Structure-based design of enzyme inhibitors and receptor ligands

Hugo Kubinyi

Address
Combinatorial Chemistry and Molecular Modelling
ZHF/G - A30
BASF AG
D-67056 Ludwigshafen
Germany
Email: HUGO.KUBINYI@MSM.BASF-AG.DE

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With the ongoing progress in protein crystallography and NMR, structure-based drug design is adopting increasing importance in the search for new drugs. Modeling starts from the 3D structure of a target protein in order to construct molecules which are complementary to a binding site, in their geometry as well as in the pattern of their physicochemical properties around the molecules. The rational design process is accompanied by 3D structure determinations of different ligand-protein complexes. Most often, significantly improved binding affinities of the ligands are observed after several cycles of 3D structure determinations, the design of compounds with appropriate structural modifications, synthesis, and testing of the new drug candidates. As an alternative, pharmacophore models are derived from the 3D structures of active analogs. A risk with lead structure optimization by structure-based design is the neglect of other important biological properties, such as bioavailability and metabolic stability. Recent applications of structure-based design, as well as success stories in the search for new, potent and selective HIV protease inhibitors, thrombin inhibitors, neuraminidase inhibitors and integrin receptor antagonists, are reviewed.

Introduction

Traditionally, leads for new drugs resulted from the accidental observation of the biological effects of natural products and from screening organic compounds; serendipity played an important role in drug research. Later, the structures of endogenous effector molecules, such as neurotransmitters and hormones, were taken as templates to design new receptor agonists and antagonists. As early as 1973, a structure-based design of protein ligands was performed. Beddell and Goodford utilized the 3D structure of the 2,3-diphosphoglycerate (2,3-DPG) complex of hemoglobin 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 analogs with significantly improved affinities to DHFR [1]. However, neither the hemoglobin ligands nor the trimethoprim analogs could be optimized to become drugs for human therapy.

The first success story in structure-based design was the antihypertensive drug, captopril (**1**, Capoten®, Lopirin®, Squibb, now Bristol-Myers Squibb; Figure 1), an angiotensin-converting enzyme (ACE) inhibitor. Its structure was derived in a rational manner from a binding site model, using the 3D information of the complex of benzylsuccinate with the closely related zinc proteinase carboxypeptidase A [2••].

With the ongoing progress in protein crystallography and multidimensional NMR studies, the 3D structures of many important proteins, especially enzymes, have been determined (commented on the Web site, **http://www.biochem.ucl.ac.uk/ bsm/pdbsum/** [3••]). This information led to the structure-based design of many other enzyme inhibitors, most of which are still in preclinical or clinical development, but some have already been introduced into human therapy, eg, the carboanhydrase inhibitor, dorzolamide (**2**, Trusopt®, Merck & Co; Figure 1), an antiglaucoma agent [4•,5•], and the HIV protease inhibitors, saquinavir (**3**, Invirase®, Hoffmann-La Roche), indinavir (**4**, Crixivan®, Merck & Co), ritonavir (**5**, Norvir®, Abbott Laboratories) and nelfinavir (**6**, Viracept®, Agouron Pharmaceuticals; Figure 1) [6•].

All major drug companies currently apply structure-based design as an important technique in their search for new drugs. Some start-up companies, such as Agouron Pharmaceuticals, Vertex [7••], and several others, exclusively select biological targets where structure-based and computer-aided drug design can be applied in order to increase the rate of success and to speed up the lead finding and optimization cycles.

