile peptide bonds. The simplest way of construction of such derivatives is replacement of peptide bond by its mimetic. There are plenty of possibilities to do this and some of them are outlined below.

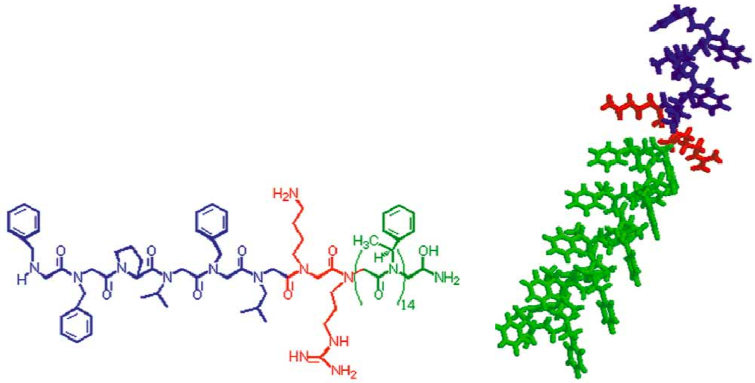


Representative mimics of peptide bond

Good example illustrating the use of this approach is replacement of all amino acids of frog-produced antibacterial agents – magainins – by β–amino acids. Magainins are family of peptides with broad-spectrum antimicrobial activity that have been isolated from the skin of the African clawed frog *Xenopus laevis.* They consist of two closely related peptides that are each 23 amino acids and differ by two substitutions. They exhibit weak haemolytic activity. When trying to improve these peptides by increasing their hydrophobicity, their ability to lyse red cells also increased. The replacement of their α–amino acids by β–amino acids resulted in the same antibacterial activity with simultaneous suppression of their action against red cells.

Somewhat different approach comes from search for artificial lung surfactants. In breathing, mammalian lungs must expand and contract repeatedly. This is mediated by alveoli - small, bubble-like structures that are joined together, which make up the lungs. The lungs must overcome the surface tension present at the alveolar surface in order to expand and also to keep the alveoli from collapsing. To enable this to happen, the surfaces of the alveoli are coated with a mixture of lipids and proteins, termed lung surfactant, which lowers the surface tension and allows proper physiological breathing. Many prematurely born babies suffer from, so called, respiratory distress syndrome. Without sufficient amounts of functional lung surfactants, they are not able to breathe without assistance. Thus, supplementation with these surfactants is necessary. Treatment of lung surfactant maladies currently involves replacement of

surfactant with animal-lung-derived compounds. This can lead to numerous health complications, such as poor antigen transfer and supply problems, making a totally synthetic solution highly desirable. Lung surfactants are composed of phospholipids, palmitic acid and surfactant proteins. Surfactant protein C is the smaller and simpler of them at just 35 monomers in length. Unfortunately, it has tendency to form amyloid-like deposits thus causing lung fibrosis. Replacement of this peptide by its mimetic obtained in such a manner that side chains of amino acids are transferred to nitrogen atom of peptide bond gave the mimetic, which captured into lipid film exhibits lung surfactant properties.



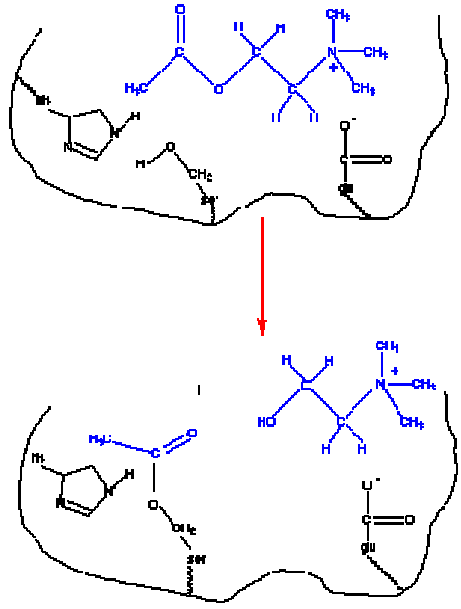
Mimetic of the surfactant protein C fragment (5-35) and a molecular model of its three-dimensional structure

Chapter 8. Inhibitors that covalently modify enzymes. Enzyme killers

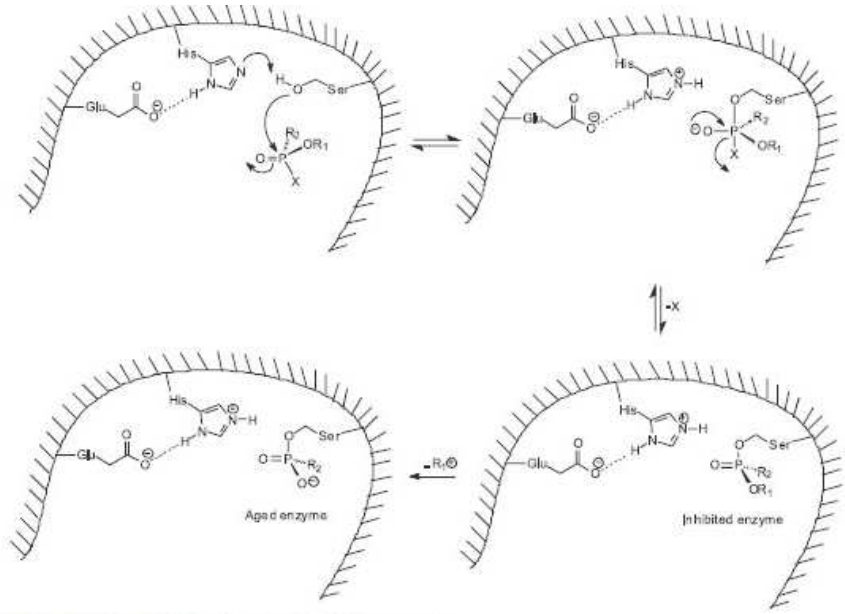
A covalent and permanent modification of a functional group of the enzyme, rendering the molecule inactive, usually results in irreversible inhibition of enzyme activity. Such inhibitors are designed by leaving in inhibitor as much structural features of the substrate as possible and adding groups reactive towards nucleophiles, which are always present in active sites of enzymes. Irreversible inhibitors are generally specific for one class of enzymes and do not inactivate all proteins. They do

not function by destroying protein structure but by specifically altering the active site of their target. Thus, the reactivity of the inhibitor with chosen moiety of the enzyme is more important here than its similarity to the substrate or product of enzymatic reaction.

The action of nerve gases on actetylcholine esterase is a good example here. This enzyme breaks down the neurotransmitter acetylcholine. Each molecule of the enzyme degrades about 25,000 molecules of acetylcholine per second. Action of nerve gas causes that acetylocholine remains at its post-synaptic receptor sites causing excessive cholinergic stimulation followed by neuromuscular paralysis through the entire body, leading to death. They phosphorylate acetylcholineesterase active site serine and form stable phosphorylenzyme conjugate. Since the modified serine residue is involved in catalysis, the enzyme became non-functional.

