Journal of Receptor and Signal Transduction Research 19 (1-4):15-39 (1999)

CHANCE FAVORS THE PREPARED MIND – FROM SERENDIPITY TO RATIONAL DRUG DESIGN

Hugo Kubinyi
Combinatorial Chemistry and Molecular Modelling, ZHF/G - A 30, BASF Aktiengesellschaft, D-67056 Ludwigshafen, Germany. hugo.kubinyi@msm.basf-ag.de

Dans les champs de l'obsérvation le hasard ne favorise que les ésprits préparés.

Louis Pasteur (1822-1895)

Introduction

In 1847, Pasteur presented two doctoral theses to the Faculty of Science in Paris, one in chemistry, on arsenite salts, the other one in physics, on the optical rotation of organic liquids. One year later, he became professor of physics in Dijon. In the same year he discovered that sodium ammonium tartrate crystallizes in two enantiomorphic forms. After mechanical separation of the different crystals, using tweezers under a microscope, their aqueous solutions rotated polarized light into different directions! The grand old man in the field of optical rotation, Jean Baptiste Biot (1774-1862), insisted that Pasteur had to repeat his experiments in public. Pasteur was successful because two fortunate circumstances worked together. First, he had selected sodium ammonium tartrate which is one of the very few salts of tartaric acid that forms enantiomorphic crystals which can be separated manually; second, he did his crystallization at temperatures below 26° C (79° F); at higher temperatures, only the racemate crystallizes [1].

Louis Pasteur was a master of experimental research. Being not so much interested in theory, he made many fundamental discoveries just by careful observation. In this context, Pasteur formulated in 1854, *"in the field of observation, chance only favors the prepared mind"*. Already hundred years earlier, Sir Horace Walpole (1717-1797), Earl of Oxford and member of the English parliament, had coined the term *Serendipity* for accidental discoveries. Walpole had a passion in writing and receiving letters and he kept copies of all his letters. To a friend, Sir Horace Mann, an English envoy to Italy, he wrote 848 letters and received the same number of replies. In one of these letters, dated January 28, 1754, Walpole wrote about a *"silly fairy tale, called The Three Princes of Serendip; ... as their highnesses travelled, they were always making discoveries, by accidents and sagacity, of things which they were not in quest of"* (Serendip, old name for Ceylon, Sri Lanka) that had made a profound impression on his life. The tale described the fate of three princes who left their home to travel through the world. Rarely they found the treasures they were looking for but ran into other ones equally great or even greater which they were not seeking [2,3].

Serendipity in Drug Research

Accidental discoveries always played an important role in science [1], especially in the search for new drugs [4-8]. Even if we do not count the traditional evaluation of plants, animal toxins and minerals for therapeutic potential, in ancient history, and the more or less systematic screening of synthetic compounds in our century, the number of serendipitous findings in drug history is legion (Table 1).

Table 1. Incomplete list of serendipitous discoveries in drug research.

Compound	Accidental Discovery	Ref.
Acetanilide	tested as internal antiseptic (instead of naphthalene)	4,8
Acetylsalicylic acid	irreversible enzyme inhibitor (*vs*. salicylic acid prodrug)	4,8
Aminoglutethimide	breast cancer treatment (instead of antiepileptic)	4
Amphetamine	stimulant (instead of nasal decongestant)	4
Chloral hydrate	prodrug of trichloroethanol (instead of chloroform)	4,8
Chlordiazepoxide	tranquillizer (unexpected chemical rearrangement)	1,4,6-9
Chlorpromazine	neuroleptic (tested to prevent surgical shock)	1,4,7,8
Cinnarizine	cardiovascular (predominant to antihistaminic) activity	4
Cisplatin	cytotoxic effect of electrolysis product	4
Clonidine	antihypertensive (instead of nasal decongestant)	4,6,8
Cromoglycate	antiallergic (accidental formation of chromone dimer)	4
Cyclosporin	immunosuppressant (instead of antifungal agent)	1,4
Dichloroisoprenaline	ß-blockade (instead of bronchodilation)	4
Dicoumarol	fatal cattle poisoning (bleeding) by moldy hay	4,5,8
Diethylstilbestrol	estrogenic impurity of anol (dimerization product)	4
Diphenhydramine	allergy treatment caused prevention of travel sickness	4,5,8
Diphenoxylate	antidiarrhoic (instead of analgesic)	4
Disulfiram	hypersensitivity to alcohol	5,8
Ether	anesthetic activity in inhalation party	1,4,8
Etomidate	anesthetic (instead of chemotherapeutic) activity	4
Griseofulvin	growth inhibition of conifers on certain soils	4
Guanethidine	antihypertensive (instead of antitrypanosomal drug)	4,6
Haloperidol	neuroleptic (instead of analgetic) activity	4,5,8
Heparin	deterioration of lipid coagulant unmasked anticoagulant	4
Imipramine	antidepressant (instead of neuroleptic) activity	1,4,5,8
Iproniazid	antidepressant (instead of tuberculostatic) activity	4,5,8
Isoniazid	tuberculostatic activity of organic intermediate	4,5,8
Levamisole	immunomodulating (instead of antiparasitic) agent	8
Lithium carbonate	antidepressant activity of lithium urate	1,4,7,8
Lysergide (LSD)	hallucinogenic (instead of cardiovascular) activity	4,7,8
Meprobamate	tranquillizer (instead of muscle relaxant)	1,4,7
Merbaphen	diuretic activity (of an antisyphilitic agent)	4,8
Methaqualone	hypnotic (instead of antimalarial activity)	4
Mifepristone	antiprogesterone (instead of glucocorticoid) activity	4
Naftifine	antifungal rearrangement product of CNS drug	4,8
Nalorphine	antagonism instead of respiratory stimulation	4
Nitrogen mustard	cytotoxicity observed after ship bombardment	1,5,8
Nitroglycerin	antianginal activity (headache after inhalation)	4
Nitrous oxide	accidental wounding in laughing gas session	1,4,8
Norethynodrel/Mestranol	estrogenic impurity in the first oral contraceptive	4
Penicillin	antibiotic activity of *Penicillium* infection	1,4,5,8
Pethidine (meperidine)	morphine agonist (instead of spasmolytic)	4,5,8
Phenylbutazone	antiinflammatory activity of solubility enhancer	4,8
Phenolphthalein	laxative (tested as label for cheap wines)	4,8
Praziquantel	antiparasitic agent (instead of antidepressant activity)	8
Prednisone	bacterial oxidation produced highly active analog	4
Propafenone	antiarrhythmic (instead of ß-blocker)	4
Sulphamidochrysoidine	prodrug of sulfanilamide (active only *in vivo*)	1,4,5,8
Sulfonamides, various	diuretic and antidiabetic side effects	4,5,8
Tamoxifen	antiestrogenic activity of *cis*-isomer	4
Urethane	hypnotic activity (instead of alcohol prodrug)	4,8
Valproic acid	anticonvulsant (solubility enhancer for various drugs)	4
Warfarin	low acute toxicity of rat poison in attempted suicide	5,8,10

"Ein glücklicher Zufall hat uns ein Präparat in die Hand gespielt" (a lucky accident played a new drug in our hands) are the first words of a publication which describes the fortunate discovery of the fever-reducing activity of acetanilide. Erroneously this compound was clinically tested, instead of naphthalene that should have been investigated as an intestinal worm-killing agent [4,8].

