Beilstein-Institut The Chemical Theatre of Biological Systems, May 24th - 28th, 2004, Bozen, Italy

CHANGING PARADIGMS IN DRUG DISCOVERY

HUGO KUBINYI

University of Heidelberg, Donnersbergstrasse 9, D-67256 Weisenheim am Sand, Germany
E-Mail: kubinyi@t-online.de

Received: 5th May 2004 / Published: 22nd July 2005

ABSTRACT

The strategies of drug design have changed significantly within the past few decades. Whereas chemistry, biological activity hypotheses and animal experiments dominated drug research, especially in its "golden age", from the sixties to the eighties of the last century, many new technologies have developed over the past 20 years. A vast amount of new drugs was expected to result from combinatorial chemistry and high-throughput screening; however, the yield of new drug candidates was relatively poor. Molecular modelling, virtual screening and 3D structure-based design support the selection and rational design of high-affinity protein ligands. But high affinity to a disease-relevant target is only one important property; in addition, a drug must be orally bioavailable, it should have favourable pharmacokinetics and no unacceptable side effects or toxicity. The following questions are discussed in detail: what are the reasons for the productivity gap between R&D costs and the number of NCE's? Is there a "druggable genome"? Is target focus always best? Is poor ADME the main problem in clinical development? Are we using the right virtual screening tools? What are the main problems in virtual screening and structure-based design? What is wrong and what could we do better?

INTRODUCTION

The strategies of drug research did not change much from the late 19th century until the seventies of the 20th century. New compounds were synthesized and tested on animals or organ preparations, following some chemical or biological hypotheses. Although synthetic output was relatively low, the real bottlenecks were the biological test models. Pharmacological experiments using dozens of animals for every new compound often needed more time for biological characterization than for chemical synthesis.

This situation started to change about 30 years ago. Slowly rational approaches developed, such as QSAR and molecular modelling. The consequence was a lower output in such projects, when certain chemical structures that were proposed by these methods had to be synthesized. On the other hand, *in vitro* test systems such as enzyme inhibition or the displacement of radio-labelled ligands in membrane preparations enabled much faster investigation of new analogues. Now chemistry was the bottleneck. About 10 to 15 years ago, another significant shift in drug discovery paradigms occurred: combinatorial chemistry suddenly flooded the biology laboratories with an overwhelming number of new compounds. It has been commented that combinatorial chemistry was the "revenge of the chemists" to the development of fast *in vitro* test models, with their large output of data within a relatively short time. However, biologists were able to compete: tens of thousands of compounds, later even more, could be investigated by automated high-throughput screening (HTS) systems in just one week.

In the past, wrong or misleading results were obtained too often just because of the use of animal models. Gene technology made an important contribution to drug discovery: the opportunity to produce almost any protein in sufficient quantities enabled biologists to test new compounds at human targets. Genetically modified animals indicate whether a certain principle could work in therapy. The action of an enzyme inhibitor can be simulated before any compounds are synthesized and tested, by a knockout of the corresponding enzyme; the action of drugs can be investigated in animals bearing a human protein. In addition, the production of larger quantities of a protein of therapeutic relevance allows the determination of its three-dimensional (3D) structure at atomic resolution by protein crystallography, alternatively by multidimensional NMR methods. As a consequence, methods developed for the structure-based design of ligands, by modelling or experimental determination of the 3D structures of protein-ligand complexes. Unfortunately, a new bottleneck resulted! Early combinatorial chemistry was guided by synthetic accessibility and the hype for large numbers. Due to this wrong focus, a huge amount of greasy, high-molecular weight compounds resulted, with all their problems in bioavailability and pharmacokinetics. Biological testing which did not produce any valuable hits or supposed hits, later failed in preclinical or clinical development. Whereas this situation has fortunately changed in recent years, due to the maturation of combinatorial chemistry to an automated parallel synthesis of designed libraries, there was still a need for the fast measurement or prediction of ADME (absorption, distribution, metabolism, excretion) properties. Indeed, ADME became the new bottleneck.

Nowadays, we have information on the sequence of the human genome; our combinatorial chemistry approaches are under control by medicinal chemists and biologists; in addition to structure-based design we apply computer-aided methods for data mining, virtual screening, docking and scoring, to predict valuable leads and optimized candidates; HTS models have developed to ultra-HTS models, with up to a million test points per 24 hours; we even have fast experimental models and prediction tools for some ADME parameters. Is there a new bottleneck? Yes, unfortunately, or better to say: yes, of course. Target validation, the proof that the modulation of a certain target by a small molecule will indeed work in therapy, is one of the new bottlenecks. The other one, even more problematic, is the fact that only for some targets can small molecules be discovered which modulate the protein or a certain protein-protein interaction in the desired manner; "druggable" defines the property of a certain target to be accessible by small molecule intervention.

In the past, serendipity played a big role in the discovery of new drugs [1-3]. Some other projects confirmed that the search for new drugs may be more efficient by establishing biological or structure-activity hypotheses and/or selecting certain scaffolds and substituents in the design of new drug candidates. The ratio of 10,000 compounds to produce one new drug is still very often cited. This applies to the situation where research starts from an endogenous ligand or any other lead structure. The "irrational approach", to test huge numbers of in-house compounds, commercially available compounds or chemistry-driven combinatorial libraries in HTS, did not deliver the expected amount [4]. Hundreds of thousands to millions of compounds have to be investigated if such a search starts from scratch, without any knowledge of an active lead, and even then there is no guarantee of success.

THE "DRUGGABLE GENOME"

The human genome project has provided information on all our genes. However, the situation is the same as the one with Egyptian hieroglyphs before the discovery of the Rosetta Stone. We read the text but we understand only a minor part. There are about 30,000 genes in the human genome but we do not know how many of them are disease-related and how many of the gene products will be druggable. It has been estimated that about 600 to 1500 druggable, disease-related targets exist, if one assumes about 10% of disease-related genes on the one hand and about 10% druggable gene products on the other hand [5]. However, this number has to be questioned because only the number of genes was considered [6].

First of all, many proteins or protein variants (estimated to be in the range of some 100,000s) are produced by alternative splicing and/or post-translational modification than there are genes in the genome. Second, proteins can form a multitude of heteromeric complexes that are made up from only a small number of different proteins, e.g. GABA and nicotinic acetylcholine (nACh) receptors, integrins and heterodimeric G protein-coupled receptors (GPCR). Third, some proteins are involved in more than one signalling chain, interacting with different proteins to modulate certain effects. And, last but not least, many therapeutically used drugs do not interact with just one target but have a balanced effect on several different targets. A striking example for such a promiscuous drug is the atypical neuroleptic olanzapine, which interacts as a nanomolar ligand with many different GPCRs [7-9].

Thus, we should neither discuss a druggable genome, nor a druggable proteome, nor a "druggable targetome", but a "druggable physiome" [6]. Our problem is that we do not yet know how to define and design a drug with the right balance of different target affinities, e.g. for depression, schizophrenia and other CNS diseases.