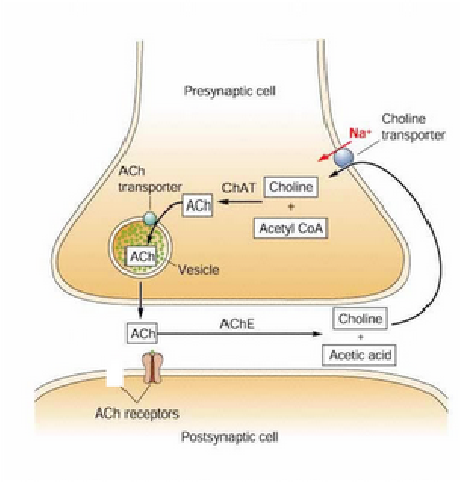


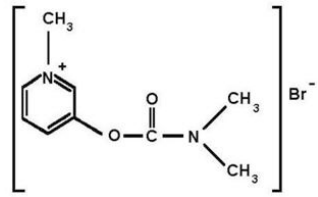
Mechanism of action of acetylocholine esterase

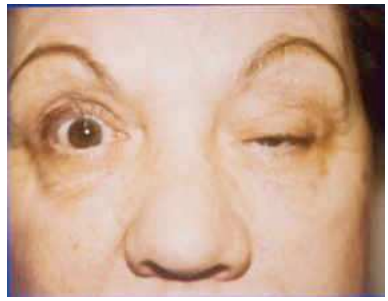


Inhibitory action of organophosphorus compounds on acetylcholine esterase

During the Gulf War of 1990-91 the United States Military conducted the largest experimental drug evaluation in its history. Pyridostigmine bromide was considered by the Pentagon as a possible prophylactic for a soldier's exposure to chemical warfare nerve agents. The rational for conducting this large-scale experimental drug test on military personnel, without informed consent, is highly questionable. Pyridostigmine is used to decrease muscle weakness resulting from myasthenia gravis. It is an immunologically acquired abnormality, but some cases result from genetic abnormalities at the neuromuscular junction. In acquired myasthenia gravis, the post-synaptic muscle membrane is distorted and simplified, having lost its normal folded shape. The concentration of acetylcholine receptors on the muscle end-plate membrane is reduced, and antibodies are attached to the membrane. Acetylcholine is released normally, but its effect on the post-synaptic membrane is thus reduced. Pyridostigmine prevents the breakdown of acetylcholine in the neuromuscular junction keeping the level of this neurotransmitter high enough to maintain more or less proper functioning of the muscle. Thus, this drug does not cure myasthenia gravis but does help to improve the symptoms.

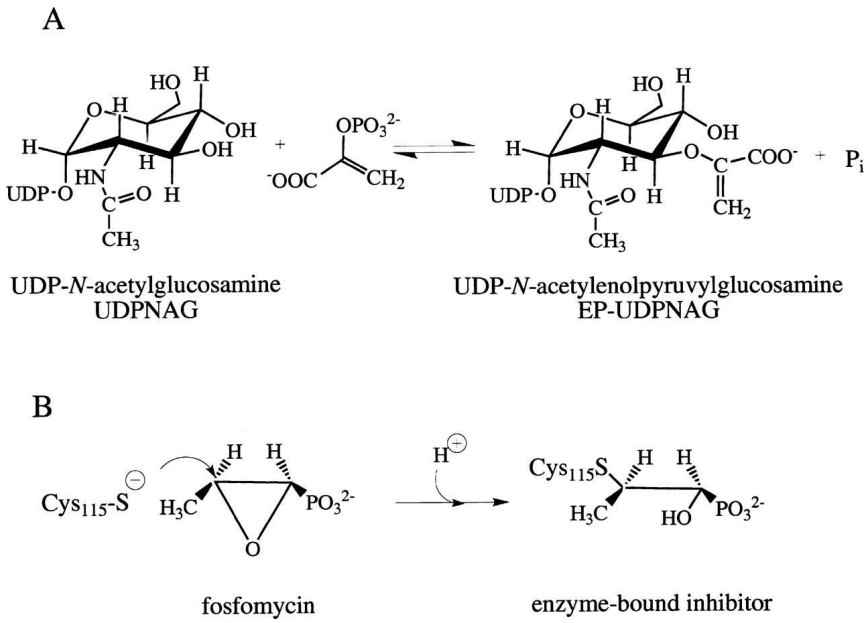






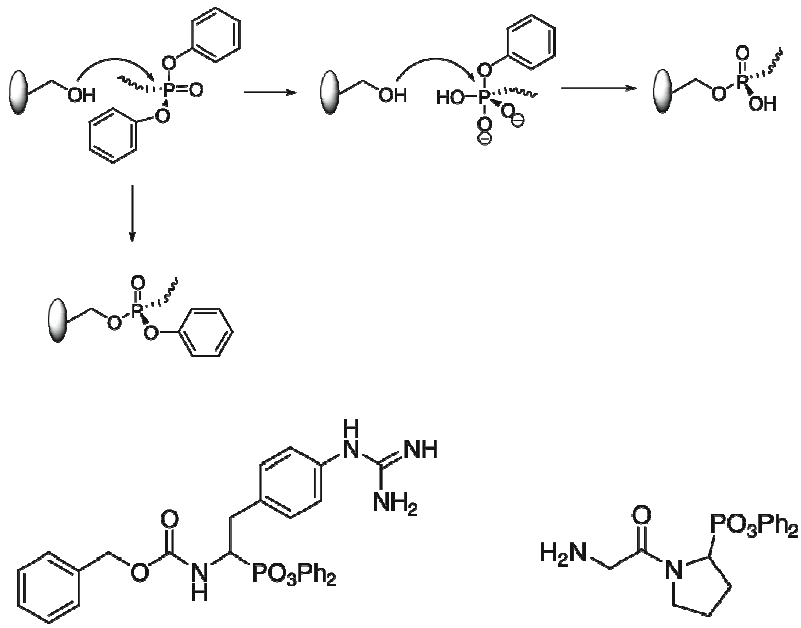
Molecular mechanism of synaptic action of acetylocholine (upper left panel), pyridostigmine (upper right) panel and symptoms of myasthenia (lower panel)

Fosfomycin is an antibiotic produced by certain *Streptomyces* species. It is used to treat urinary tract infections and cystitis (bladder infection) in women. It inhibits bacterial UDP-*N*-acetylglucosamine enolpyruvyl transferase (MurA), an enzyme that catalyzes the transfer of enolpyruvate from phosphoenolpyruvate to uridine diphospho-*N*-acetylglucosamine. This is the first committed step of bacterial cell wall biosynthesis. Fosfomycin is considered as phosphoenolpuruvate analogue acting by alkylation of an enzyme active site cysteine.



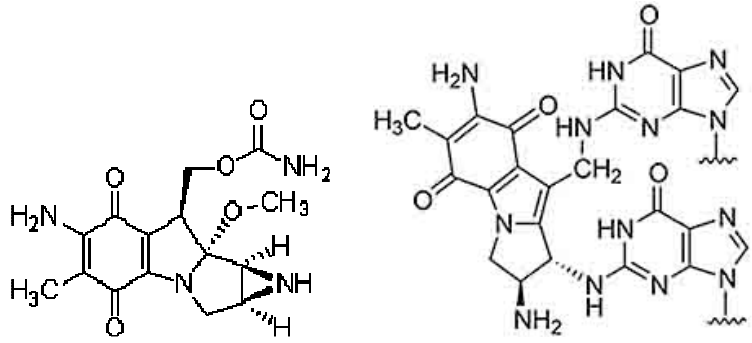
Reaction catalyzed by UDP-*N*-acetylglucosamine enolpyruvyl transferase (panel A) and mechanismof fosfomycin inhibition

Serine proteases are a group of enzymes with a wide range of activity involved in many physiological states of pathological disorders. Their uncontrolled activity often leads to serious diseases like emphysema, cystic fibrosis, or cancer development and progression. Reactive phosphonate- and peptidyl-phosphonate diphenyl esters have been applied successfully to covalently modify members the of serine hydrolase superfamily. They act as active site directed irreversible inhibitors, which after formation of the normal enzyme–substrate complex, covalently phosphonylate the active site serine. The mechanism of action of these inhibitors presumably involves nucleophilic attack of serine hydroxyl at the phosphorus atom, which results in the formation of phosphonylated enzyme. In this complex, the phosphorus atom resembles the tetrahedral intermediate formed during the hydrolysis of the peptide bond. Single diphenyl phosphonates are usually weak inhibitors and enhancement of their activity is achieved by their introduction into the peptide chain. The composition of the peptide chain also ensures their selectivity towards the chosen enzyme.



Mechanism of inhibitory action of diphenylphosphionate inhibitors and structures of inhibitors of urokinase-type plasminogen activator (potential anticancer agent) and of seprase (antimelanotic drug)

Mitomycin is one of the older chemotherapy drugs, which has been around and in use for decades against many cancer types and is also used in a number of procedures to prevent scar formation. This antibiotic was isolated from *Streptomyces caespitosus*. Drug is in a form of clear blue or purple liquid that is injected into a vein. Mitomycin is a potent DNA crosslinker, covalently binding two strains of DNA. A single crosslink per genome has shown to be effective in killing bacteria.