The two best known examples of serendipitous findings are the discovery of the antibiotic effect of a certain *Penicillium* strain by Sir Alexander Fleming, which led to the development of penicillin and its synthetic derivatives [1,4,5,8], and the discovery that Chlordiazepoxide, which resulted from an unexpected chemical rearrangement, is a potent tranquillizer [1,4,6-9]. The latter example is also one of the very rare cases that the first lead became a blockbuster drug.

The hallucinogenic activity of Lysergide (LSD) is another well-known serendipitous discovery. Albert Hofmann synthesized lysergic acid diethylamide to combine cardiovascular and respiratory stimulatory effects. First pharmacological experiments did not show any valuable effects. Five years later, he prepared the compound once again; this time he experienced hallucinations, after accidental intake or inhalation of minute amounts of this highly potent compound [1,4,7,8].

Acetylsalicylic acid was originally designed as a prodrug of salicylic acid to treat headache, fever and rheumatic diseases. Much later it turned out to be an irreversible cyclooxygenase inhibitor, preventing blood coagulation by the inhibition of thrombocyte aggregation [4,8].

The potent antitumor compound Cisplatin was discovered when biophysicists investigated the effect of an electric current on the growth of *Escherichia coli*. A careful inspection of the reasons for the observed cytotoxic effect led to the surprising result that it was due to the action of ammonium and chloride ions on the platinum electrode, forming Cisplatin in the electrolysis medium [4].

Cyclosporin was developed because of its antifungal activity. Sandoz was already going to stop the program when the compound turned out to be an immuno-suppressant, highly valuable to prevent the rejection of organ transplants [1,4].

The story of the anticoagulants Dicoumarol and Warfarin is full of serendipitous findings. First, cattle bleeded to death after they were fed with moldy hay. The toxic agent Dicoumarol was isolated and introduced into human therapy. Because of its narrow therapeutic range and its frequent side effects it was abandoned after a short period. The Wisconsin Alumni Research Foundation developed the dicoumarol analog Warfarin as a rat poison. New clinical trials started when a US army cadet unsuccessfully attempted to commit suicide. Warfarin is now the drug of choice to protect against stroke and other acute thrombotic events [5,8,10]. Recently it was recognized as a valuable lead for the development of potent HIV protease inhibitors.

Diethylstilbestrol was a minor impurity of the *p*-allylphenol anol; only the dimerization product proved to be estrogenic [4]. The first oral contraceptive Norethynodrel contained a minor estrogenic impurity when it was clinically tested. About 1% of the unreacted starting material Mestranol turned out to be a prodrug of ethinylestradiol [4]. Pure Norethynodrel caused some undesired pregnancies; only the fortuitous combination of Norethynodrel and Mestranol proved to be a safe contraceptive.

The antidepressant activity of Lithium salts was discovered because it was suspected that manic-depressive illness could be caused by an abnormal metabolism of uric acid. Application of a water-soluble salt, lithium urate, led to the serendipitous discovery of the beneficial effect of lithium salts [1,4,7,8]. Phenylbutazone and Valproic acid were designed as solubility enhancers for other drugs. However, both compounds turned out to be valuable drugs on their own [4,8].

Phenolphthalein was discovered to be a potent laxative when it was tested as a possible marker to label cheap Hungarian wines [4,8]. The three most important artificial sweeteners, saccharine, cyclamate and aspartame, were also serendipitous

discoveries. Chemists experienced the sweet taste when licking their fingers or smoking a cigarette [1,8].

An important discovery in receptor research was also a case of serendipity. The second messenger cyclic AMP was discovered in 1957, adenylate cyclase in 1958. Fluoride ions activated adenylate cyclase but the mechanism of this surprising stimulation of enzymatic activity could not be explained for the next 24 years. Adenylate cyclase originally consisted of two components, the cyclase and a regulatory unit, the G protein. Fluoride activation of the G protein was observed in disposable glass tubes or in the presence of tap water, but not with distilled water in plastic tubes [11]. Further systematic investigation of these confusing results made clear that fluoride ions activate the G proteins only in the presence of minute amounts of aluminum ions. Whereas a GDP-G protein complex is inactive, the GTP-G protein or GDP-fluoroaluminate-G protein complexes activate adenylate cyclase: it was supposed that the fluoroaluminate ion mimics the outer phosphate group of GTP [11], a hypothesis, which was confirmed in 1994 by the X-ray crystallographic investigation of a GDP-fluoroaluminate-Gα protein complex [12]. In the presence of millimolar fluoride concentrations and trace amounts of aluminum ions (also beryllium or large concentrations of magnesium ions) many other phosphatases, phosphorylases, and kinases, e.g. actin, tubulin, and myosin, form ground state or transition state analog complexes by assembling with the fluoroaluminate ion.

Protein 3D Structure-Based Drug Design

Allosteric effectors of Hemoglobin and Dihydrofolate reductase inhibitors, related to Trimethoprim, were the very first biologically active molecules that were derived from protein 3D structures [13]. The antihypertensive Captopril (Squibb; Figure 1) was the first therapeutically used drug that resulted from a structure-based design [14]. The 3D structure of Angiotensin-converting enzyme was (and still is) not available; thus, a binding site model was derived from the related dipeptidase Carboxypeptidase A and used for the design.

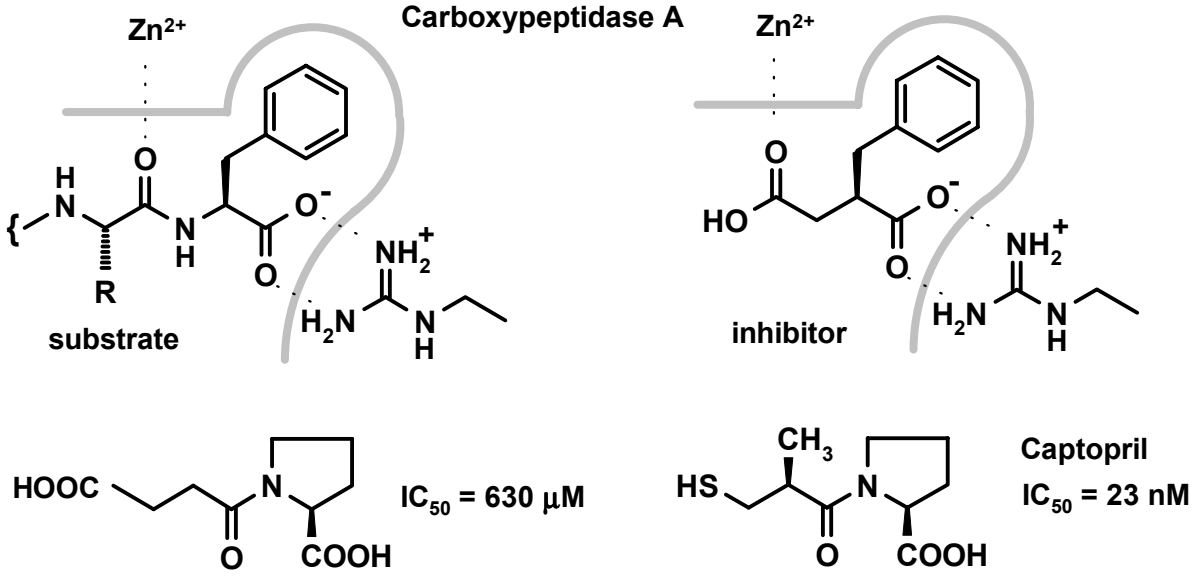

Figure 1. The structure of Captopril was designed with the help of a model which was derived from the 3D structure of Carboxypeptidase A. The structures below show the 30,000-fold improvement in inhibitory activity, from the first lead *N*-succinoyl-prolin to Captopril.