VIRTUAL SCREENING

Several new strategies have been developed for the structure-based and computer-aided design of active compounds. Drug research has often been compared with the search for a needle in a haystack. If neither active leads nor the 3D structure of the biological target are known, HTS seems to be the only reasonable approach. But much useful information can be derived from virtual screening [10], which reduces the size of the haystack. First of all, reactive compounds and other compounds with undesirable groups can be eliminated by so-called "garbage filters" [11]. In a next step, the Lipinski (Pfizer) rule of five may be applied to estimate the potential for oral bioavailability; this set of four rules demands that the molecular weight of a molecule should be lower than 500; the lipophilicity, expressed by log P (P = calculated octanol/water partition coefficient), should be smaller than 5; the number of hydrogen bond donors should not be larger than 5; the sum of oxygen and nitrogen atoms in the molecule (as a rough approximation of the number of hydrogen bond acceptors) should not exceed 10 [12]. A high risk of insufficient oral bioavailability is assumed if more than one of these conditions is violated. Often the rule of five-compatible molecules is erroneously called "drug-like" [6]. However, most of the compounds of the ACD (Available Compounds Directory) [13] would get this label if only the Lipinski rules are applied. "Drug-like" or "non-drug-like" character can

only be attributed by neural nets that have been trained with drugs and chemicals [14-16]. In this context it is important to notice that filters are valuable and efficient in the enrichment of interesting candidates out of large libraries. Single compounds should not be evaluated by such filters because the relatively large error rate of about 20% false positives and 20% false negatives would too often provide misleading results.

The situation in drug discovery is much better if a certain number of active and inactive ligands of a target are already known. If a chemical series belongs to a common scaffold or to some related scaffolds, 2D or 3D similarity methods, QSAR and 3D QSAR approaches, and pharmacophore approaches can be applied to derive structure-activity hypotheses (some of the problems of pharmacophore generation will be discussed in the next section). The results of such analyses are proposals for new syntheses or selections of compounds from a library. A highly valuable tool in this respect is feature tree similarity comparisons [17,18], where the molecules are coded as strings with nodes, to which the pharmacophoric properties of the corresponding functional group, ring or linker are attributed. Due to this simple representation of the molecules, similarity searches can be performed extremely fast. In this manner, screening hits can be compared in their similarity to a whole in-house compound library, to libraries of commercially available compounds, e.g. the MDL Screening Compounds Directory [19], and to even larger virtual libraries.

If the 3D structure of a new target is known from experimental determination or from reliable homology modelling, the situation seems to be better but in reality it is not. There remains a high degree of uncertainty about the 3D structure of the protein in the bound state if no information on protein-ligand complex 3D structures is available. Relatively often, the protein itself and its ligand complexes have significantly different 3D structures, the most prominent example being HIV protease. In addition, the relatively low resolution of most protein 3D structures does not allow one to differentiate between the side chain rotamers of asparagine, glutamine, threonine and histidine; the protonation state of histidine remains unclear; water molecules, which are important for the binding of a ligand, are sometimes neglected in protein 3D structures.

All these problems exist only to a minor extent if several protein-ligand complexes can be inspected, which leads to the fourth and best situation in ligand design: not only the protein but also some protein-ligand complexes are known. Molecular modelling and docking aids in the design of new ligands with hopefully improved binding affinity and/or selectivity with respect to other targets.

It should be emphasized that structure-based design can result in a high-affinity ligand but affinity is only a necessary property of a drug, not a sufficient one. In addition, a drug has to be bioavailable, it must have a proper biological half-life time and it must not be toxic, among other important properties.

PHARMACOPHORES

The definition of a pharmacophore is simple [20]. A 3D pharmacophore corresponds to an arrangement of hydrogen bond donor and acceptor, lipophilic and aromatic groups in space, in such a manner that these moieties can interact with a binding site at the target protein; in addition, steric exclusion volumes can be defined. However, the identification of a pharmacophore within a congeneric group of compounds is far from being trivial. Although there are computer programs for the automated derivation of pharmacophores from series of active and less active analogues [21], a better and more reliable method seems to be a "construction by hand" [22]. Four independent problems have to be considered:

- the different pharmacophoric properties of oxygen atoms,
- the protonation and deprotonation of ionizable groups,
- the consideration of tautomeric forms, and
- the superposition of flexible molecules.

Oxygen atoms are strong hydrogen bond acceptors, as long as they are either connected to a carbon atom by a double bond (e.g. in aldehydes, ketones, carboxylic acids, carbonyl group of esters) or substituted by hydrogen and/or aliphatic residues (water, aliphatic alcohols and aliphatic ethers). They are weak or even not acceptors at all (e.g. the sp^3 oxygen atom of an ester group) if their directly neighbouring atoms are connected to another atom by a double bond or if they are part of an aromatic system, as in oxazoles and isoxazoles (Fig. 1) [23,24].

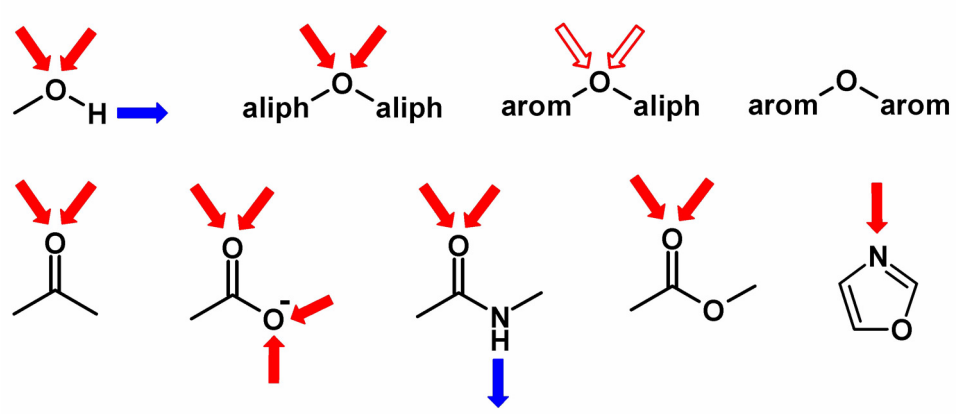

Figure 1. The oxygen atoms of alcohols, aliphatic ethers, aldehydes, ketones, carboxylates and amides are strong hydrogen bond acceptors. The oxygen atoms of mixed aliphatic-aromatic ethers are weaker acceptors and the oxygen atoms of aromatic ethers and heterocycles are more or less without acceptor properties. The same applies to the sp3 atom of an ester group, because of the electron-withdrawing effect of the carbonyl group, which itself is a strong hydrogen bond acceptor, and to oxygen in aromatic systems.

Ionizable groups must be recognized and defined in the right manner to end up with correct pharmacophores. As this is still a mainly unsolved problem for many compounds that are not simple acids, phenols or anilines (at least considering the speed that is needed in the virtual screening of large libraries), a rule-based system has recently been proposed [25]. In this set of rules, all carboxylic acids, the strongly basic amidines and guanidines, and quaternary ammonium compounds are permanently charged. Neutral and protonated forms are generated and investigated in parallel for amines, imidazoles, pyridines and other nitrogen-containing heterocycles. For tetrazoles, thiols, hydroxamic acids, and activated sulfonamides, neutral and deprotonated forms should be investigated in parallel. Certain rules restrict the number of generated species, to avoid combinatorial explosions: there are definitions of the maximum number of charges in a molecule and no identical charges are allowed in adjacent positions of the molecule. Although this approach is definitely better than using all molecules in their neutral form, refined prediction models are urgently needed. An even more difficult problem arises from the fact that ionizable amino acid side chains in proteins may significantly change their pK_a value in dependence of their environment [26,27].