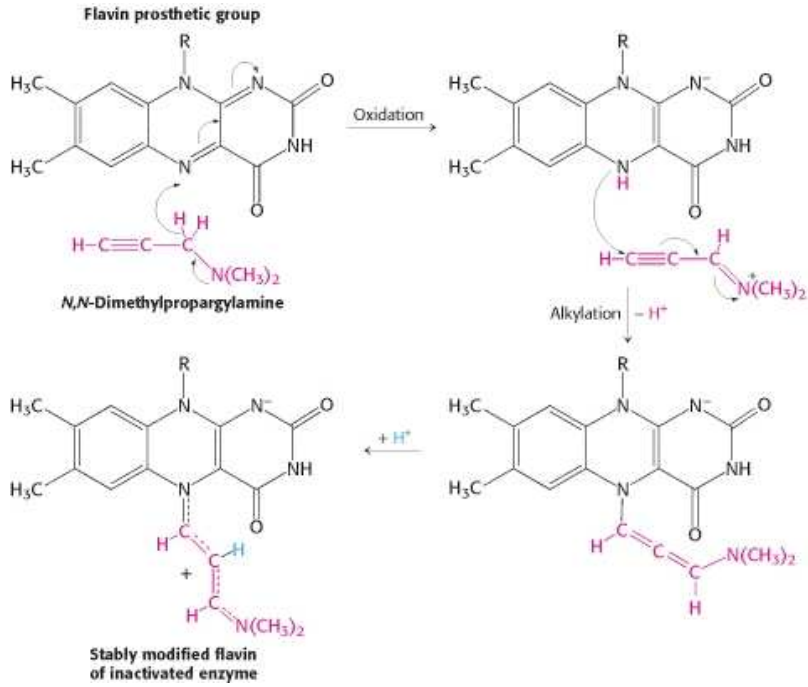


Mitimycin (left panel) and its conjugate with DNA (right panel)

Chapter 9. Suicide substrates – Trojan horses of enzymatic reaction

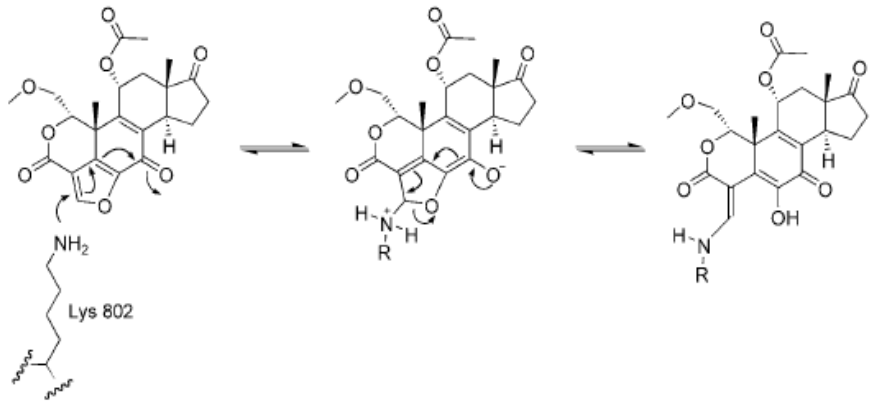
A suicidal substrate (or suicidal inhibitor) is a compound that is not of itself toxic to an enzyme, but which resembles a normal metabolite closely enough that it undergoes metabolic transformation to a product that does inhibit a crucial enzyme. When suicide substrates inactivate enzymes during catalysis, formation of product and inactivation of enzyme proceed concurrently. The concept that a pharmacologically important target enzyme can generate, via its normal catalytic mechanism, an irreversible inhibitor from an innocuous substrate analogue and hence commit suicide offers a highly selective approach to drug design.

One example of such an inhibitor is *N*,*N*-dimethylpropargylamine. A flavin prosthetic group of monoamine oxidase (MAO) oxidizes the *N*,*N*-dimethylpropargylamine, which in turn inactivates the enzyme by covalently modifying the flavin prosthetic group by alkylating N-5 atom of the cofactor. Monoamine oxidase deaminates neurotransmitters such as dopamine and serotonin, lowering their levels in the brain. Parkinson disease is associated with low levels of dopamine, and depression is associated with low levels of serotonin. The drug (-)deprenyl, which is used to treat Parkinson disease and depression, is a suicide inhibitor of monoamine oxidase. Deprenyl acts virtually by the same mechanism as *N*,*N*-dimethylpropargylamine, however its overall mechenism of action is more complex. It is the first selective inhibitor of MAO-B ever discovered, it is the only one used in clinical practice, and it remains the scientific reference standard for B-type inhibition after more than 40 years.



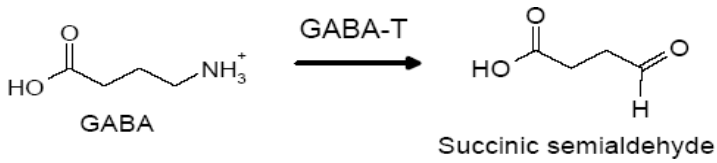
Mechanism of inhibitory action of *N,N*-dimethylpropargylamine

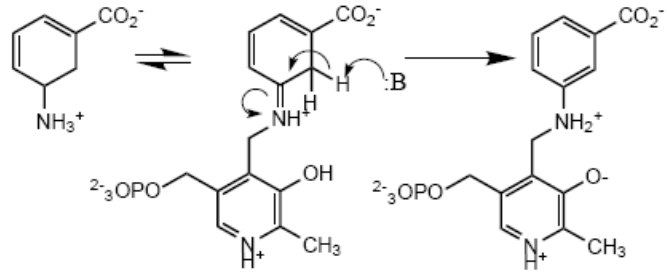
Another example is a antifungal antibiotic wortmannin, which was isolated from *Penicillium wortmanii*. Wortmannin and its structural relative viridin are potent cell-permeable inhibitors of the lipid kinases – phosphatidyl-inositol 3-kinases. These kinases are involved in a large number of fundamental cellular processes, including apoptosis, proliferation, cell motility, and adhesion, and have been implicated in the malignant transformation of cells. Thus, wortmannin and its structural relatives are considered to have potential as therapeutic agents for the treatment of human neoplasms and other disease states such as diabetes, inflammation, platelet aggregation, atherosclerosis and osteoporosis. The mechanism of inhibition was shown to involve covalent attack of lysine present within the ATP-binding site of the enzyme at an electrophilic site on this antibiotic. This causes a sequence of reactions and irreversible binding of the drug to the enzyme.



Molecular mechanism of inhibitory action of wortmanin

Gabaculine is another example of this type of inhibition. γ-Aminobutyric acid (GABA) is perhaps the most comprehensively studied inhibitory neurotransmitter in the mammalian central nervous system. It has been estimated that about 40% of synapses in the brain are GABAergic. Most anxiolytics and hypnotic-sedative drugs such as benzodiazepines and barbiturates exert their pharmacological actions *via* interactions with a discrete neuronal site on the $GABA_A$ receptor. Gabaculine is a naturally occurring neurotoxin first isolated from the bacteria *Streptomyces toyacaensis*. It acts as a potent and irreversible GABA transaminase inhibitor and seems to be a useful tool for investigating the GABA function in the brain. Its action is somewhat unique in that it involves generation of an aromatic compound (aromatization is the driving force of the inhibition). After gabaculine binds to the active site of transaminase it forms the Schiff base with pyridoxal phosphate. This causes the aromatization of the formed conjugate and total inactivation of the cofactor.