Dorzolamide, an anti-glaucoma agent (Merck & Co; Figure 2), was the first drug in human therapy (market introduction 1995) which resulted from a mere structure-based design [15]. Also the HIV protease inhibitors Saquinavir (Hoffmann-La Roche), Indinavir (Merck & Co), Ritonavir (Abbott Laboratories), and Nelfinavir (Agouron Pharmaceuticals) resulted from structure-based design (Figure 3) [16].

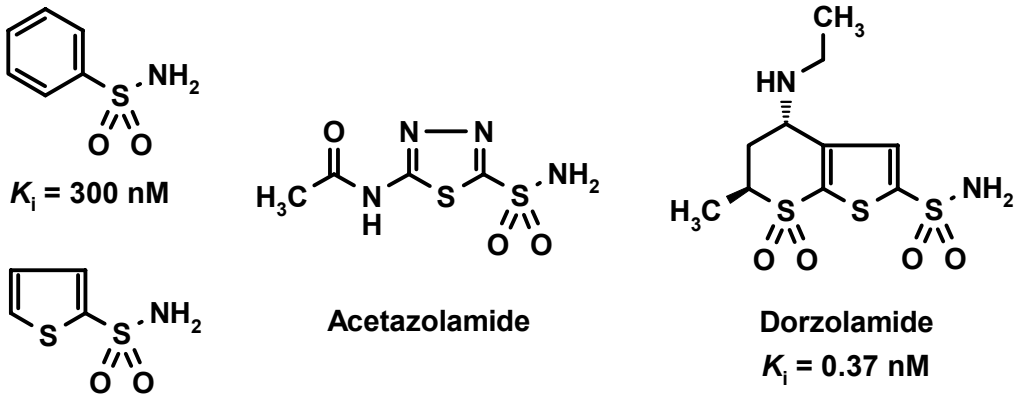

Figure 2. Benzene and thiophene sulfonamides are moderately active inhibitors of Carbonic anhydrase. Acetazolamide has only systemic activity. Dorzolamide, being about 3 decades more active than the original lead, resulted from a structure-based design; it is topically applied in the form of eye drops.

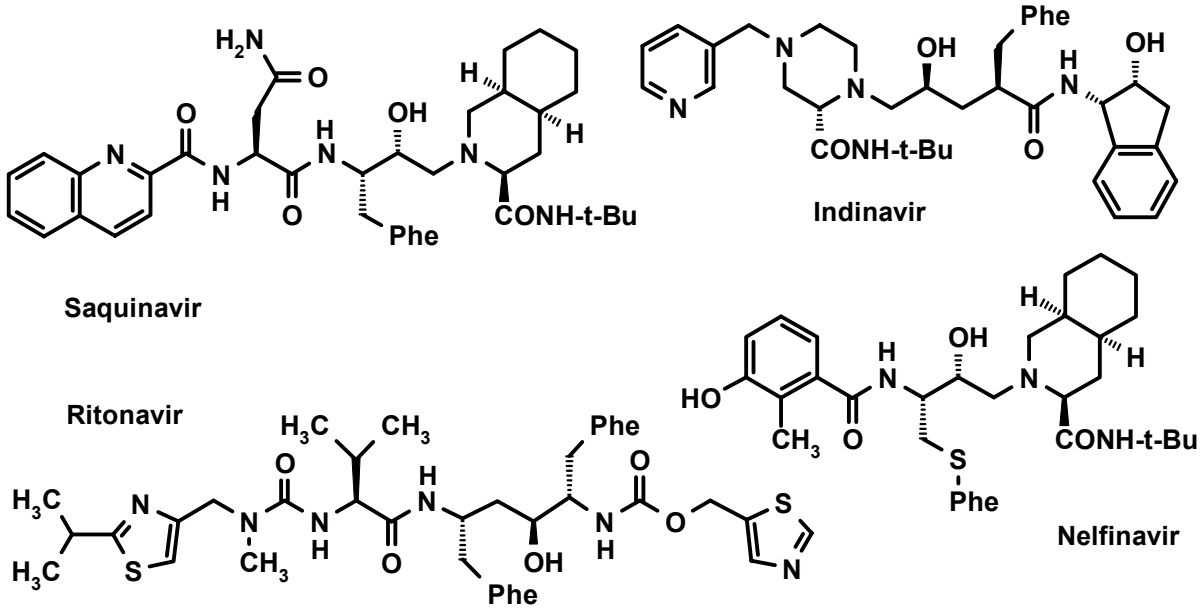

Figure 3. The HIV-1 protease inhibitors Saquinavir, Indinavir, Ritonavir, and Nelfinavir, all being developed by structure-based design, were introduced into human therapy in the years 1995-1997.

Neuraminidase inhibitors offer a new chance for the treatment of influenza. Analysis of the 3D structure of the complex of neuraminidase with the weak inhibitor Neu5Ac2en (K_i = 1 μM) with the computer program GRID showed that the introduction of a guanidino group into the 4-position should enhance inhibitory activity. This was indeed the case; Zanamivir (Monash University; Figure 4) is not only highly active *in vitro* (K_i = 0.1 nM) [17,18] but also systemically available after nasal application. The drug is now in clinical development (Glaxo-Wellcome). Aromatic

analogs of Zanamivir gave a first hint that removal or replacement of the glycerol side chain could yield active analogs [19]. Synthesis of a carbocyclic Neu5Ac2en analog with a branched alkoxy residue produced the nanomolar neuraminidase inhibitor GS 4071 (IC_{50} = 1 nM); its orally active prodrug GS 4104 is in clinical development (Gilead Sciences/Hoffmann-La Roche; Figure 4) [20].