Protomers and tautomers constitute another serious problem in virtual screening and docking (Fig. 2) [28,29]. 1,3-Diketones, acetoacetic esters, hydroxypyridines, oxygen-substituted pyrimidines and purines, and many other compounds may exist in several tautomeric forms that have to be recognized and considered.

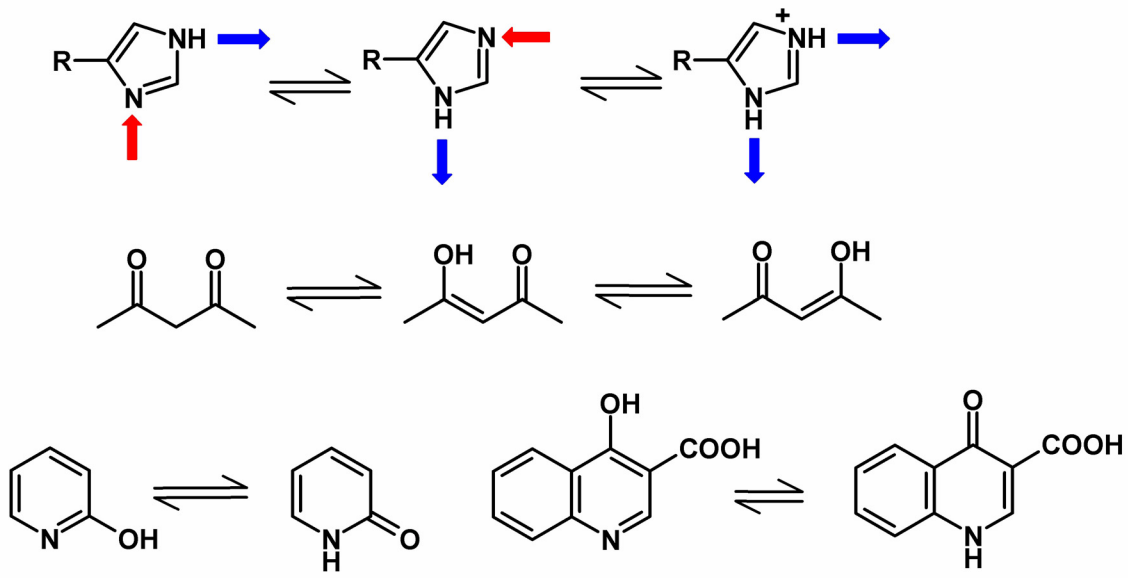

Figure 2. The two different protomers of imidazole (upper left) present their donor and acceptor moieties in different positions; as imidazole has a pK_a value around 7, also the charged form with two donor functions has to be considered (upper right). The other compounds are typical examples of tautomeric forms of molecules, where donor and acceptor functions change their position.

For the purine base guanine, 15 different tautomers can be formulated [29]. In this context it is interesting to note that for a long time Watson and Crick had problems in deriving the correct 3D structure of DNA because they only considered the enol tautomers of guanine and thymine, instead of the keto forms (Fig. 3). When their colleague Donohue corrected this error, they immediately arrived at the correct base pairing [30]. Sometimes enol forms of a ligand are induced by the binding site, as is the case for the binding of the barbiturate Ro 200-1770 to a matrix metalloprotease (Fig. 3) [31]. Computer programs for the generation of all possible tautomers have been described [29,32].

If all these topics are considered in an appropriate manner, the next step is an alignment of the molecules. With rigid molecules, this is most often no problem. But even with steroids the question arises, whether a 3-keto-17-hydroxy-steroid and a 3-hydroxy-17-keto-steroid should be aligned according to their molecular skeleton (which puts the hydrogen bond donor groups of both molecules far apart) or whether a head-to-tail superposition is more favourable; the steric superposition is about as good in this latter case as in the conventional superposition [33]. For flexible molecules, the most rigid active species should be used as a template onto which, step by step, the other molecules are superimposed.

This can be done by visual inspection or by field fit methods, such as SEAL [34,35]; most often several different conformations have to be considered. FlexS is a computer program for flexible superposition; one molecule is used as a rigid template and all other molecules are superimposed in a flexible manner onto this template [36,37]. 3D QSAR methods, such as CoMFA (comparative molecular field analysis) [38,39] or CoMSIA (comparative molecular similarity index analysis) [40], surprisingly do not depend on knowledge of the bioactive conformation. If all conformations are "wrong" to the same extent, the result of an analysis may nevertheless be useful. Another difficulty in the alignment of molecules arises from different binding modes of seemingly similar molecules; there are no general rules on how to recognize such situations.

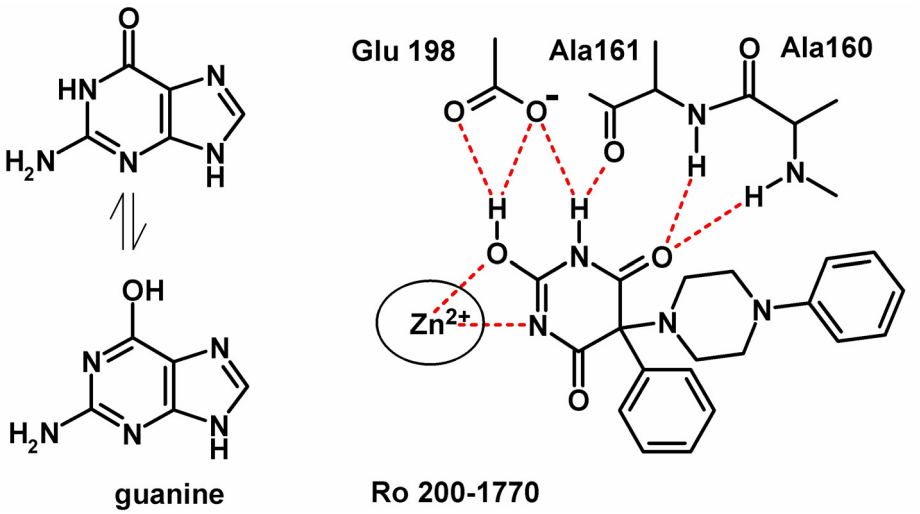

Figure 3. The guanine tautomer shown in the upper left is the predominant one of 15 possible tautomers, whereas Watson and Crick, worked for a long time, with the tautomer shown in the lower left. Ro 200-1770 is a matrix metalloprotease inhibitor. Only one tautomer can bind with high affinity; the carbonyl form or the other enol form will not form favourable hydrogen bond networks.

Once a pharmacophore hypothesis has been derived, 3D searches can be performed, using commercial software [21,41]. However, it must be emphasized that 3D searches are only meaningful if all the structures of a database are defined according to their correct pharmacophoric properties; otherwise such searches are just useless.

STRUCTURE-BASED LIGAND DESIGN

In the 1970s, the first structure-based design of ligands was performed. The 3D structure of the 2,3-diphosphoglycerate (2,3-DPG) haemoglobin complex was used to derive simple aromatic dialdehydes which mimicked the function of 2,3-DPG as an allosteric effector molecule. Another early example was the structure-based design of trimethoprim analogues with significantly improved affinities to dihydrofolate reductase. However, neither the haemoglobin ligands nor the trimethoprim analogues could be optimized to become drugs for human therapy [42,43]. The first real success story was the structure-based design of the antihypertensive drug captopril, an angiotensin-converting enzyme (ACE) inhibitor. The structure of captopril was derived in a rational manner from a binding site model, using the 3D information of an inhibitor complex of the closely related zinc protease carboxypeptidase A [44].