Reaction catalyzed by GABA transferase and molecular mechanism of action of gabaculine

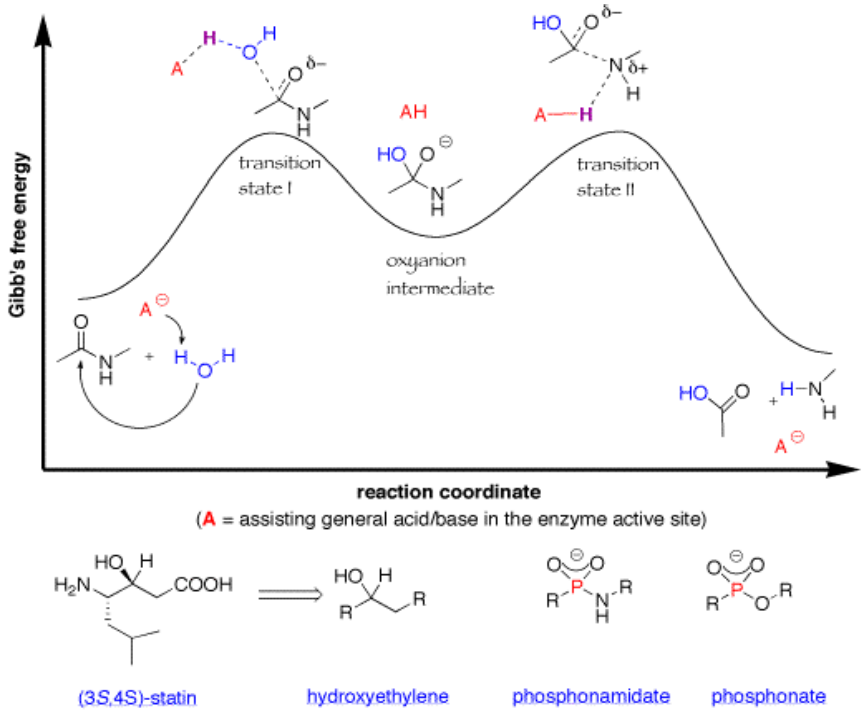
Chapter 10. Transition state analogues

All chemical transformations pass through an unstable structure called the transition state, which is poised between the chemical structures of the substrates and products. The transition states for chemical reactions are proposed to have lifetimes near 10^{-13} sec, the time for a single bond vibration. Thus, the transition state is the critical configuration of a reaction system situated at the highest point of the most favorable reaction path on the potential-energy surface, its characteristics governing the dynamic behavior of reacting systems decisively. It is used primarily to understand qualitatively how chemical reactions take place.

Yet transition state structure is central to understanding catalysis, because enzymes function by lowering activation energy. An accepted view of enzymatic catalysis is tight binding to the unstable transition state structure was coiled in 1949 by Linus Pauling. Because reaction rate is proportional to the fraction of the reactant in the transition state complex, the enzyme was proposed to increase the concentration of these reactive species. This proposal was further formalized by Wolfenden and coworkers, who hypothesized that the rate increase imposed by enzymes is proportional to the affinity of the enzyme for the transition state structure relative to

the Michaelis complex. Thus, a substance, which structurally resembles transition state (or other energetically high species) are considered as excellent enzyme inhibitors.

First inhibitors were designed for proteolytic enzymes. The design was based on the resemblance of transition state of phosphorus atom to the sp^3 transition state of the hydrolysis of peptide bond. This idea was particularly attractive in construction of wide range of potent and selective metralloprotease inhibitors.

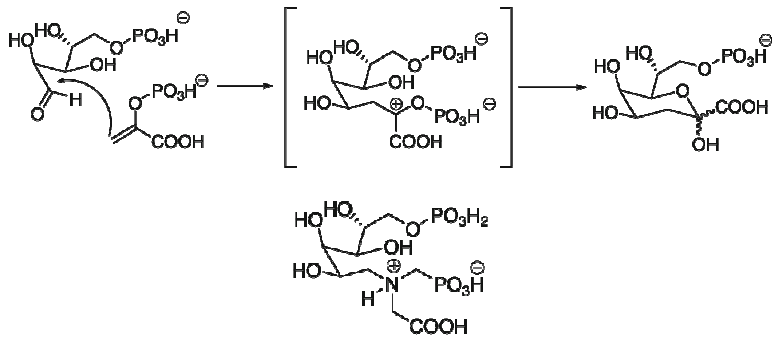


Phosphonates as transition state analogues of proteolytic hydrolysis of peptide bond.

One of the first discovered and most widely studied aminopeptidases with respect to sequence, composition, structure and mechanism of action is cytosolic leucine aminopeptidase. It is also perhaps an enzyme for which the most systematic and detailed computational studies regarding enzyme–inhibitor interactions have been performed. This enabled, amongst other, to select effective and potent inhibitors of the enzyme from *Plasmodium palcifarum.* These inhibitors appeared to be promising antimalarial agents as shown in animal studies. Their binding by the enzyme was

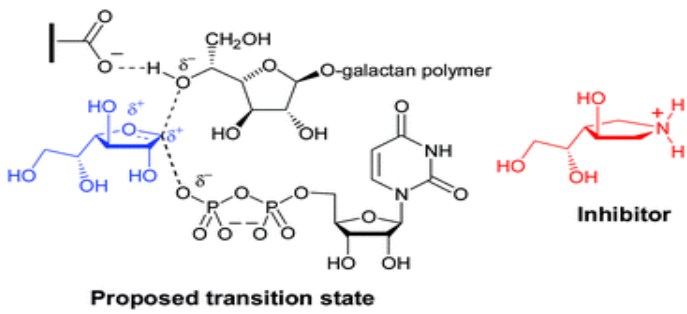
evaluated crystallographically and confirmed that tetrahedral phosphinic acid moiety acts as analogue of transition state of the enzymatic reaction.

Compounds with a positively charged amino group mimicking the carbocationic high-energy intermediate of S_N1 reactions, are also used in inhibitor design. A good example is the design and synthesis of an inhibitor of 3-deoxy-*D-manno* -2-oculosonate-8-phosphate (KDOP) synthase. This enzyme plays an essential role in the assembly process of the lipopolysachcharides of most Gram- negative bacteria, and is therefore an attractive target for the design of novel antibacterial drugs.



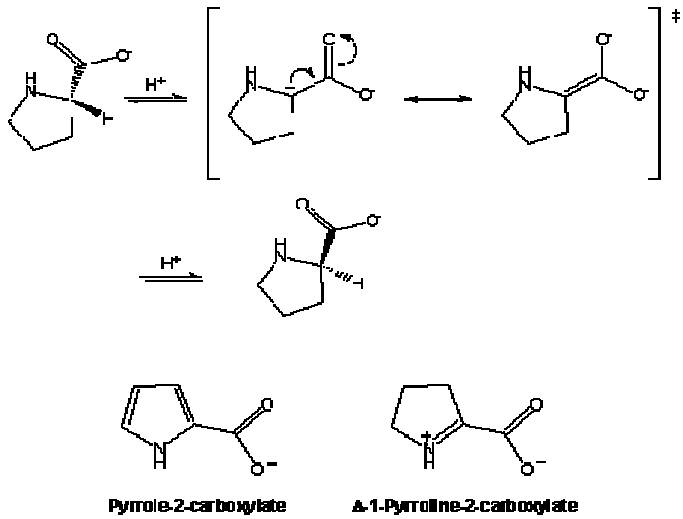
Mechanism of the reaction catalyzed by KDOP synthase and its transition state inhibitor

D-Galactan is an O-antigenic polymer with the repeat unit structure that is found in the lipopolysaccharide of *Klebsiella pneumoniae* and other Gram-negative bacteria. One of the enzymes involved in its synthesis is UDP-glalactosylsynthase. Similar approach as above was used for the synthesis of this enzyme inhibitors and thus compounds with potential antibacterial activity.