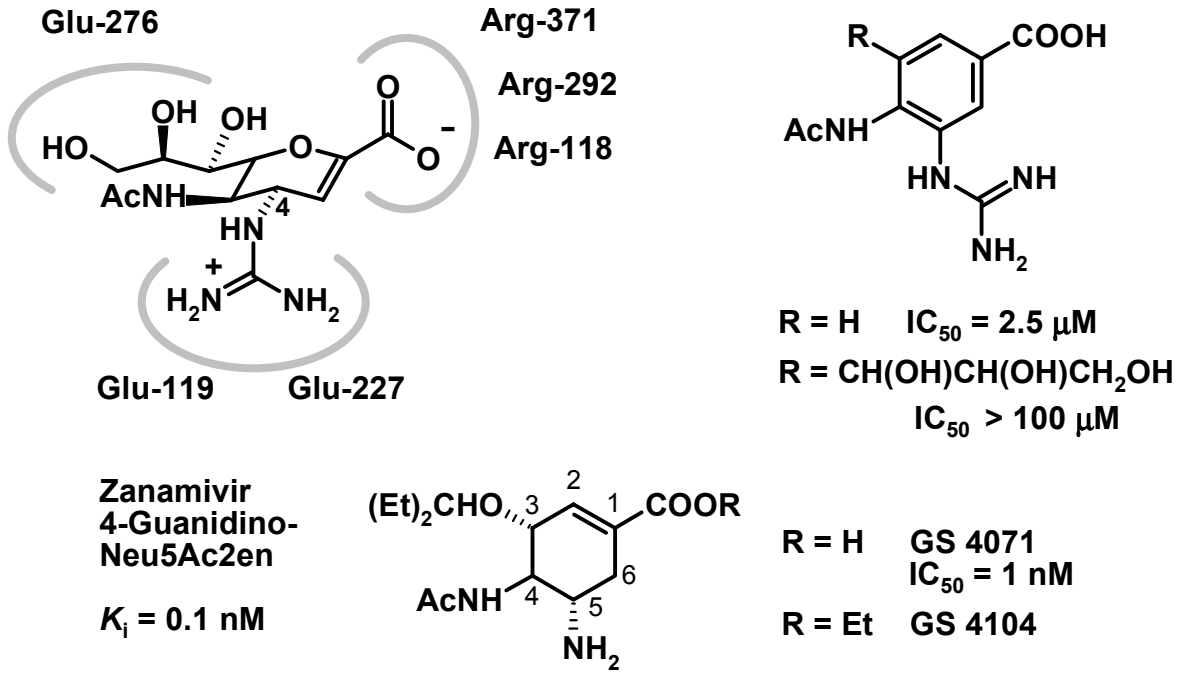

Figure 4. Design of the anti-influenza drugs Zanamivir and GS 4104.

Protein 3D structure-based approaches have been applied in the design of various enzyme inhibitors, e.g. Aldose reductase, Carbonic anhydrase, Cysteine protease, Dihydrofolate reductase, Elastase, Factor Xa, HIV protease, β-Lactamase, Matrix metalloprotease, Neuraminidase (sialidase), Protein kinase, Purine nucleoside phosphorylase, Renin, Reverse transcriptase, Thrombin, and Thymidylate synthase inhibitors, as well as in the design of FKBP-binding protein and Rhinoviral coat protein ligands [8, 10, 15, 16, 21-26].

Ligand 3D Structure-Based Drug Design

With the exception of ligand binding domains of some soluble cytosolic receptors, 3D structures of receptors with atomic resolution are not yet available. Whilst 3D models, derived from bacteriorhodopsin provide valuable information for site-directed mutation and for functional analysis, they are not yet suited for structure-based design. However, several receptor ligands and also inhibitors of enzymes with unknown 3D structures have been designed using the structural information from conformationally constrained ligands [8, 22, 25, 26].

Cholecystokinin (CCK) is a peptide hormone that exerts manifold activities within the central nervous system and in the intestinal tract. The CCK antagonist Asperlicin (IC_{50} = 1,4 μM; Figure 5) was isolated from the fungus *Aspergillus alliaceus*. Chemists recognized benzodiazepine- and tryptophan-related partial structures; broad structural variation, starting from this hypothesis, led to the much simpler analog Devazepide (MK-329, Merck & Co) that showed a more than 10,000-fold increase in affinity (IC_{50} = 80 pM) [8, 10, 14, 22, 27].

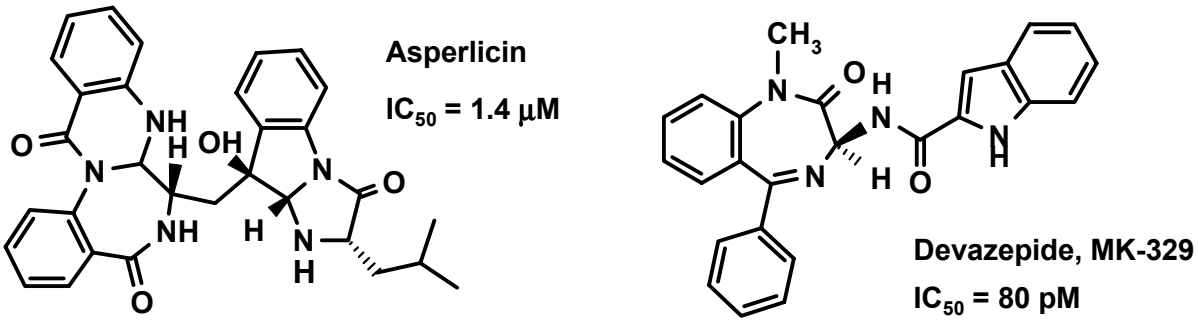

Figure 5. Design of the CCK antagonist Devazepide (MK-329) from the natural product Asperlicin.

A striking example for the potential of ligand 3D structure-based design is the research on integrin receptors, performed at SmithKline Beecham (Figure 6) [28-31]; α2bβ3- and αvβ3-integrin receptor antagonists offer interesting therapeutic potential as antithrombotics, anticancer agents, angiogenesis inhibitors and drugs for the treatment of osteoporosis. The natural ligands of the fibrinogen receptor (GPIIb/IIIa receptor, α2bβ3 receptor) and the vitronectin receptor (αvβ3 receptor) contain an identical binding motif, the RGD (arginine, glycine, aspartate) sequence.