With the ongoing progress in protein crystallography and multidimensional NMR techniques, the 3D structures of many important proteins, especially enzymes, have been determined. This information led to the structure-based design of several therapeutically useful enzyme inhibitors, most of which are still in preclinical or clinical development. Marketed drugs that have resulted from structure-based design are e.g. the antiglaucoma drug dorzolamide (Merck) and the newer HIV protease inhibitors nelfinavir (Agouron Pharmaceuticals, now Pfizer) and amprenavir (Vertex Pharmaceuticals; developed and marketed by GSK).

Neuraminidase is an interesting target for the structure-based design of anti-influenza drugs. In a very elegant study, Mark von Itzstein used the computer program GRID to estimate interaction energies of the neuraminic acid binding site of this enzyme with different probe atoms or small groups [45]. He realized that the introduction of basic groups, like $-NH_2$, $-C(=NH)NH_2$ or $-NH-C(=NH)NH_2$, into the relatively weak inhibitor neu5ac2en should significantly improve inhibitory activities. This is indeed the case: the neuraminidase inhibitor zanamivir is about 4 orders of magnitude more active than its 4-hydroxy-analogue neu5ac2en. Due to its polar character, zanamivir (Relenza®, GSK) is orally inactive; it must be applied by inhalation [46].

Scientists at Gilead Sciences started from the observation that the glycerol side chain of certain zanamivir analogues does not contribute to affinity. In a series of carbocyclic analogues, strongest inhibitor activity was observed for a pent-3-yl ether. Its ethyl ester prodrug oseltamivir (GS 4104, Tamiflu®, Hoffmann-La Roche; Fig. 4) shows good oral bioavailability [46,47]. Several other success stories of structure-based design have been published [43,48-51].

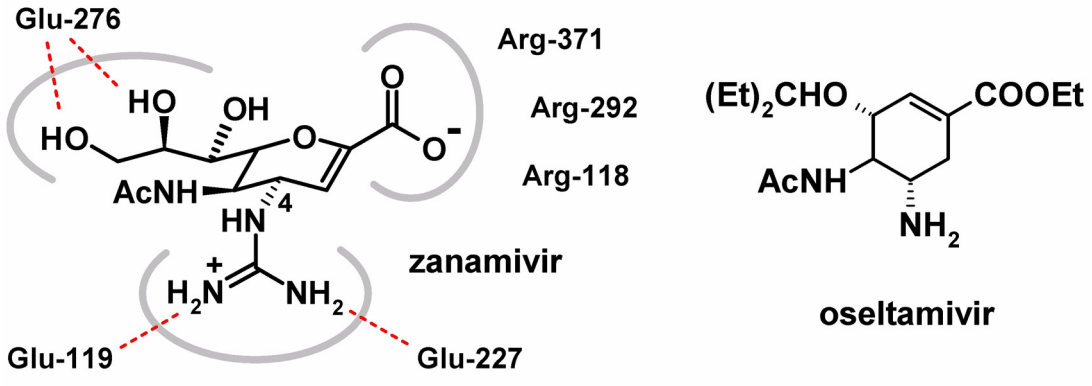

Figure 4. Schematic presentation of the binding mode of the 4-desoxy-4-guanidino-analogue of neu5ac2en, zanamivir, to neuraminidase (left); chemical structure of the orally available prodrug oseltamivir (right).

COMPUTER-AIDED LIGAND DESIGN

Whereas structure-based design can be regarded as the predominant strategy of the last two decades, several computer-assisted methods have been developed more recently. If thousands of candidates and even larger structural databases are to be tested for their suitability to be ligands of a certain binding site, this can no longer be performed by visual inspection. The design process has to be automated with the help of the computer.

The very first computer-based approaches, to search for ligands of a certain binding site, were the programs DOCK [52] and GROW. The *de novo* design program LUDI, developed by Hans-Joachim Böhm at BASF in the early 1990s, was a significant improvement over these early prototypes [53,54]. After the definition of a binding site region by the user, the program automatically identifies all the hydrogen bond donor and acceptor sites, as well as aliphatic and aromatic hydrophobic areas of this part of the protein surface. From the program-implemented information on the geometry of the interaction of such groups with a ligand, the program creates vectors and regions in space, where the complementary groups of a ligand should be located. In the next step, LUDI searches any database of 3D structures of small and medium-sized molecules for potential ligands. Every candidate is tested in a multitude of different orientations and interaction modes, optionally also in different conformations. After a rough evaluation, by counting the number of favourable interactions and by checking for unfavourable van der Waals overlap between the ligand and the protein, the remaining candidates are prioritized by a simple but efficient scoring function [55].

This scoring function estimates interaction energies on the basis of charged and neutral hydrogen bonding energies, hydrophobic contact areas, and the number of rotatable bonds of the ligand. In a last step, the program is able to attach groups, fragments and/or rings to a hit or to an already existing lead structure. The flexible docking of ligands onto a rigid binding site can be achieved by the programs DOCK 4.0 [56], GOLD [57], FlexX [58,59], and the public domain program AutoDock [60,61], to mention those which are the most prominent; more docking programs and several success stories of computer-assisted drug design have been reviewed by Schneider and Böhm [62]. The FlexX modifications FlexE [63] and Flex-Pharm [64] allow a flexible ligand docking into an ensemble of different binding site conformations and the definition of pharmacophore constraints, respectively. Of course, the pharmacophoric properties of all molecules must also be defined in a correct manner in structure-based and computer-aided design.

FRAGMENT-BASED AND COMBINATORIAL LIGAND DESIGN

Several other methods for the design of new ligands have been described in the past, e.g. needle screening [65,66], which starts from a collection of small drug-like ligands and attempts to extend the best ones to larger ligands. In the binding of biotin to avidin, some molecular fragments have only micromolar affinities, whereas biotin itself binds with femtomolar affinity [67]. This principle has recently been used in the rational design of a nanomolar enzyme inhibitor from two low-affinity natural products which bind to different sites of the protein [68]. The SAR by NMR method [69-71] searches for small, low-affinity ligands of proteins which bind to adjacent areas of the binding site. A linker combines both molecules to a nanomolar ligand. Some other NMR-based techniques for ligand discovery have been developed [72-78].

Fragment-based ligand design has been applied for combinatorial techniques [79]. Up to 10,000 low-molecular weight ligands can be tagged onto a gold-coated glass surface [80]; binding of any protein to these microarrays of immobilized ligands is detected by surface plasmon resonance, in this manner avoiding the development of a specific screening method for a new protein. The dynamic assembly of ligands [81-84] generates new molecules from fragments which reversibly react with each other in the presence of a protein.

Molecules that fit the binding site are preferentially formed and afterwards trapped by a reaction which freezes the equilibrium. Some other approaches for the combinatorial design of new leads have been described [85-89].