Positively charged transition state inhibitor of UDP-glalactosyl synthase

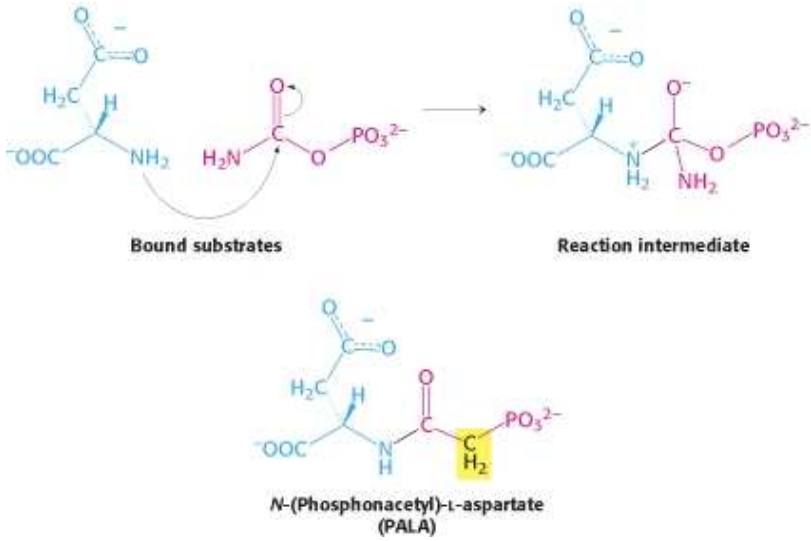
Another simple example is proline racemase. This enzyme catalyzes the conversion of *L*-proline into its *D*-isomer and generates a planar transition state in the course of this interconversion. Therefore, compounds that have planar structure are going to be potent inhibitors of the enzyme. Thus, pyrrole-2-carboxylate and Δ-1-pyrroline-2-carboxylate are both competitive inhibitors of the enzyme from *Clostridiium stiklandii* and bind with 160-foldgreater affinity than the substrate to the protein.



Reaction catalyzed by proline racemase proceeds through planar transition state. Thus planar analogues of substrate are good inhibitors of the enzyme.

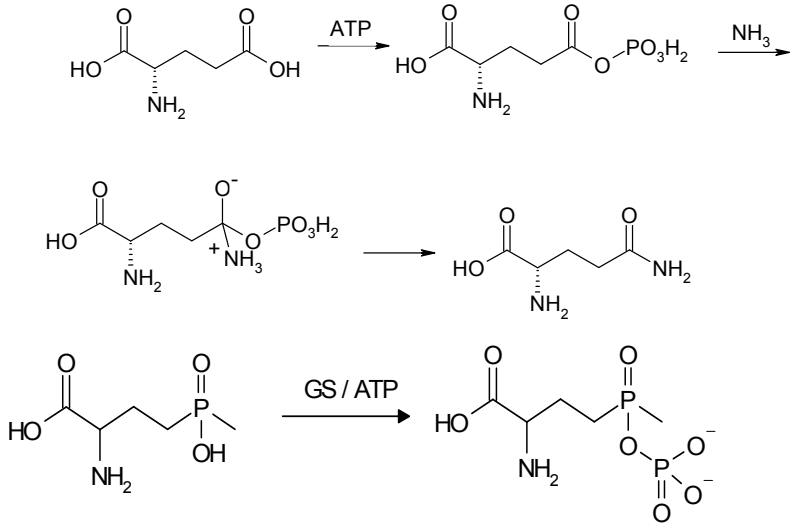
Non-typical inhibitor of this class is *N*-phosphonoacetyl-*L*-aspartate (PALA) invented as far as in 1971. It is an inhibitor of aspartate transcarbamoylase, an enzyme that catalyzes the first step in the biosynthesis of pyrimidines that are components of nucleic acids. The reaction catalyzed is the condensation of aspartate and carbamoyl phosphate to form *N*-carbamoylaspartate and orthophosphate. PALA is a bisubstrate analog (an analog of the two substrates) that resembles an intermediate along the pathway of catalysis. It is a potent competitive inhibitor of aspartate transcarbamoylase, which binds to and blocks its active sites. It was discovered on the premise that it will act as a strong anticancer agent. However, its *in vivo* activity does not reflect the inhibitory potency at the enzymatic level. This is most likely due to the

poor transportation of this compound through cell membranes.



Reaction catalyzed by aspartate carbamoyltrasferase and its inhibitor PALA. Taken from http://www.ncbi.nlm.nih.gov

An interesting combination of suicide substrate with transition state analogue is *L*-phosphinithricin (also known as glufosinate, sold under trade names BASTA, Buster and Liberty), a potent inhibitor of glutamine synthetase isolated from *Streptomyces*. Glutamine synthetase is of importance in ammonia assimilation and regulation of nitrogen metabolism, both in plants and bacteria. Phosphinothricin is commonly used in plant molecular biology and plant tissue culture. This herbicide acts as suicide inhibitor, which is due to phosphorylation of the inhibitor by the enzyme, which in turn results in transition state analogue of the second reaction catalyzed by the enzyme, namely amonolysis of the mixed anhydride formed between phosphinotrhicin and phosphate. Inhibition of glutamine synthetase and subsequent accumulation of ammonia cause plant death.



Mechanism of the reaction catalyzed by glutamine synthetase and activation of phosphinothricin yielding transition state analogue of the second reaction.

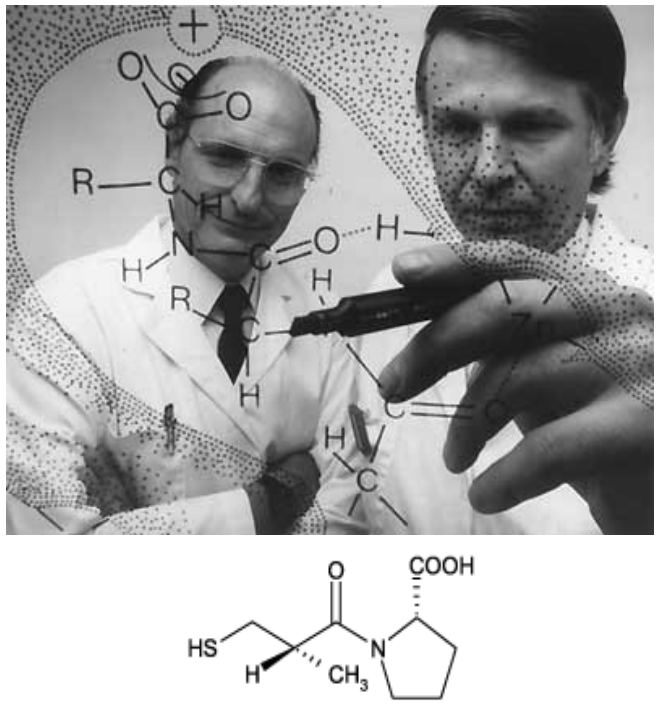
Chapter 11. Inhibitors of metalloenzymes

Metalloproteins have many different functions in cells since they are involved in a variety of biologically important processes, including oxygen transport, biosynthesis, electron transfer, biodegradation, drug metabolism, proteolysis, and hydrolysis of amides and esters, environmental sulfur and nitrogen cycles, and disease mechanisms. Indeed, about one quarter to one third of all proteins require metals to carry out their functions.

Metalloenzymes are a diverse class of enzymes that require a catalytic metal ion for activity. These enzymes are widely distributed in nature. They catalyze a variety of reactions ranging from electron transfer and hydrolysis of peptide bond to the insertion of oxygen into carbon-hydrogen bonds. In performing these action the metal can be alone, in a cluster, or associated with a porphyrin. The metal ion-dependent mechanism of these enzymes makes them attractive targets, as many potent inhibitors of metalloenzymes have been described. The introduction to the inhibitor molecule metal binding moieties is a major technique to obtain their potent effectors.

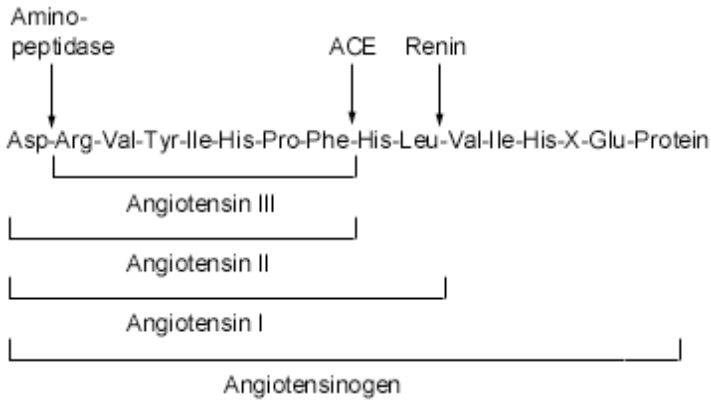
Captorpril is the first drug obtained basing on mechanism of enzymatic action. It is an

inhibitor of angiotensin converting enzyme (ACE), thus being a potent antihypertensive agent. David Cushman and Miguel Ondetti share the 1999 Albert Lasker Award in Clinical Medical Research for the discovery and development of captopril, the first orally active ACE inhibitor—the parent drug to what is today a pharmaceutical treasure trove of newer drugs in cardiovascular medicine