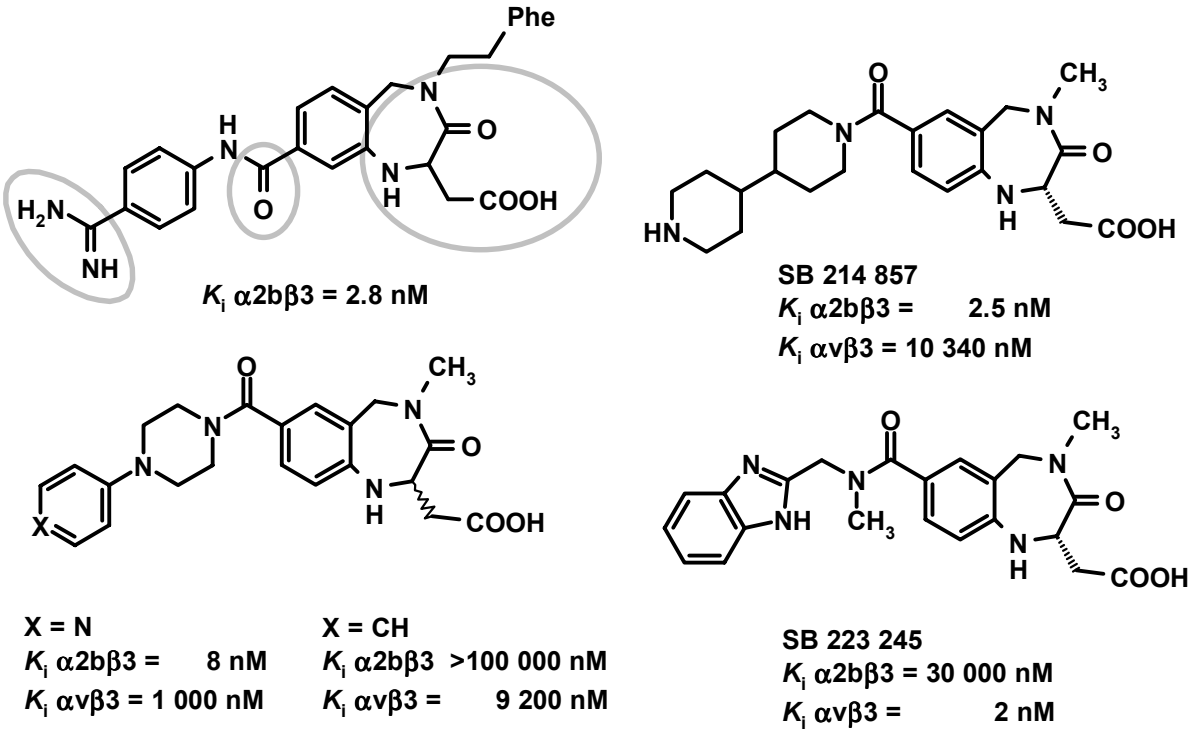

Figure 6. A peptidomimetic lead structure (upper left) was derived from the 3D structure of a cyclic RGD peptide; the basic side chain of Arg, the amide carbonyl of Gly, and the Asp of the RGD motif can still be recognized. The presence or absence of a nitrogen atom in the structurally related benzodiazepines (lower left) produces significantly different receptor selectivities. The α2bβ3-selective receptor antagonist SB 214 857 and the αvβ3-selective receptor antagonist SB 223 245 were derived from this observation.

Highly selective $\alpha 2b\beta 3$ and $\alpha v\beta 3$-selective receptor ligands resulted from the observation that cyclic peptides, bearing this motif in slightly different conformations, had different receptor selectivities. Whereas cyclo-(Arg-Gly-Asp-Phe-D-Val), RGDFv (v = D-Val), is a high-affinity ligand of the $\alpha 2b\beta 3$ receptor (K_i = 2 nM), its isomer RGDfV (f = D-Phe) is a specific $\alpha v\beta 3$ receptor antagonist (K_i $\alpha 2b\beta 3$ = 42,000 nM; K_i $\alpha v\beta 3$ = 10 nM). The further development of the receptor-selective antagonists SB 214 857 and SB 223 245 [28-31] is illustrated in Figure 6. Both analogs differ in their selectivity by nearly eight orders of magnitude, despite their close chemical relationship.

Combinatorial and Computational Approaches in Drug Design

In Part 3 of *"Gulliver´s Travels"*, Jonathan Swift (1667-1745) describes the Academy of Sciences of Lagado where, in addition to other curious inventors, the projectors in speculative learning reside [32]. One of the professors was employed *"in a project for improving speculative knowledge by practical and mechanical operations"*. With the help of an engine (Figure 7), *"the most ignorant person at a reasonable charge, and with little bodily labour, may write books in philosophy, poetry, politics, law, mathematics and theology, without the least assistance from genius or study"*.

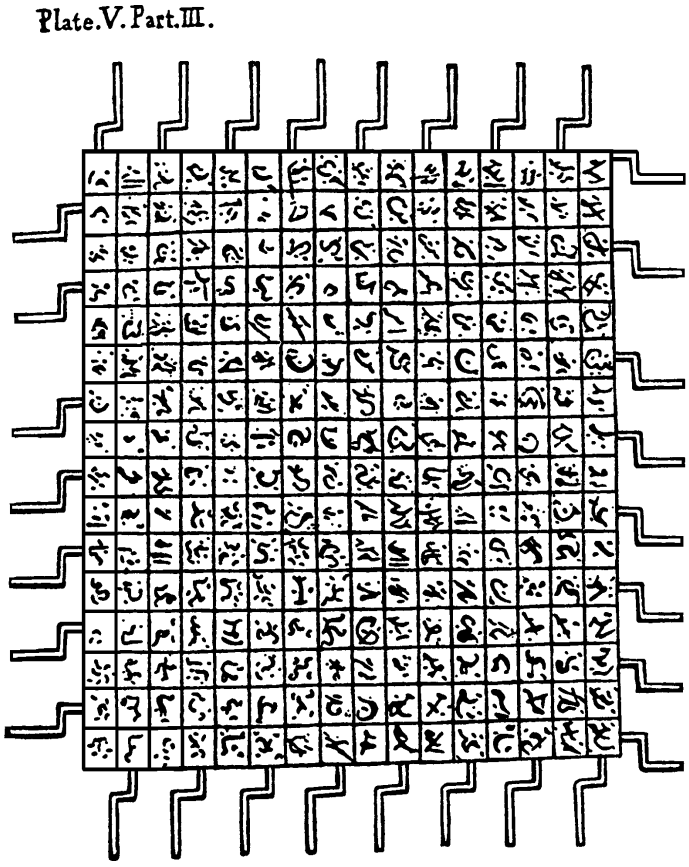

Figure 7. The frame for speculative learning is made up from pieces of wood, linked together by tires. A vocabulary of words in several moods, tenses and declensions, but without any order, is written on the squares of the wooden bits. The pupils of the professor turn around the handles and the disposition of the words is entirely changed. Then they *"read the several lines softly as they appeared upon the frame; and where they found three or four words together that might make part of a sentence, they dictated to the ... boys who were scribes. This work was repeated three or four times, and at every turn the engine was so contrived, that the words shifted into new places, as the square bits of wood moved upside down"*.

The professor showed several volumes of already collected broken sentences, which he intended to piece together *"to give the world a complete body of all arts and sciences; which however might be still improved, and much expedited, if the public would raise a fund for making and employing five hundred such frames .."*.

This seems to be the most amusing description of an evolutionary approach, by generating random sequences of words and selecting the best results by human

intuition. Raimundus Lullus (1235-1315; Ars combinatoria), Cornelius Agrippa (1486-1535), and other philosophers had already formulated such concepts earlier. Swift´s engine is supposed to ridicule a pamphlet, published in 1678 by the English mathematician John Peters, called *"Artificial Versifying: A New Way to Make Latin Verses"*.

Many more drug discoveries than listed in Table 1 are based on serendipitous observations. Screening, especially automated high-throughput screening (HTS), can be considered as a systematic approach to benefit from mere chance. Nowadays, combinatorial chemistry and screening *in computro* add further important components to this strategy. Whereas the very first syntheses of huge libraries of peptides, peptide derivatives, peptoids, and other peptidomimetics followed more or less Swift's principle of a random combination of building blocks, the design of focused libraries is now in the foreground [33-36].