There are also several computer-assisted techniques for the combinatorial combination of fragments to new leads. The program CombiGen [90] designs libraries with a high percentage of drug-like compounds by assembling privileged and/or user-defined fragments and optionally modifying the resulting structures; virtual screening procedures eliminate molecules with undesired properties. TOPAS [91,92] dissects lead structures into fragments and assembles new molecules by re-combining a chemically similar scaffold with fragments that are similar to the originals; cleavage and assembly of the molecules follow chemical reactions that are defined by a RECAP-like procedure [93]. In this manner, new chemistry is generated by "scaffold hopping" [94]. In principle, the docking program FlexX [58,59], which performs an incremental construction of a ligand within the binding site, could also arrive at new analogues, if many different building blocks are used instead of the original building blocks; no virtual library of millions of potential candidates needs to be constructed, only favourable intermediate solutions and final candidates are generated. The only unsolved problem in this respect is the lack of reliability of the scoring functions [95].

SUMMARY AND CONCLUSIONS

Virtual screening and fragment-based approaches are powerful techniques in the search for new ligands [10,62,96]; promising candidates can be enriched in compound collections and virtual libraries. The integration of protein crystallography, NMR techniques, and virtual screening will "significantly enhance the pace of the discovery process and the quality of compounds selected for further development" [97].

The similarity principle, that similar compounds should exert similar biological activities, has always been a most successful approach in drug research, despite many exceptions to this general concept [98,99]. Chemogenomics is a new term for the dedicated investigation of certain compound classes in target families, such as the G protein-coupled receptors (GPCR), kinases, phosphodiesterases, serine proteases, ion channels, etc. [100-104].

An analogous concept, the "selective optimization of side activities" (SOSA approach), attempts to develop new drugs in the direction of a side-effect of a certain drug [105,106]. Historical examples for the validity of this approach are e.g. antitussive and constipating morphine analogues, diuretic and antidiabetic sulfonamides, and many others [107]; some very recent examples have been reviewed [106,107].

As already mentioned, good ligands are not necessarily good drugs. High-throughput screening of in-house libraries, which originally contained large numbers of reactive, degraded, coloured, fluorescent, and highly lipophilic molecules, and screening of combinatorial libraries of large, lipophilic molecules produced hits that could not be optimized to drug candidates. Awareness of the real problems came only after Lipinski had defined his set of rules [12]. On the other hand, the massive increase of screening failures due to such inappropriate compound collections or libraries turned this awareness of ADME problems into a hype. Prior statistics of 40% failure in clinical investigation (the most expensive phase of drug development), due to ADME problems [108,109] are cited in the literature, again and again; a closer inspection of the data shows that the ADME-related failure can be neglected if anti-infectives are removed from the original sample [6,109]. This is an indication that medicinal chemists have always considered, the importance of ADME properties. Only HTS and early combinatorial chemistry have generated so many problems in this direction. In addition to the Lipinski rules, several *in vitro* and *in silico* techniques are now available for the estimation of ADME properties [110].

With respect to biological testing, Horrobin has raised the question as to whether we are already living in Castalia, the famous virtual land of Hermann Hesse's novel "The Glass Bead Game", where the masters organize and play the most sophisticated, complex and brilliant games - without any context to reality [111]. Sometimes, this is also the case in modelling and drug design [6]. The new tools of drug research are extremely powerful but they will be successful only if the most important factors, some of which have been reviewed here, are considered in the right manner. In the fascinating search for better and safer drugs, the new paradigms of drug discovery have to merge with traditional medicinal chemistry experience [2,112,113].

REFERENCES

[1] Roberts, R. M. (1989) *Serendipity. Accidental Discoveries in Science*. John Wiley & Sons, New York.

[2] Sneader, W. (1996) *Drug Prototypes and Their Exploitation.* John Wiley & Sons, Chichester.

[3] Kubinyi, H. (1999) Chance favors the prepared mind. From serendipity to rational drug design. *J. Recept. Signal Transduct. Res.* **19**:5-39.

[4] Lahana, R.(1999) How many leads from HTS? *Drug Discov. Today* **4**:47-448.

[5] Hopkins, A. L., Groom, C. R. (2002) The druggable genome. *Nature Rev. Drug Discov.* **1**:727-730.

[6] Kubinyi, H. (2003) Drug research: myths, hype and reality. *Nature Rev. Drug Discov.* **2**:665-668.

[7] Schaus, J. M., Bymaster, F. P. (1998) Dopaminergic approaches to antipsychotic agents. *Ann. Rep. Med. Chem.* **33**:1-10.

[8] Bymaster, F. P., Calligaro, D. O., Falcone, J. F., Marsh, R. D., Moore, N. A., Tye, N. C., Seeman P., Wong, D. T. (1996) Radioreceptor binding profile of the atypical antipsychotic olanzapine. *Neuropsychopharmacology* **14**:87-96.

[9] Bymaster, F. P., Nelson, D. L., DeLapp, N. W., Falcone, J. F., Eckols, K., Truex, L. L., Foreman, M. M., Lucaites V. L., Calligaro, D. O. (1999) Antagonism by olanzapine of dopamine D1, serotonin2, muscarinic, histamine H1 and alpha 1-adrenergic receptors *in vitro*. *Schizophr. Res.* **37**:107-122.

[10] Böhm, H.-J., Schneider, G. (Eds) (2000) Virtual Screening for Bioactive Molecules. *Methods and Principles in Medicinal Chemistry.* (Mannhold, R., Kubinyi, H., Timmerman, H., Eds), Vol.10. Wiley-VCH, Weinheim.

[11] a) Rishton, G. M. (1997) Reactive compounds and in vitro false positives in HTS. *Drug Discov. Today* **2**:382-384; b) Rishton, G. M. (2003) Nonleadlikeness and leadlikeness in biochemical screening *Drug Discov. Today* **8**:86-96.

[12] Lipinski, C. A., Lombardo F., Dominy, B. W., Feeney, P. J. (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **23**: 3-26.

[13] MDL Available Compounds Directory, MDL Information Systems Inc., San Leandro, CA, U.S.A.; www.mdl.com.

[14] Ajay, Walters, W. P., Murcko, M. A. (1998) Can we learn to distinguish between "drug-like" and "nondrug-like" molecules? *J. Med. Chem.* **41**:3314-3324.

[15] Sadowski, J., Kubinyi, H. (1998) A scoring scheme for discriminating between drugs and nondrugs. *J. Med. Chem.* **41**:3325-3329.

[16] Walters, W. P., Murcko, M. A. (2002) Prediction of 'drug-likeness'. *Adv. Drug Deliv. Rev.* **54**: 255-271.

[17] Rarey, M., Dixon, J. S. (1998) Feature trees: a new molecular similarity measure based on tree matching. *J. Comput.-Aided Mol. Design* **12**: 471-490; www.biosolveit.de.

[18] Rarey, M., Stahl, M. (2001) Similarity searching in large combinatorial chemistry spaces. *J. Comput.-Aided Mol. Design* **15**: 497-520.

[19] MDL Screening Compounds Directory, MDL Information Systems Inc., San Leandro, CA, U.S.A.; www.mdl.com.

[20] Güner, O. F.(Ed.) (2000) *Pharmacophore Perception, Development and Use in Drug Design*. International University Line, La Jolla, CA.

[21] Catalyst, Accelrys Inc., San Diego, CA, U.S.A.; www.accelrys.com.