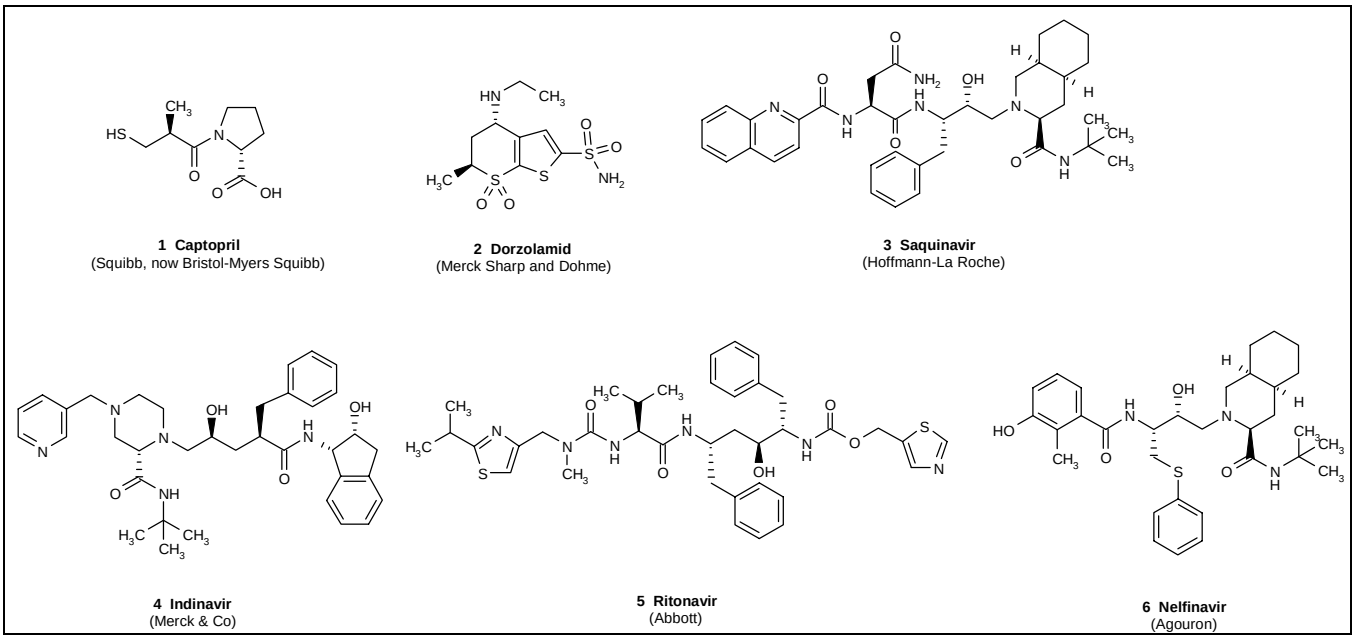
Protein 3D structure-based drug design

The most important factors for a favorable interaction between a drug and its specific biological target, most often a protein, are a perfect geometric fit of the ligand to the binding site, both being in low-energy conformations, a correspondence of the molecular electrostatic potentials, the formation of charged and/or neutral hydrogen bonds between functional groups, and hydrophobic interactions between lipophilic surfaces [8••]. Whilst the hydrophobic interactions always increase binding affinities (sometimes reducing aqueous solubility), the contribution of hydrogen bonds to the overall free binding energy depends on the balance of the desolvation energies and the energies of the newly formed hydrogen bonds. Changing a single functionality of a ligand may have very complex consequences [8••,9••,10••].

In the design cycle, the information from the 3D structure of the target protein or, even better, from ligand-protein complexes, is used to design new ligands with improved binding affinities. After synthesis and testing, the underlying hypotheses on the structure-activity relationships are modified and a new design cycle starts. In optimal cases (compare with the later section on thrombin inhibitors), ligands with nanomolar affinities result after several design cycles. A weak point of the structure-based ligand design is the fact that other important properties of a drug are neglected; the mere optimization of ligand affinities may lead to compounds with insufficient bioavailability or metabolic stability.

The focus of this review is not so much on a comprehensive description of all of the different applications of structure-

Figure 1.