A highly attractive NMR method has been developed for the stepwise construction of ligands from building blocks, the SAR by NMR method [37,38]. In this approach, libraries of small molecules are screened against a certain protein. Binding of ligands to a subsite is observed by shifts of the corresponding amide proton signals of the ^{15}N-labeled protein. In the next step, the protein is saturated with the highest affinity ligand for this site and a different library is screened to search for ligands, which bind to a proximal subsite. Both ligands are then combined with an appropriate linker to obtain a high-affinity ligand (Figure 8) [37-39].

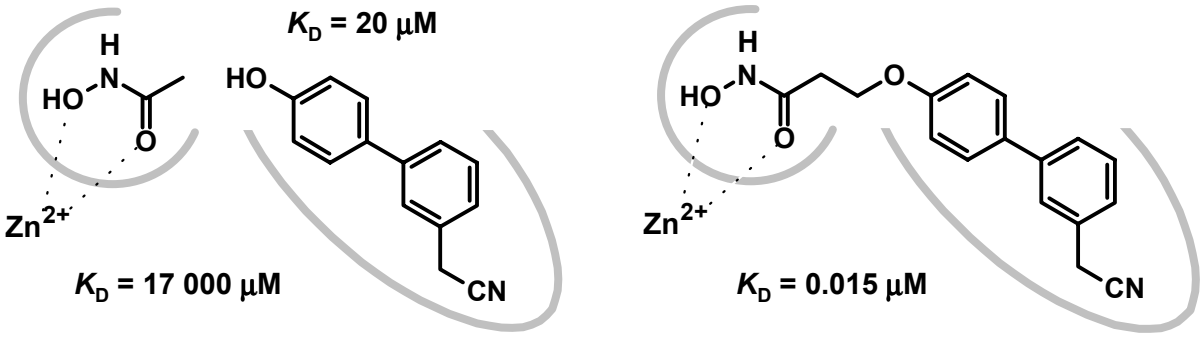

Figure 8. SAR by NMR discovers ligands that bind to proximal subsites of a protein. Acetohydroxamic acid and 3-(cyanomethyl)-4'-hydroxybiphenyl are only low-affinity ligands of the matrix metalloprotease stromelysin; combining them with an appropriate linker produces a high-affinity stromelysin inhibitor [39].

Structure-based drug design is supported by computer programs for the automated superposition (alignment) of molecules, for flexible docking of ligands and for *de novo* design of ligands that fit a binding site in shape and complementarity of their physicochemical properties [40-47]. FlexS [48] and FlexX [49] are such programs for the flexible superposition and docking of ligands. First, a molecule is dissected into rigid fragments that are re-assembled by a tree-search procedure, to achieve the best mutual alignment to another molecule (FlexS) or to obtain the best fit to a protein binding site (FlexX). Other programs for flexible ligand docking have been described [50-52].

The *de novo* design program LUDI [53-55] has been adapted to a combinatorial design of ligands from appropriate building blocks [56, 57]. The MCSS (multiple copy simultaneous search) method is another interesting realization of the concept of combinatorial docking [58, 59]. This approach searches for preferred locations of certain functional groups or small ligands in the binding site. The corresponding positions are analyzed and selected ligand orientations are connected with linkers to

build molecules whose structures are optimized within the binding site. Work is in progress in several other research institutes and companies to develop programs for *in vitro* screening of large virtual combinatorial libraries, for the combinatorial docking of ligands, and for the assembly of ligands from smaller building blocks within the protein binding site. All these experimental (SAR by NMR) and computational approaches are modern realizations of Swift's engine for the improvement of speculative knowledge.

Outlook

In the last decades we have witnessed a decline in the number of new drugs that were introduced into therapy. Sometimes this fact is discussed as an argument against the contribution of modern drug design strategies. However, the reason for this decline are manifold; even neglecting the slight increase in the number of newly marketed drugs in the very last years [60], it should be allowed to conclude that the situation would be much worse without the progress in

- gene technology for the identification of new targets and the production of human proteins for testing and structural analysis,
- combinatorial chemistry for the synthesis of large series of compounds, for lead structure search and optimization,
- high-throughput screening for the rapid identification of new leads from large in-house, external or combinatorial libraries,
- X-ray crystallography and NMR for the determination of protein 3D structures and the identification of ligands,
- computational chemistry for molecular property calculation and modelling, and
- structure-based and computer-aided drug design for the search for new leads and their rational optimization.

There are still unsolved problems in structure-based and computer-aided drug design [61]. Our knowledge of the effects of solvation and desolvation does not allow us to estimate the strength of newly formed hydrogen bonds; the same applies to entropy changes due to freezing conformational degrees of freedom and to the release of water molecules at hydrophobic parts of the protein surface. Different approaches have been developed for the estimation of binding energies of protein ligands [62]. However, minor changes of the chemical structure of a ligand may change its binding mode; even in favorable cases, where high-resolution protein 3D structures are available, surprising results are sometimes obtained [63-66]. And, worst of all, many ligands resulting from a structure-based design lack sufficient bioavailability and metabolic stability.

In a recent lecture, the Nobel laureate Rolf Zinkernagel gave a witty characterization of the chances of different research strategies [67]. Having no working hypotheses and performing no experiments is definitely the cheapest approach but will not lead to any results. To start from a hypothesis and to do only theoretical work is another relatively cheap method. However, also this approach nearly always fails to generate meaningful results. Doing experiments without any working hypotheses may produce, as we have seen, serendipitous discoveries but also here the chance of success is relatively small. In fact, this kind of research is most often a total waste of money. The usual approach, being also expensive, is to generate working hypotheses and to perform experimental studies. Sometimes the expected results are obtained which comes at no surprise. However, one has to be aware of unexpected, accidental discoveries. These are the real breakthroughs in science, giving evidence for results that were up to this discovery unimaginable to the human mind.

Are there other factors that determine the success in drug research? The late George de Stevens formulated *"The* [drug] *discovery process is at times slow, somewhat tedious, always exciting and requiring patience, tenacity, objectivity and above all intellectual integrity. Therefore, scientists, to be innovative, must work in a corporate environment in which the management not only recognizes these factors but makes every effort to let their importance be known to the scientists. The people in research don't have a need to be loved but they do need to feel that they are understood and supported and not to be manipulated according to short-term business cycles. ... Drug discoveries are made by scientists practicing good science. By and large these discoveries are usually made in a company with any enlightened management which encourages its scientists with freedom of action, freedom to think widely and to challenge dogma, and freedom in risk-taking. Moreover, important drug discoveries are not made by committees but by individual scientists working closely together, sharing ideas, testing hypotheses, looking for new solutions to difficult problems, accepting negative results and learning from these results so that the next group of compounds synthesized and tested will open the door to new and improved therapy"* [6].