[22] Höltje, H.-D., Sippl, W., Rognan, D., Folkers, G. (2003) *Molecular Modelling. Basic Principles and Applications*, 2nd Edn. Wiley-VCH, Weinheim.

[23] Lommerse, J. P. M., Price, S. L., Taylor, R. (1997) Hydrogen bonding of carbonyl, ether, and ester oxygen atoms with alkanol hydroxyl groups. *Comput. Chem*. **18**:757-774.

[24] Böhm, H.-J., Brode, S., Hesse, U., Klebe, G. (1996) Oxygen and nitrogen in competitive situations: which is the hydrogen-bond acceptor? *Chem. Eur. J.* **2**:1509-1513.

[25] Sadowski, J. (2002) A tautomer and protonation pre-processor for virtual screening, Lecture presented at the 224th ACS National Meeting, Boston.

[26] Nielsen, J. E., Vriend, G. (2001) Optimizing the hydrogen-bond network in Poisson-Boltzmann equation-based pKa calculations. *Proteins* **43**:403-412.

[27] Nielsen, J. E., McCammon, J. A. (2003) Calculating pKa values in enzyme active sites. *Protein Sci.* **12**:1894-1901.

[28] Pospisil, P., Ballmer, P., Scapozza. L., Folkers, G. (2003) Tautomerism in computer-aided drug design. *J. Recept. Signal Transduct. Res*. **23**:361-371.

[29] Trepalin, S. V., Skorenko, A. V., Balakin, K. V., Nasonov, A. F., Lang, S. A., Ivashchenko, A. A., Savchuk, N. P. (2003) Advanced exact structure searching in large databases of chemical compounds. *J. Chem. Inf. Comput. Sci.* **43**:852-860.

[30] a) Watson, J. D. (1968) *The Double Helix: A Personal Account of the Discovery of the Structure of DNA*. Atheneum Press, New York; b) Watson, J. D., Berry, A. (2003) DNA. *The Secret of Life*. William Heinemann, London; c) www.phy.cam.ac.uk/camphy/dna/dna13_1.htm and ... /dna14_1.htm.

[31] Brandstetter, H., Grams, F., Glitz, D., Lang, A., Huber, R., Bode, W., Krell, H. W., Engh, R. A. (2001) The 1.8-A crystal structure of a matrix metalloproteinase 8-barbiturate inhibitor complex reveals a previously unobserved mechanism for collagenase substrate recognition. *J. Biol. Chem.* **276**:17405-17412.

[32] Pospisil, P., Ballmer, P., ETH Zurich, Switzerland; www.pharma.ethz.ch/pc/Agent2/.

[33] Kubinyi, H., Hamprecht, F. A., Mietzner, T. (1998) Three-dimensional quantitative similarity-activity relationships (3D QSiAR) from SEAL similarity matrices. *J. Med. Chem.* **41**:2553-2564.

[34] Kearsley, S. K., Smith, G. M. (1990) An alternative method for the alignment of molecular structures: maximizing electrostatic and steric overlap. *Tetrahedron Comput. Methodol.* **3**:615-633.

[35] Klebe, G., Mietzner, T., Weber, F. (1994) Different approaches toward an automatic structural alignment of drug molecules: applications to sterol mimics, thrombin and thermolysin inhibitors. *J. Comput.-Aided Mol. Design* **8**:751-778.

[36] Lemmen, C., Lengauer, T. (1997) Time-efficient flexible superposition of medium-sized molecules. *J. Comput.-Aided Mol. Design* **11**:357-368; www.biosolveit.de; www.tripos.com.

[37] Lemmen, C., Lengauer, T. (2000) Computational methods for the structural alignment of molecules. *J. Comput.-Aided Mol. Design* **14**:215-232; www.biosolveit.de; www.tripos.com.

[38] Cramer III, R. D., Patterson, D. E., Bunce, J. D. (1988) Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. *J. Am. Chem. Soc.* **110**:5959-5967.

[39] a) Kubinyi, H. (Ed.)(1993) *3D QSAR in Drug Design*. Theory, Methods and Applications. ESCOM Science Publishers B.V., Leiden; b) Kubinyi, H., Folkers, G., Martin, Y. C. (Eds) (1998) *3D QSAR in Drug Design*. Ligand-Protein Complexes and Molecular Similarity, Vol.2. Kluwer/ESCOM, Dordrecht; also published as *Persp. Drug Discov. Design* 9-11: 1-416; c) Kubinyi, H., Folkers, G., Martin, Y. C. (Eds) (1998) *3D QSAR in Drug Design*. Recent Advances, Vol. 3. Kluwer/ESCOM, Dordrecht; also published as Persp. Drug Discov. Design 12-14: 1-352.

[40] Klebe, G., Abraham, U., Mietzner, T. (1994) Molecular similarity indices in a comparative analysis (CoMSIA) of drug molecules to correlate and predict their biological activity. *J. Med. Chem.* **37**:4130-4146.

[41] Sybyl / Unity, Tripos Inc., St. Louis, MO, U.S.A.; www.tripos.com.

[42] Goodford, P. J. (1984) Drug design by the method of receptor fit. *J. Med. Chem.* **27**:558-564.

[43] Kubinyi, H. (1998) Structure-based design of enzyme inhibitors and receptor ligands. *Curr. Opin. Drug Discov. Devel.* **1**:4-15.

[44] Redshaw, S. (1993) Angiotensin-converting enzyme (ACE) inhibitors and the design of Cilazapril. In: *Medicinal Chemistry. The Role of Organic Chemistry in Drug Research*. (Ganellin, C. R., Roberts, S. M. Eds), 2nd Edn, pp. 163-185. Academic Press, London.

[45] 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., Penn, C. R. (1993) Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature* **363**:418-423.

[46] Abdel-Magid, A. F., Maryanoff, C. A., Mehrman, S. J. (2001) Synthesis of influenza neuraminidase inhibitors. *Curr. Opin. Drug Discov. Devel.* **4**:776-791.

[47] 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., Stevens, R. C. (1997) 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.

[48] Veerapandian, P. (Ed.) (1997) *Structure-Based Drug Design*. Marcel Dekker, New York.

[49] Babine, R. E., Bender, S. L. (1997) Molecular recognition of protein-ligand complexes: applications to drug design. *Chem. Rev.* **97**:1359-1472.

[50] Gubernator, K., Böhm, H.-J.(Eds) (1998) Structure-Based Ligand Design. *Methods and Principles in Medicinal Chemistry.* (Mannhold, R., Kubinyi, H., Timmerman, H., Eds), Vol. 6. Wiley-VCH, Weinheim.

[51] Babine R. E., Abdel-Meguid, S. S. (2004) Protein Crystallography in Drug Discovery. *Methods and Principles in Medicinal Chemistry.* (Mannhold, R., Kubinyi, H., Folkers, G., Eds), Vol.20. Wiley-VCH, Weinheim.

[52] DesJarlais, R. L., Sheridan, R. P., Seibel, G. L., Dixon, J. S., Kuntz, I. D., Venkataraghavan, R.(1988) Using shape complementarity as an initial screen in designing ligands for a receptor binding site of known three-dimensional structure. *J. Med. Chem.* **31**:722-729.

[53] Böhm, H.-J. (1992) The computer program LUDI: a new method for the de novo design of enzyme inhibitors. *J. Comput.-Aided Mol. Design* **6**:61-78.