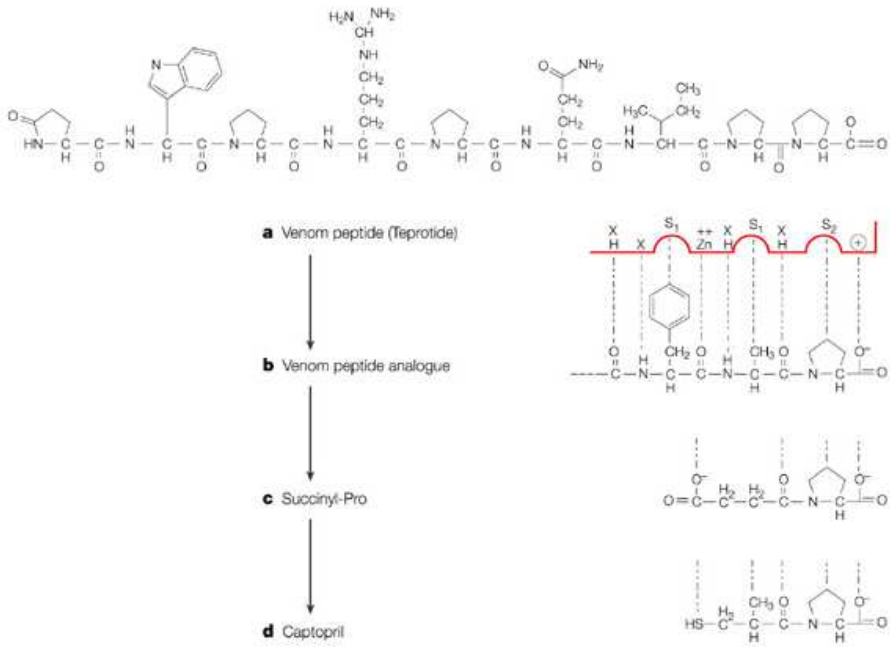
D. Cushman and M. Ondettii and the structure of captopril.
Taken from http://www.bms.com/ourcompany

Angiotensin converting enzyme is an exopeptidase that participates in the body's rennin-angiotensin hormone system, which regulates blood pressure and water (fluid) balance. When blood volume is low, the kidneys secrete renin. Renin stimulates the production of angiotensin, which causes blood vessels to constrict, resulting in increased blood pressure. Angiotensin also stimulates the secretion of the hormone aldosterone from the adrenal cortex. Aldosterone causes the tubules of the kidneys to increase the reabsorption of sodium and water into the blood. This increases the volume of fluid in the body, which also increases blood pressure.



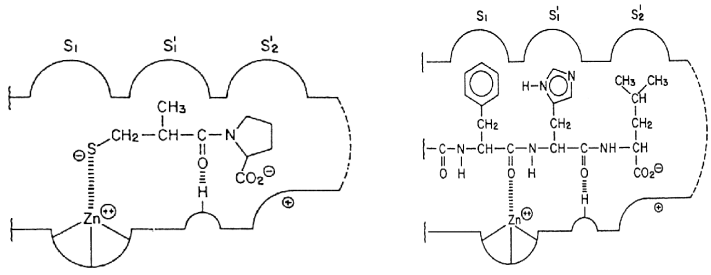
Stepwise cleavage of human angiotensinogen and the enzymes involved in this process

The breakthrough in the development of captopril came from the finding that one of the peptides extracted from the venom of a Brazilian viper, *Bothrops jararaca*, by Brazilian pharmacologist Sergio Ferreira, inhibit ACE, thus preventing formation of the hypertensive peptide angiotensin II. This might play some role in enhancing the lethal action of the snake's venom, which kills quickly by causing a rapid, catastrophic plunge in blood pressure. Within a couple of years Cushman and Ondetti isolated several peptides including a nine-amino-acid peptide, Teprotide was an effective ACE inhibitor, but it was also a large molecule - too large to be absorbed when given orally. Although it did lower blood pressure in hypertensive patients who received the drug by injection, proving once and for all the medical utility of ACE inhibition. Reduction of teprotide structure and introduction of thiol moiety able to complex zinc ion present in the active site site of the enzyme led to captopril. This is a small, orally active compound, easily absorbed into the bloodstream and therefore an ideal drug.

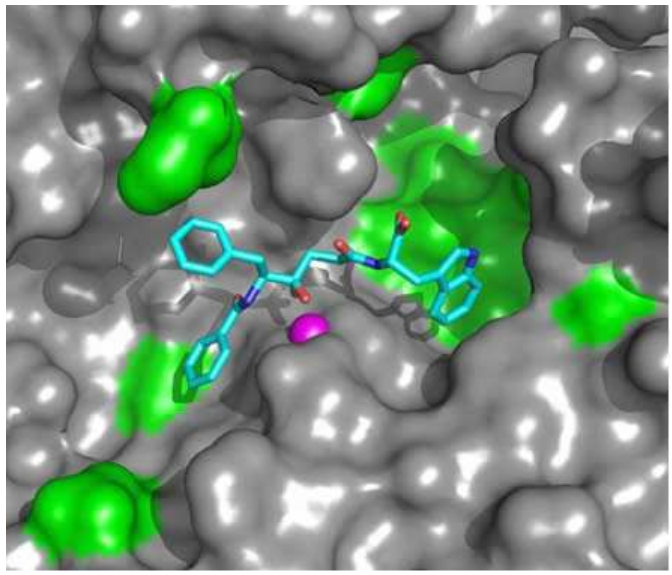


Design of captopril by stepwise reduction of the structuie of teprotide.
Taken from http://www.nature.com

The following years brought hundreds of ACE inhibitors designed basing on the structure of angiotensin converting enzyme active site and the neighboring binding domains, which form the scaffold for the design process. Today millions of patients are treated with structurally variable inhibitors of this enzyme.

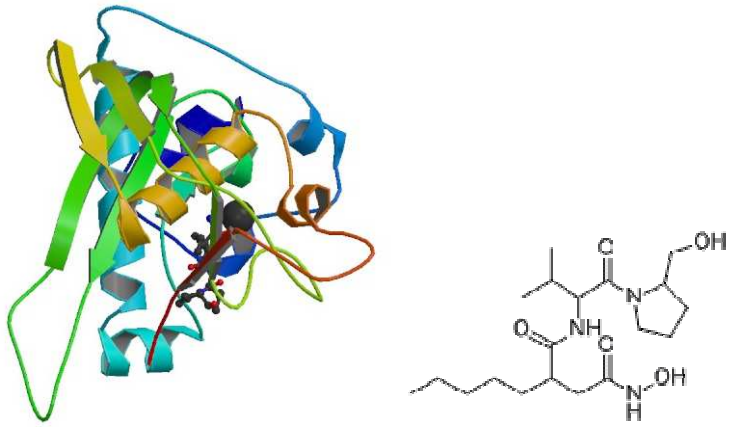


Binding domaind of ACE and binding modes of captopril (left panel) and substrate (right panel)



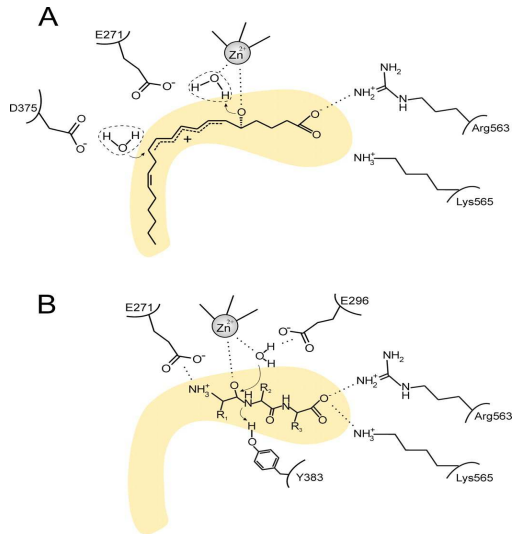
An example of inhibitor (phosphinic acid) bound within the domains of ACE.