Captopril (**1**) was developed in the 1970s, using a binding site model derived from the 3D structure of the benzylsuccinate-carboxypeptidase A complex. The carboanhydrase inhibitor, dorzolamide (**2**, Merck & Co), is the first drug for human therapy (market launch 1995), which resulted from a mere structure-based design; the HIV-1 protease inhibitors, saquinavir (**3**), indinavir (**4**), ritonavir (**5**) and nelfinavir (**6**), followed in the years 1995 to 1997.

based design (compare eg, [9••,11•]), but on a presentation of several typical, and successful, examples from recent literature. An excellent comprehensive review [9••] and three books [5•,10••,11•] on structure-based design have been published within the last two years; some other reviews [12,13•,14,15•,16•] discuss important aspects of structure-based design. Table 1 gives an overview of recent applications of these techniques in the rational design of enzyme inhibitors and other protein ligands, including some examples of structure-based design without knowledge of the protein 3D structure (compare with the later section on drug design based on ligand 3D structures).

HIV-1 protease inhibitors

HIV-1 protease is one of the most important proteins involved in the replication of the AIDS virus. It processes two of the three gene products of the AIDS virus to functionally active proteins. Inactivation of HIV protease by site-directed mutagenesis leads to non-infectious HIV mutants. In the few years since the first 3D structures of HIV protease were published in 1989, the inhibitors, **3-6**, were developed by structure-based design, preclinically and clinically tested, and introduced into human therapy (Figure 1) [6•].

Several fortunate circumstances came together to achieve this success. Research on AIDS therapy had much publicity and was generously supported by governmental funds. For many years, drug companies had searched for inhibitors of the aspartic protease, renin [5•,10••,11••]. As soon as the 3D structure of HIV protease [58•] became available, several companies shifted their activities to this new, rewarding target. The first HIV protease inhibitors had structural similarity to the peptide sequence of the substrate, bearing the statin partial structure, >N-CH(R)-CH(OH)-CH_2-N<, instead of a scissile amide bond. The second generation inhibitors, **3-6**, are true peptido-mimetics, with fewer amide bonds. However, some structural resemblance to the peptide leads can still be recognized. In addition, they all contain elements of the statin partial structure.

Research carried out by DuPont Merck demonstrates an example of a straightforward rational design, starting from a pharmacophore hypothesis and a 3D database search for analogs bearing this pharmacophore (Figure 2) [19••,59••]. Compound **7** was a hit, which suggested that a methoxy group could replace a structural water molecule, the so-called 'flap water', in the HIV protease complex. The initial concept for the design of a nonpeptide inhibitor was structure **8**. Analogs **9** and **10** (Figure 2) followed as leads, providing additional hydrogen bonds between the inhibitor and the enzyme; P1, P1', P2 and P2' are benzyl, substituted benzyl and naphth-2-yl-methyl groups. Several clinical candidates with nanomolar affinities and favorable pharmacokinetic properties resulted from this approach [19••].

A serious problem in AIDS therapy is the large genetic variability of the virus, leading to approximately one error per 10,000 base pairs per virus replication cycle. Thus, the emergence of resistance is a serious problem and limits the therapeutic usefulness of such drugs. One strategy to solve the problem, the combined application of two or even three drugs with different mechanisms of action, is already employed. Another attractive approach is the development of AIDS drugs which are less sensitive to the development

Table 1. Some recent applications of structure-based drug design.

LIGANDS AND PROTEINS	REFERENCES
PROTEIN 3D STRUCTURE-BASED DESIGN	
Aspartic proteinase inhibitors	
Renin	5•,10••,11•
HIV protease	2••,4•,5•,6•,9••, 10••,11•,13•,15•, 17,18,19••,20, 21
Serine proteinase inhibitors	
Thrombin	5•,9••,10••,11•, 22-24,25••
Factor Xa	11•,23, 26,27•
Elastase	2••,5•,9••,10••, 28
β-Lactamase	5•,9••
Cysteine proteinase inhibitors	9••,29•,30••
Matrix metalloproteinase inhibitors	9••,10••,11•,31, 32•,33•
Other enzyme inhibitors	
Aldose reductase	11•,34,35
Carbonic anhydrase	4•,5•,9••,10••, 13•
Dihydrofolate reductase	1,10••
Glycolytic enzymes	1,11•
Neuraminidase (sialidase)	5•,9••,11•,13•, 15•, 35,36•,37,38•
Protein kinases	11•
Purine nucleoside phosphorylase	2••,9••,11•,13•, 39••,40•,41,42•
Reverse transcriptase	5•,11•,43
Thymidylate synthase	2••,4•,10••,13•, 41,44,45
Various parasitic proteinases	29•,46
Other protein ligands	
FKBP-binding protein	9••,47
Rhinoviral coat proteins	11•
PHARMACOPHORE MODEL-DERIVED AND LIGAND 3D STRUCTURE-BASED DESIGN	
Metalloproteinase inhibitors	
Angiotensin-converting enzyme	2••,10••,13•,48
Neutral endopeptidase 24.11	2••,13•,48
Endothelin-converting enzyme	49,50
Other enzyme inhibitors	
Protein tyrosine kinases	51•
HIV-1 integrase	11•,52
Receptor antagonists	
Integrin receptors	53-55,56•,57