[54] Böhm, H.-J.(1992) LUDI: rule-based automatic design of new substituents for enzyme inhibitor leads. *J. Comput.-Aided Mol. Design* **6**:593-606.

[55] Böhm, H.-J. (1994) The development of a simple empirical scoring function to estimate the binding constant for a protein-ligand complex of known three-dimensional structure. *J. Comput.-Aided Mol. Design* **8**:243-256.

[56] Ewing, T. J., Makino, S., Skillman, A. G., Kuntz, I. D. (2001) DOCK 4.0: search strategies for automated molecular docking of flexible molecule databases. *J. Comput.-Aided Mol. Design* **15**:411-428.

[57] Jones, G., Willett, P., Glen, R. C., Leach, A. R., Taylor R. (1997) Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **267**:727-748.

[58] Rarey, M., Kramer, B., Lengauer, T., Klebe, G. (1996) A fast flexible docking method using an incremental construction algorithm. *J. Mol. Biol.* **261**:470-489; www.biosolveit.de; www.tripos.com.

[59] Lengauer, T., Rarey, M. (1996) Computational methods for biomolecular docking. *Curr. Opin. Struct. Biol.* **6**:402-406.

[60] Goodsell, D. S., Olson, A. J. (1990) Automated docking of substrates to proteins by simulated annealing. *Proteins* **8**:195-202.

[61] Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K., Olson, A. J. (1998) Automated docking using a Lamarckian genetic algorithm and and empirical binding free energy function. *J. Comput. Chem.* **19**:1639-1662.

[62] Schneider, G., Böhm, H.-J. (2002) Virtual screening and fast automated docking methods. *Drug Discov. Today* **7**:64-70.

[63] Claussen, H., Buning, C., Rarey, M., Lengauer, T. (2001) FlexE: efficient molecular docking considering protein structure variations. *J. Mol. Biol.* **308**:377-395; www.biosolveit.de; www.tripos.de.

[64] Hindle, S. A., Rarey, M., Buning, C., Lengauer, T. (2002) Flexible docking under pharmacophore type constraints. *J. Comput.-Aided Mol. Design* **16**:129-149; www.biosolveit.de; www.tripos.de.

[65] Hilpert, K., Ackermann, J., Banner, D. W., Gast, A., Gubernator, K., Hadvary, P., Labler, L., Müller, K., Schmid, G., Tschopp, T. B., van de Waterbeemd, H. (1994) Design and synthesis of potent and highly selective thrombin inhibitors. *J. Med. Chem.* **37**:3889-3901.

[66] Boehm, H.-J., Boehringer, M., Bur, D., Gmuender, H., Huber, W., Klaus, W., Kostrewa, D., Kuehne, H., Luebbers, T., Meunier-Keller, N., Mueller, F. (2000) Novel inhibitors of DNA gyrase: 3D structure based biased needle screening, hit validation by biophysical methods, and 3D guided optimization. A promising alternative to random screening. *J. Med. Chem.* **43**:2664-2674.

[67] Green, N. M. (1975) *Avidin. Adv. Protein Chem.* **29**: 85-133.

[68] Hanessian, S., Lu, P.-P., Sanceau, J.-Y., Chemla, P., Gohda, K., Fonne-Pfister, R., Prade, L., Cowan-Jacob, S. W. (1999) An enzyme-bound bisubstrate hybrid inhibitor of adenylosuccinate synthetase. *Angew. Chem. Int. Ed. Engl.* **38**:3159-3162.

[69] Shuker, S. B., Hajduk, P. J., Meadows, R. P., Fesik, S. W. (1996) Discovering high-affinity ligands for proteins: SAR by NMR. *Science* **274**:1531-1534.

[70] Hajduk, P. J., Meadows, R. P., Fesik, S. W. (1997) Discovering high-affinity ligands for proteins. *Science* **278**:497-499.

[71] 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., Fesik, S. W. (1997) Discovery of potent nonpeptide inhibitors of stromelysin using SAR by NMR. *J. Am. Chem. Soc.* **119**:5818-5827.

[72] Zerbe, O. (Ed.) (2003) BioNMR in Drug Research. *Methods and Principles in Medicinal Chemistry.* (Mannhold, R., Kubinyi, H., Folkers, G., Eds), Vol. 16. Wiley-VCH, Weinheim.

[73] Fejzo, J., Lepre, C. A., Peng, J. W., Bemis, G. W., Ajay, Murcko, M. A., Moore, J. M. (1999) The SHAPES strategy: an NMR-based approach for lead generation in drug discovery. *Chem. Biol.* **6**:755-769.

[74] Lepre, C. A., Peng, J., Fejzo, J., Abdul-Manan, N., Pocas, J., Jacobs, M., Xie, X., Moore, J. M. (2002) Applications of SHAPES screening in drug discovery. *Comb. Chem. High Throughput Screen* **5**:583-590.

[75] Diercks, T., Coles, M., Kessler, H. (2001) Applications of NMR in drug discovery. *Curr. Opin. Chem. Biol.* **5**:285-291.

[76] Pellecchia, M., Sem, D. S., Wüthrich, K. (2002) NMR in drug discovery. *Nature Rev. Drug Discov.* **1**:211-219.

[77] Jahnke, W., Floersheim, P., Ostermeier, C., Zhang, X., Hemmig, R., Hurth, K., Uzunov, D. P. (2002) NMR reporter screening for the detection of high-affinity ligands. *Angew. Chem. Int. Ed. Engl.* **41**:3420-3423.

[78] Meyer, B., Peters, T. (2003) NMR spectroscopy techniques for screening and identifying ligand binding to protein receptors. *Angew. Chem. Int. Ed. Engl.* **42**:842-890.

[79] Kubinyi, H. (1998) Combinatorial and computational approaches in structure-based drug design. *Curr. Opin. Drug Discovery Dev.* **1**:16-27.

[80] Metz, G., Ottleben, H., Vetter, D. (2003) Small molecule screening on chemical microarrays. In: Protein-Ligand Interactions. From Molecular Recognition to Drug Design, (Böhm, H.-J., Schneider, G., Eds) *Methods and Principles in Medicinal Chemistry.* (Mannhold, R., Kubinyi, H., Folkers, G., Eds), Vol.19, pp.213-236. Wiley-VCH, Weinheim.

[81] Huc, I., Lehn, J.-M. (1997) Virtual combinatorial libraries: dynamic generation of molecular and supramolecular diversity by self-assembly. *Proc. Natl. Acad. Sci. USA* **94**:2106-2110; erratum in *Proc. Natl. Acad. Sci. USA* **94**:8272.

[82] Lehn, J.-M., Eliseev, A. V. (2001) Dynamic combinatorial chemistry. *Science* **291**: 2331-2332.

[83] Ramström, O., Lehn, J.-M. (2002) Drug discovery by dynamic combinatorial libraries. *Nature Rev. Drug Discov.* **1**:26-36.

[84] Hochgürtel, M., Kroth, H., Piecha, D., Hofmann, M. W., Nicolau, C., Krause, S., Schaaf, O., Sonnenmoser, G., Eliseev, A. V. (2002) Target-induced formation of neuraminidase inhibitors from in vitro virtual combinatorial libraries. *Proc. Natl. Acad. Sci. USA* **99**:3382-3387.