Inhibitors of methionine deformylase might be considered as another good example. The activity of the enzyme rely on ribosomal protein synthesis in prokaryotes, where new peptides are initiated with an N-formylated methionine. This residue is commonly removed in a multi-step process beginning with deformylation. Inactivation of deformylase activity is lethal and developes as anticancer and antibiotic strategy. Enzyme employs a metal ion as a Lewis acid catalyst of hydrolysis (predominantly Zn^{2+}, or Ni^{2+}, however Fe^{2+} - containing form is 1000 x more active). Therefore the short peptides designed to ensure the selectivity against this enzyme and containing moieties are able to bound these ions tightly and are good inhibitors of the enzyme. Actinonin, isolated in 1962 from actinomycetes, is an example of such inhibitor. Actinonin, being a hydroxamic acid and thus very strong complexing agent for zinc ions, also appeared to be a strong inhibitor of leucine aminopeptidase.



Structure of actinonin and binding mode of this inhibitor by methionine deformylase. Inhibitor is shown as ball and sticks and zinc ion is shown as a grey sphere.

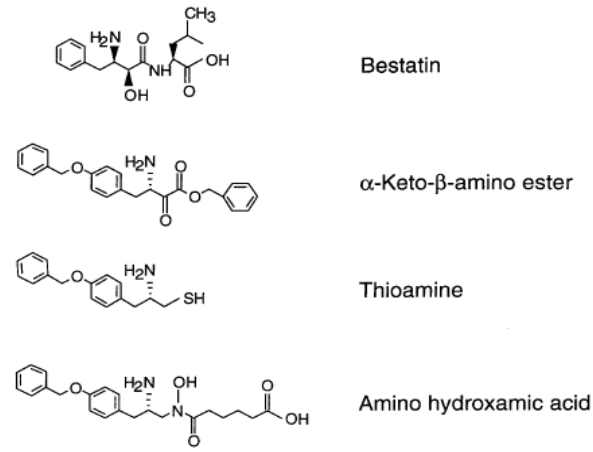
The leukotrienes are a family of lipid mediators that play important roles in a variety of allergic and inflammatory reactions. Leukotriene A_4 hydrolase (LTA4H) is a zinc-dependent epoxide hydrolase involved in synthesis of leukotriene B_4, which is a potent leukocyte chemoattractant and is involved in a number of inflammatory processes. Sequence comparisons between LTA4H and several zinc hydrolases such as aminopeptidase N and thermolysin, led to the discovery of identities in their catalytic zinc sites.



Leukotriene hydrolase acting as (A) epoxide hydrolase and (B) aminopeptidase

Thus, it may be also considered as an aminopeptidase. If so, the typical inhibitors of

aminopeptidases might be applied as inhibitors of this enzyme.



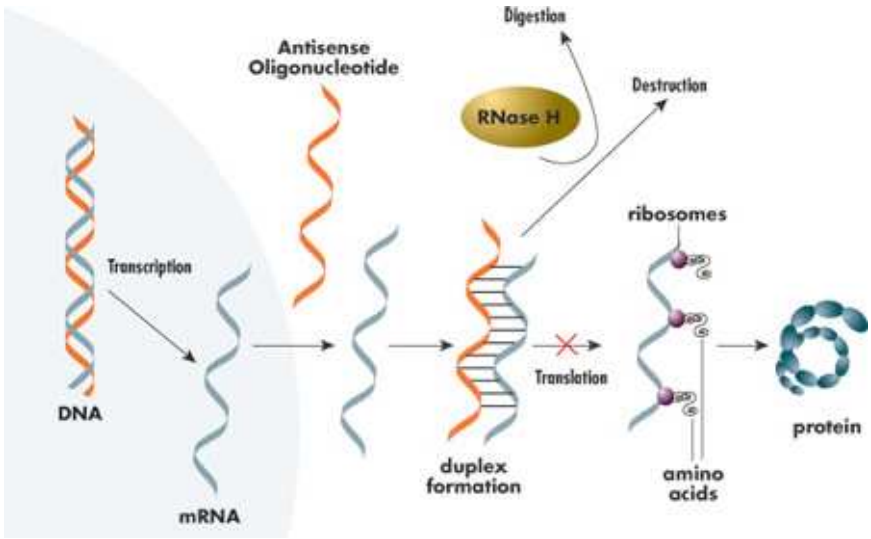
Potent inhibitors of leukotriene hydrolase. This set includes also bestatin, the most commonly used inhibitor of aminopeptidases.

This is indeed the fact and the structures of the most potent inhibitors of leukotriene hydrolase were designed using the techniques commonly applied for the desidnof inhibitors of aminopeptidases. The most important was the design of functional moiety effectively binding zinc ion present in the active site of the enzyme.

Chapter 12. Antisense and related therapies

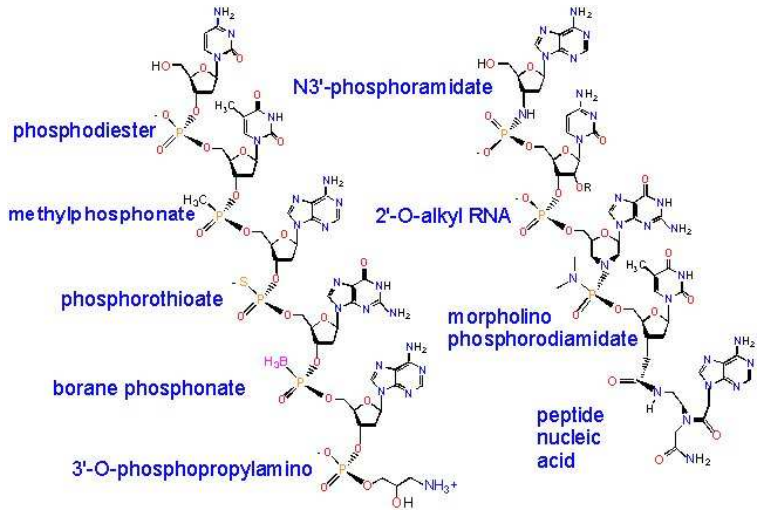
Antisense therapy is a method of treatment for genetic disorders or infections. Antisense therapy is not strictly a form of gene therapy, but is a genetically-mediated therapy and is often considered together with other methods**.** When the genetic sequence of a gene causative of a particular disease is known, it is possible to synthesize a strand of nucleic acid or its that will bind to mRNA produced by that gene and inactivate it. Such a synthetic strand is effectively turning that gene "off". At present, the main focus of antisense therapy is in oncology and involves the use of approximately 20 nucleotides (oligonucleotide) synthesized to be complementary to the specific “sense” (5’ to 3’orientation) mRNA sequence responsible for coding of the targeted protein.

Short antisense strands of DNA can be introduced into cells. Once introduced into a cell, the "antisense" oligonucleotide hybridizes to the corresponding mRNA sequence through Watson-Crick binding, forming a heteroduplex. When the duplex is formed translation of the protein coded by the sequence of bound mRNA is inhibited. Antisense DNA is currently an approved therapy for cytomegalovirus infections of the eye, under the trade name Vitravene. Vitravene targets two different viral proteins. Antisense DNA is also being explored for therapy of HIV, some cancers, and other diseases.



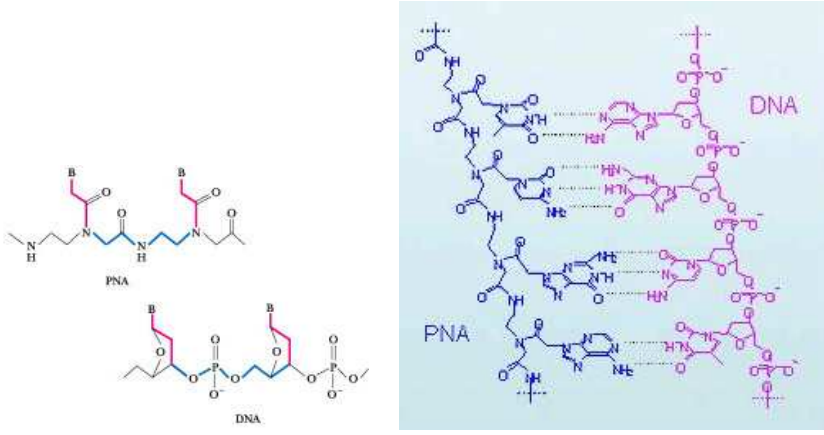
Schematic presentation of antisense therapy.