of viral resistance because the ligand interacts with the protein backbone and the catalytic aspartates which form the invariant parts of the protease. An interesting application of this concept resulted from research at DuPont Merck [60,61]. Out of a total of 14 hydrogen bonds in the complex of the inhibitor, **11**, to HIV-1 protease (Figure 2), eight interactions are to backbone -NH- and >C=O groups, and four are to the catalytic aspartate side chains of the HIV protease. In addition, there are numerous favorable van-der-Waals contacts between the aromatic rings and the hydrophobic parts of the binding site. Inhibitors of this type are highly active against wild-type HIV and maintain the same, or even improved, levels of potency against a range of HIV mutant strains with resistance to a wide variety of other HIV protease inhibitors [60]. Due to the good steric fit and the excellent complementarity, the six-membered cyclic urea, **11**, has a picomolar affinity to HIV protease [61].

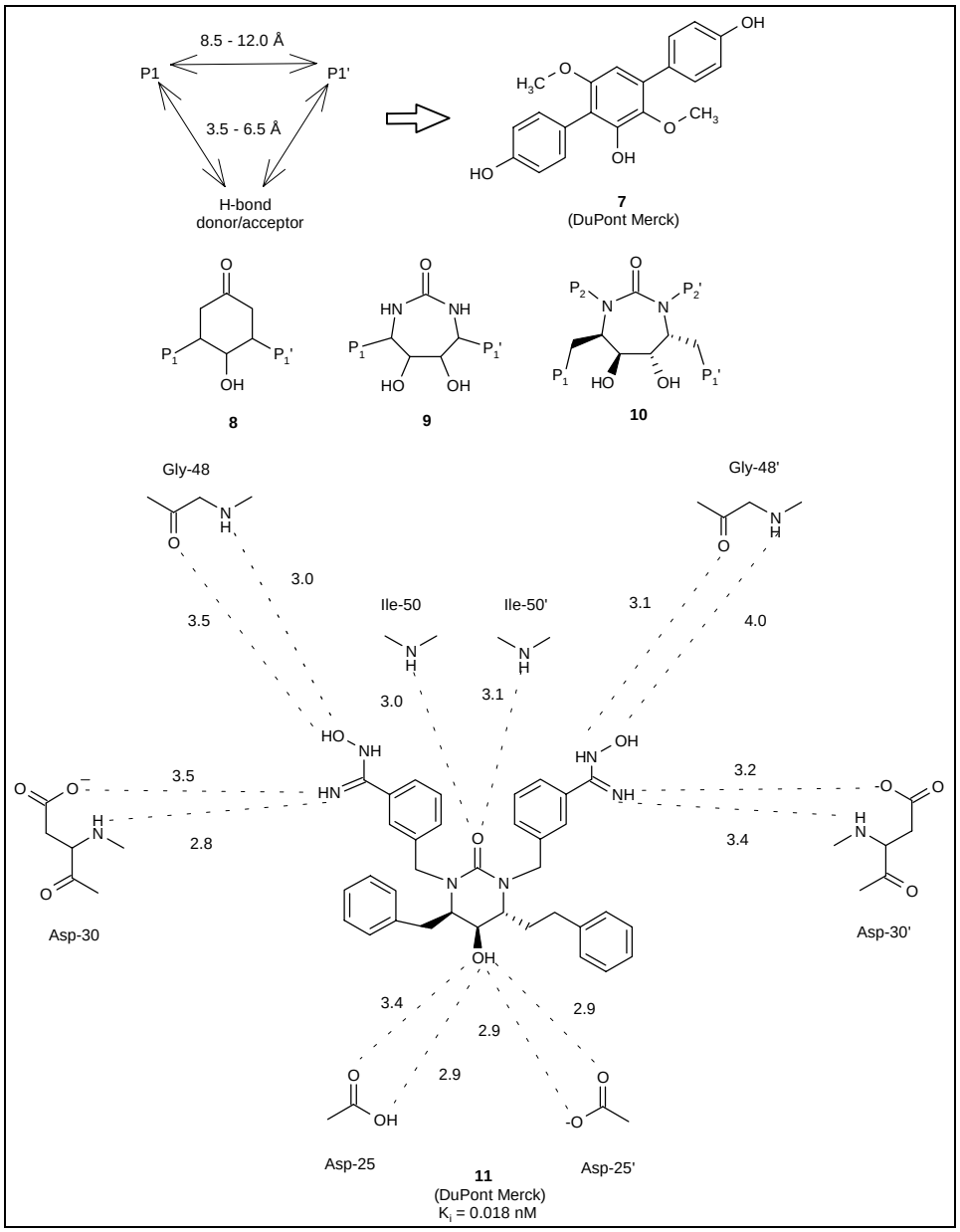
Thrombin inhibitors

Several aspects of structure-based and computer-aided drug research can be illustrated by the search for potent, selective and bioavailable thrombin inhibitors. Thrombin plays a central role in blood coagulation, by mediating the cleavage of fibrinogen to fibrin which together with blood platelets and erythrocytes results in the formation of an insoluble clot. This physiological process is desirable in wound healing, but is life-threatening in stroke, cardiac insult and other diseases with an increased tendency to blood coagulation.

Although the first thrombin inhibitors were derived from the structures of different substrates by classical strategies, all recent efforts are based on the thrombin 3D structure [5•,9••,10••,11•,22-24,25••]. Scientists at Merck & Co started with a natural product which was isolated from the marine sponge *Theonella*. Cyclotheonamide (**12**) [22,24] is a cyclic peptide with a Pro-Arg sequence and a β-diketone moiety (Figure 