References

1. Roberts, R.M. Serendipity. Accidental discoveries in science. John Wiley & Sons, New York, 1989.
2. Lewis, S.L., Ed. Horace Walpole's Correspondence. 31 Vols., Yale University Press, New Haven, 1937 ff.
3. Remer, T.G. Serendipity and the three princes. From the Peregrinaggio of 1557. University of Oklahoma Press, Norman, 1965. Translated from the book Peregrinaggio di tre giovani, figliuoli del re di Serendippo; tradotto dalla lingua persiana in lingua italiana do M. Christoforo Armeno. Venice, Michele Tramezzino, 1557.
4. Sneader W. Drug prototypes and their exploitation, John Wiley & Sons, Chichester, 1996.
5. Burger, A. A guide to the chemical basis of drug design. John Wiley & Sons, New York, 1983.
6. de Stevens, G. Serendipity and structured research in drug discovery, Progr. Drug Res. 30, 189-203, 1986.
7. Restak, R.M. Receptors, Bantam Books, New York, 1994.
8. Böhm, H.-J.; Klebe, G. and Kubinyi, H. Wirkstoffdesign. Spektrum Akademischer Verlag, Heidelberg, 1996.
9. Sternbach, L.H. The benzodiazepine story. Progr. Drug Res. 22, 229-266, 1978.
10. Wolff, M.E., Ed. Burger's medicinal chemistry and drug discovery. Volume 1: Principles and practice. John Wiley & Sons, New York, 1995.
11. Sternweis, P.C. and Gilman, A. G. Aluminum: a requirement for activation of the regulatory component of adenylate cyclase by fluoride. Proc. Natl. Acad. Sci. USA 79, 4888-4891, 1982.
12. Sondek, J.; Lambright, D.G.; Noel, J.P.; Hamm, H.E. and Sigler P.B. GTPase mechanism of Gproteins from the 1.7-Å crystal structure of transducin α.GDP.AlF_4^-. Nature 372, 276-279, 1994.
13. Goodford, P.J. Drug design by the method of receptor fit. J. Med. Chem. 27, 557-564, 1984.

14. Ganellin, C.R. and Roberts, S.M. Medicinal chemistry. The role of organic chemistry in drug research. Second Edition, Academic Press, London, 1993.
15. Greer, J.; Erickson, J.W.; Baldwin, J.J. and Varney, M.D. Application of the three-dimensional structures of protein target molecules in structure-based drug design. J. Med. Chem. 37,1035-1054, 1994.
16. Vacca, J.P. and Condra, J.H. Clinically effective HIV-1 protease inhibitors. Drug Discovery today 2, 6-18, 1997.
17. von Itzstein, M.; Wu, W.Y.; Kok, G.B.; Pegg, M.S.; Dyason, J.C.; Jin, B.; Phan, T.V.; Smythe, M.L.; White, H.F.; Oliver, S.W.; Colman, P.M.; Varghese, J.N.; Ryan, D.M.; Woods, J.M.; Bethell, R.C.; Hotham, V.J.; Cameron, J.M. and Penn, C.R. Rational design of potent sialidase-based inhibitors of influenza virus replication. Nature 363, 418-423, 1993.
18. von Itzstein, M.; Dyason, J.C.; Oliver, S.W.; White, H.F.; Wu, W.Y.; Kok, G.B. and Pegg, M.S. A study of the active site of influenza virus sialidase: an approach to the rational design of novel anti-influenza drugs. J. Med. Chem. 39, 388-391, 1996.
19. Chand, P.; Babu, Y.S.; Bantia, S.; Chu, N.; Cole, L.B.; Kotian, P.L.; Laver, W.G.; Montgomery, J.A.; Pathak, V.P.; Petty, S.L.; Shrout, D.P.; Walsh, D.A. and Walsh, G.M. Design and synthesis of benzoic acid derivatives as influenza neuraminidase inhibitors using structure-based drug design. J. Med. Chem. 40, 4030-4052, 1997.
20. Kim. C.U.; Lew, W.; Williams, M.A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M.S.; Mendel, D.B.; Tai, C.Y.; Laver, W.G. and Stevens, R.C. Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. J. Am. Chem. Soc. 119:681-690, 1997.
21. Beddell, C.R., Ed. The design of drugs to macromolecular targets. John Wiley & Sons, Chichester, 1992.
22. Wermuth, C.G., Ed. The practice of medicinal chemistry. Academic Press, London, 1996.
23. Bohacek, R.S.; McMartin, C. and Guida, W.C. The art and practice of structure-based drug design: a molecular modeling perspective. Med. Res. Rev. 16, 3-50, 1996.
24. Babine, R.E. and Bender, S.L. Molecular recognition of protein-ligand complexes: applications to drug design. Chem. Rev. 97, 1359-1472, 1997.
25. Veerapandian, P., Ed. Structure-based drug design. Marcel Dekker, Inc., New York, 1997.
26. Gubernator, K. and Böhm, H.-J., Eds. Structure-based drug design. Wiley-VCH, Weinheim, 1998.
27. Evans, B.E.; Bock, M.G.; Rittle, K.E.; DiPardo, R.M.; Whitter, W.L.; Veber, D.F.; Anderson, P.S. and Freidinger, R.M. Design of potent, orally effective, nonpeptidal antagonists of the peptide hormone cholecystokinin. Proc. Natl. Acad. Sci. USA 83, 4918-4922, 1986.
28. Samanen, J. GPIIb/IIIa antagonists. Annu. Rep. Med. Chem. 31, 91-100, 1996.
29. Engleman, V.W.; Kellogg, M.S. and Rogers, T.E. Cell adhesion integrins as pharmaceutical targets. Annu. Rep. Med. Chem. 31, 191-200, 1996.
30. Samanen, J.M.; Ali, F.E.; Barton, L.S.; Bondinell, W.E.; Burgess, J.L.; Callahan, J.F.; Calvo, R.R.; Chen, W.; Chen, L.; Erhard, K.; Feuerstein, G.; Heys, R.; Hwang, S.M.; Jakas, D.R.; Keenan, R.M.; Ku, T.W.; Kwon, C.; Lee, C.P.; Miller, W.H.; Newlander, K.A.; Nichols, A.; Parker, M.; Peishoff, C.E.; Rhodes, G.; Ross, S.; Shu, A.; Simpson, R.; Takata, D.; Yellin, T.O.; Uzsinskas, I.; Venslavsky, J.W.; Yuan, C.K. and Huffman, W.F. Potent, selective, orally active