[85] Erlanson D. A., Braisted, A. C., Raphael, D. R., Randal, M., Stroud, R. M., Gordon, E. M., Wells, J. A. (2000) Site-directed ligand discovery. *Proc. Natl. Acad. Sci. USA* **97**: 9367-9372.

[86] Erlanson, D. A., Lam, J. W., Wiesmann, C., Luong, T. N., Simmons, R. L., DeLano, W. L., Choong, I. C., Burdett, M. T., Flanagan, W. M., Lee, D., Gordon, E. M., O'Brien, T. (2003) *In situ* assembly of enzyme inhibitors using extended tethering. *Nature Biotechnol.* **21**:308-314.

[87] Maly, D. J., Choong, I. C., Ellman, J. A. (2000) Combinatorial target-guided ligand assembly: identification of potent subtype-selective c-Src inhibitors. *Proc. Natl. Acad. Sci. USA* **97**:2419-2424.

[88] Swayze, E. E., Jefferson, E. A., Sannes-Lowery, K. A., Blyn, L. B., Risen, L. M., Arakawa, S., Osgood, S. A., Hofstadler, S. A., Griffey. R. H. (2002) SAR by MS: a ligand based technique for drug lead discovery against structured RNA targets. *J. Med. Chem.* **45**:3816-3819.

[89] Lewis, W. G., Green, L. G., Grynszpan, F., Radic, Z., Carlier, P. R., Taylor, P., Finn M. G., Sharpless, K. B. (2002) Click chemistry *in situ*: acetylcholinesterase as a reaction vessel for the selective assembly of a femtomolar inhibitor from an array of building blocks. *Angew. Chem. Int. Ed. Engl.* **41**:1053-1057.

[90] Wolber, G., Langer, T. (2001) Combigen: a novel software package for the rapid generation of virtual combinatorial libraries. In: *Rational Approaches to Drug Design*. (Höltje, H.-D., Sippl, W., Eds), pp.390-399. (Proceedings of the 13th European Symposium on Quantitative Structure-Activity Relationships, Düsseldorf, 2000. Prous Science, Barcelona.

[91] Schneider, G., Clement-Chomienne, O., Hilfiger, L., Schneider, P., Kirsch, S., Boehm, H.-J., Neidhart, W. (2000) Virtual screening for bioactive molecules by evolutionary de novo design. *Angew. Chem. Int. Ed. Engl.* **39**:4130-4133.

[92] Schneider, G., Lee, M. L., Stahl, M., Schneider, P. (2000) De novo design of molecular architectures by evolutionary assembly of drug-derived building blocks. *J. Comput.-Aided Mol. Design* **14**:487-494.

[93] Lewell, X. Q., Judd, D. B., Watson, S. P., Hann, M. M. (1998) RECAP -retrosynthetic combinatorial analysis procedure: a powerful new technique for identifying privileged molecular fragments with useful applications in combinatorial chemistry. *J. Chem. Inf. Comput. Sci.* **38**:511-522.

[94] Schneider, G., Neidhart, W., Giller, T., Schmid, G. (1999) "Scaffold-Hopping" by Topological Pharmacophore Search: A Contribution to Virtual Screening. *Angew. Chem. Int. Ed. Engl.* **38**:2894-2896.

[95] Wang, R., Lu, Y., Wang, S. (2003) Comparative evaluation of 11 scoring functions for molecular docking. *J. Med. Chem.* **46**:2287-2303.

[96] Bleicher, K. H., Böhm, H.-J., Müller, K., Alanine, A. I. (2003) Hit and lead generation: beyond high-throughput screening. *Nature Rev. Drug Discov.* **2**: 369-378.

[97] Muchmore, S. W., Hajduk, P. J. (2003) Crystallography, NMR and virtual screening: integrated tools for drug discovery. *Curr. Opin. Drug Discov.Dev.* **6**:544-549.

[98] Kubinyi, H. (1998) Similarity and dissimilarity: a medicinal chemist's view, In: 3D QSAR in Drug Design. *Ligand-Protein Interactions and Molecular Similarity.* (Kubinyi, H., Folkers G., Martin, Y. C., Eds), Vol. II, pp.225-252. Kluwer/ESCOM, Dordrecht,; also published in *Persp. Drug Design Discov.* 9-11:225-252.

[99] Martin, Y. C., Kofron, J. L., Traphagen, L. M. (2002) Do structurally similar molecules have similar biological activity? *J. Med. Chem.* **45**:4350-4358.

[100] Caron, P. R., Mullican, M. D., Mashal, R. D., Wilson, K. P., Su, M. S., Murcko, M. A. (2001) Chemogenomic approaches to drug discovery. *Curr. Opin. Chem. Biol.* **5**:464-470.

[101] Bleicher, K. H. (2002) Chemogenomics: bridging a drug discovery gap. *Curr. Med. Chem.* **9**:2077-2084.

[102] Jacoby, E., Schuffenhauer, A., Floersheim, P. (2003) Chemogenomics knowledge-based strategies in drug discovery. *Drug News Perspect.* **16**:93-102.

[103] Müller, G. (2003) Medicinal chemistry of target family-directed masterkeys. *Drug Discov. Today* **8**: 681-691.

[104] Kubinyi, H., Müller, G. (Eds) (2004) Chemogenomics in Drug Discovery - A Medicinal Chemistry Perspective. *Methods and Principles in Medicinal Chemistry.* (Mannhold, R., Kubinyi, H., Folkers, G. Eds), Vol.22. Wiley-VCH, Weinheim.

[105] Wermuth, C. G. (2001) The "SOSA" aproach: an alternative to high-throughput screening. *Med. Chem. Res.* **10**:431-439.

[106] Wermuth, C. G. (2004) Selective optimization of side activities: another way for drug discovery. *J. Med. Chem.* **47**:1303-1314.

[107] Kubinyi, H. (2004) Drug discovery from side effects. In: *Chemogenomics in Drug Discovery - A Medicinal Chemistry Perspective* (Kubinyi, H., Müller, G., Eds)f Methods and Principles in Medicinal Chemistry. (Mannhold, R., Kubinyi, H., Folkers, G., Eds), Vol.22, pp.43-67. Wiley-VCH, Weinheim.

[108] Prentis, R. A., Lis, Y., Walker, S. R. (1988) Pharmaceutical innovation by the seven UK-owned pharmaceutical companies (1964-1985). *Br. J. Clin. Pharmac.* **25**:387-396.

[109] Kennedy, T. (1997) Managing the drug discovery/development interface. *Drug Discov. Today* **2**:436-444.

[110] van de Waterbeemd, H., Gifford, E. (2003) ADMET in silico modelling: towards prediction paradise? *Nature Rev. Drug Discov.* **2**:192-204.

[111] Horrobin, D. F. (2003) Modern biomedical research: an internally self-consistent universe with little contact with medical reality? *Nature Rev. Drug Discov.* **2**:151-154.

[112] Ryan, J. F. (Ed.) (2000) *The Pharmaceutical Century. Ten Decades of Drug Discovery.* Supplement to ACS Publications, American Chemical Society, Washington, DC.

[113] Wermuth, C. G. (Ed.) (2003) *The Practice of Medicinal Chemistry*, 2nd Edn. Academic Press, London.