Nucleotides are normally linked together through 5'-3' phosphodiester bonds and are unstable in body fluids. However, chemical synthesis can modify either the orientation of the linkages or the chemical structure of the linkage. Methylphosphonate-linked DNA oligonucleotides were the first to provide resistance to enzymatic degradation but were found to afford limited efficacy and poor aqueous solubility. In an effort to overcome such limitations, a number of other structural types have been developed. One of these, phosphorothioate-linked have come to dominate the antisense field, being easily prepared and moderately resistant to enzymatic degradation while providing higher efficacies and much better aqueous solubility



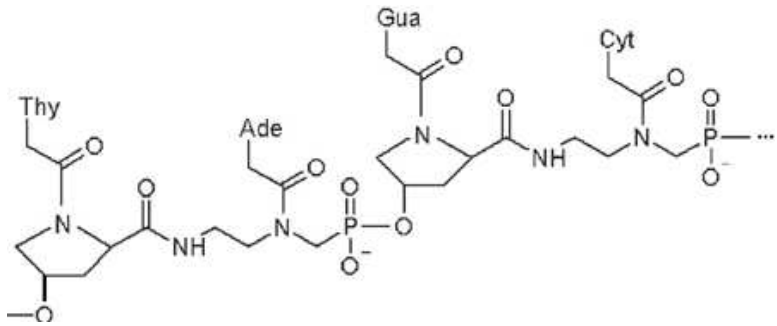
A variety of chemically modified antisense oligonucleotides shown as two strains of hybrid compounds. Taken from http://www.dddmag.com/article-Antisense-technology-knocked-down-or-knocked-out.

Peptide nucleic acids (PNAs) form the next class of antisense substrances. They were designed in order to mimic an oligonucleotide binding to double stranded DNA via Hoogsteen base pairing in the major groove. Thus the nucleobases of DNA were retained, but the deoxyribose phosphodiester backbone of DNA was replaced by a pseudo-peptide backbone that, according to computer model building, was homomorphous with the DNA backbone. In theory, a neutral (peptide) backbone should improve the triplex binding capability.



Comparison of the structures of PNA and DNA

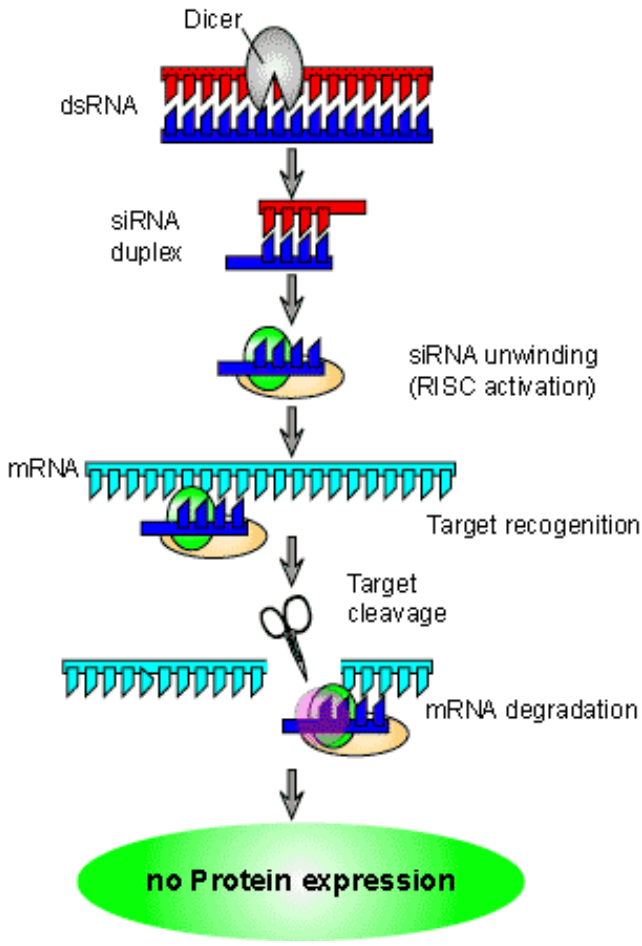
In an attempt to improve physico-chemical and biological properties of peptide nucleic acids, particularly water solubility and cellular uptake, the synthesis of a wide variety of phosphono peptide nucleic acids was accomplished and their usefulness was evaluated showing that they are promising mimetics of antisense nucleotides. The availability of such a wide variety of structures enables also to construct various hybrids of antisense drugs.



Hybrid antisense peptide nucleic acid composed of hydroxyprioline derivative and phosphono peptide nucleic acid.

Small pieces of nucleic acid, known as siRNAs (short interfering RNAs), can turn off a production of specific proteins, a property that makes them one of the more promising new classes of anticancer drugs in development. Indeed, siRNA-based anticancer therapies, both delivered to tumors as intact compounds or using nanoparticles, have begun human clinical trials.

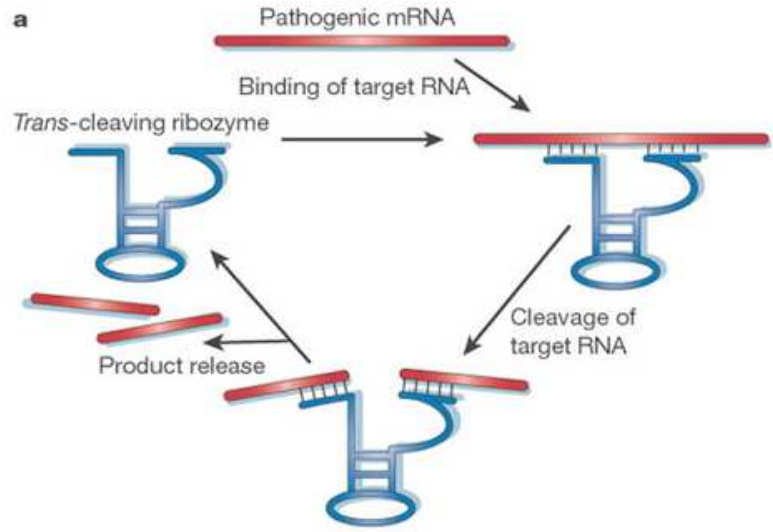
SiRNAs is a class of double stranded RNA molecules composed of 20-25 nucleotides. They interfere with the expression of specific gene being able to knock down essentially any gene of interest by degrading appropriate RNA. Because disease processes also depend on the activity of multiple genes, it is expected that in some situations turning off the activity of a gene with siRNA could produce a therapeutic benefit. However, applying siRNAs to living animals, especially humans, poses many challenges and therefore, despite many efforts, such a therapy has not yet been introduced to the practice.



siRNA mediated cleavage of RNA molecule. Dicer is an endoribonuclease, which produces siRNAs.

Ribozymes are RNA molecules that possess the dual properties of RNA: sequence-specific recognition and site-specific cleavage of sugar-phosphate bonds of other RNA molecules. These properties provide powerful tools for studies requiring gene inhibition, when the DNA sequence is known. Being capable of targeting and cleaving specific RNA molecules they appear to offer a promise in the development of experimental treatments for a variety of diseases ranging from inborn metabolic disorders to viral infections and acquired diseases such as cancer. Ribozymes can be used both to downregulate and to repair pathogenic genes. In some cases, short-term exogenous delivery of stabilized RNA is desirable, but many treatments will require

viral-mediated delivery to provide long-term expression of the therapeutic catalyst. Although ribozymes are exciting candidates for human therapy, none of them has been introduced yet.



Mechanism of RNA cleavage mediated by ribozymes

Afterword

Drug design, sometimes also referred to as rational drug design, is an inventive process of finding new medications based on the knowledge of the biological target. Most commonly it is a small organic molecule, which activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. The design of *in vitro* effective drugs is only the first step of the whole process of drug development. The next step is an elaboration of suitable systems for drug targeting and drug delivery. These are formulation technologies that modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. However, these problems are out of scope of this book.