3-oxo-1,4-benzodiazepine GPIIb/IIIa integrin antagonists. J. Med. Chem. 39, 4867-4870, 1996.
31. Keenan, R.M.; Miller, W.H.; Kwon, C.; Ali, F.E.; Callahan, J.F.; Calvo, R.R.; Hwang, S.M.; Kopple, K.D.; Peishoff, C.E.; Samanen, J.M.; Wong, A.S.; Yuan, C.K. and Huffman, W.F. Discovery of potent nonpeptide vitronectin receptor ($\alpha v\beta 3$) antagonists. J. Med. Chem. 40, 2289-2292, 1997.
32. Gulliver's Travels, published anonymously, Motte, London, 1726; Swift, J. Gulliver's Travels. Part III. A voyage to Laputa, Balnibarbi, Glubbdubdrib, Luggnagg and Japan, Chapter 5. Penguin Classics, Penguin Books Ltd., London, 1985, pp. 223-231.
33. Dolle, R.E. Discovery of enzyme inhibitors through combinatorial chemistry. Molecular Diversity 2, 223-236, 1996.
34. Cowley, P.M. and Rees, D.C. Applications of solid-phase synthesis to drug discovery. Curr. Med. Chem. 4, 211-227, 1997.
35. Bone, R. and Salemme, F.R. The integration of structure-based design and directed combinatorial chemistry for new pharmaceutical discovery. In Structure-based drug design, Veerapandian, P., Ed. Marcel Dekker, New York, 1997, pp. 525-539.
36. Myers, P.L. Will combinatorial chemistry deliver real medicines? Curr. Opin. Biotechnol. 8, 701-707, 1997.
37. Shuker, S.B.; Hajduk, P.J.; Meadows, R.P. and Fesik, S.W. Discovering high-affinity ligands for proteins: SAR by NMR. Science 274, 1531-1534, 1996.
38. Hajduk, P.J.; Meadows, R.P. and Fesik, S.W. Discovering high-affinity ligands for proteins. Science 278, 497-499, 1997.
39. Hajduk, P.J.; Sheppard, G.; Nettesheim, D.G.; Olejniczak, E.T.; Shuker, S.B.; Meadows, R.P.; Steinman, D.H.; Carrera Jr., G.M.; Marcotte, P.A.; Severin, J.; Walter, K.; Smith, H.; Gubbins, E.; Simmer, R.; Holzman, T.F.; Morgan, D.W.; Davidsen, S.K.; Summers, J.B. and Fesik, S.W. Discovery of potent nonpeptide inhibitors of stromelysin using SAR by NMR. J. Am. Chem. Soc. 119, 5818-5827, 1997.
40. Müller, K., Ed. *De novo* design. Persp. Drug Discov. Design 3, 1-209, 1995.
41. Höltje, H.D. and Folkers, G. Molecular modeling. Basic principles and applications. VCH, Weinheim, 1996.
42. Bamborough, P. and Cohen, F.E. Modeling protein-ligand complexes. Curr. Opin. Struct. Biol. 6, 236-241, 1996.
43. Böhm, H.-J. Computational tools for structure-based ligand design. Prog. Biophys. Molec. Biol. 66, 197-210, 1996.
44. Böhm, H.-J. Current computational tools for *de novo* ligand design. Curr. Opin. Biotechnol. 7, 433-436, 1996.
45. Lengauer, T. and Rarey, M. Computational methods for biomolecular docking. Curr. Opin. Struct. Biol. 6, 402-406, 1996.
46. Marrone, T.J.; Briggs, J.M. and McCammon, J.A. Structure-based drug design: computational advances. Annu. Rev. Pharmacol. Toxicol. 37, 71-90, 1997.
47. Parrill, A. Recent advances in computer-aided drug design methods. Exp. Opin. Ther. Patents 7, 937-945, 1997.
48. Lemmen, C. and Lengauer, T. Time-efficient flexible superposition of medium-sized molecules. J. Comput.-Aided Mol. Design 11, 357-368, 1997. See also http://cartan.gmd.de/flex-bin/FlexS.
49. Rarey, M.; Kramer, B.; Lengauer, T. and Klebe, G. A fast flexible docking method using an incremental construction algorithm. J. Mol. Biol. 261, 470-489, 1996. See also http://cartan.gmd.de/flex-bin/FlexX.

50. Makino, S. and Kuntz, I.D. Automated flexible ligand docking method and its application for database search. J. Comput. Chem. 18, 1812-1825, 1997.
51. Westhead, D.R.; Clark, D.E. and Murray, C.W. A comparison of heuristic search algorithms for molecular docking. J. Comput.-Aided Mol. Design 11, 203-228, 1997.
52. Dixon, J.S. Evaluation of the CASP2 docking section. Proteins 27 Suppl. 1, 198-204, 1997, and following articles in this special issue on the "Second Meeting on the Critical Assessment of Techniques for Protein Structure Prediction" (CASP2), Asilomar, CA, USA, December 13-16, 1996.
53. Böhm, H.-J. The computer program LUDI: a new method for the *de novo* design of enzyme inhibitors. J. Comput.-Aided Mol. Design 6, 61-78, 1992.
54. Böhm, H.-J. LUDI: rule-based automatic design of new substituents for enzyme inhibitor leads. J. Comput.-Aided Mol. Design 6, 593-606, 1992.
55. Böhm, H.-J. Ligand design. In: 3D QSAR in drug design. Theory, methods and applications. Kubinyi, H., Ed. ESCOM Science Publishers B.V., Leiden (1993), 386-405.
56. Böhm, H.-J. Towards the automatic design of synthetically accessible protein ligands: peptides, amides and peptidomimetics. J. Comput.-Aided Mol. Design 10, 265-272, 1996.
57. Böhm, H.-J. Combinatorial docking. In Computer-assisted lead finding and optimization (Proceedings of the 11th European symposium on quantitative structure-activity relationships, Lausanne, 1996), van de Waterbeemd, H.; Testa, B. and Folkers, G., Eds. Verlag Helvetica Chimica Acta and VCH, Basel and Weinheim, 1997, pp. 123-133.
58. Caflisch, A. and Karplus, M. Computational combinatorial chemistry for *de novo* ligand design: review and assessment. Persp. Drug Discov. Design 3, 51-84, 1995.
59. Caflisch, A. and Ehrhardt, C. Structure-based combinatorial ligand design. In Structure-based drug design, Veerapandian, P., Ed. Marcel Dekker, New York, 1997, pp. 541-558.
60. Hubbard, R.E. Can drugs be designed? Curr. Opin. Biotechnol. 8, 696-700, 1997.
61. Graul, A.I. The year's new drugs. Drug News Perspect. 11, 15-32, 1998.
62. Kubinyi, H.; Folkers, G. and Martin, Y.C., Eds. 3D QSAR in drug design. Volume 2. Ligand-protein interactions and molecular similarity, Kluwer Academic Publishers, Dordrecht, 1998.
63. Mattos, C. and Ringe, D. Multiple binding modes. In: 3D-QSAR in drug design. Theory, methods and applications. Kubinyi, H., Ed. ESCOM Science Publishers B.V., Leiden, 1993, pp. 226-254.
64. Meyer, E.F.; Botos, I.; Scapozza, L. and Zhang, D. Backward binding and other structural surprises. Persp. Drug. Discov. Design 3, 168-195, 1995.
65. Böhm, H.-J. and Klebe, G. What can we learn from molecular recognition in protein-ligand complexes for the design of new drugs? Angew. Chem. Int. Ed. Engl. 35, 2589-2614, 1996.
66. Kubinyi, H.: Similarity and dissimilarity: a medicinal chemist's view. In: 3D QSAR in drug design. Ligand-protein interactions and molecular similarity. Kubinyi, H.; Folkers, G. and Martin, Y. C., Eds. Kluwer Academic Publishers, Dordrecht, 1998, pp. 225-252.
67. Zinkernagel, R.M. Lecture "Immunität gegen Viren", presented at the Symposium "Pharmazie. Die Wissenschaft vom Arzneimittel" of the German and Swiss Pharmaceutical Societies, Zurich, Switzerland, October 02-05, 1997.