3), which readily forms a hemiketal with the hydroxyl group of the catalytically active Ser-195 of thrombin [62]. A first lead structure, **13** (Figure 3) [24,63], including several of these structural elements in a much simpler molecule, was not only very active but also highly selective, compared to its action on the homologous serine protease, trypsin. Removal of the β-diketone partial structure led to a significant reduction in biological activity which was, nevertheless, acceptable because it could be compensated by other structural variations. Analog **14** is a noncovalent inhibitor and, in addition, its low molecular weight made it a valuable lead for analogs with oral bioavailability [63]. In the next step of lead structure optimization, combinatorial chemistry was applied. Amide formation with 200 different organic acids, selected from a total of 8,000 candidate molecules, gave, within a few months, the hydroxyfluorene carbonamide, **15** [24,64], as the most promising analog, with almost no oral activity in the rat, but excellent bioavailability in the dog (Figure 3). However, the development of **15** was discontinued. In the next step, a systematic search for alternative P1 elements was performed, which resulted in several highly active 2-amino-pyridyl and non-basic analogs [65,66]. The replacement of proline by a pyridone ring system, a structural modification which had already proved successful in the design of elastase inhibitors [2••,5•,9••,10••], led to the inhibitor, **16** [67-69]. The 2-amino-6-methylpyridine analog, **17** [69], is a chemically stable, selective, subnanomolar thrombin inhibitor with good oral bioavailability; in contrast to many other analogs, it contains no chiral center.

Research scientists from the Korean company, LG Chemical, started their project from the observation that in certain series of thrombin inhibitors the introduction of an additional amino group into the amidine reduces biological activity, whereas in others, the so-called TAPAP series,

Figure 2.

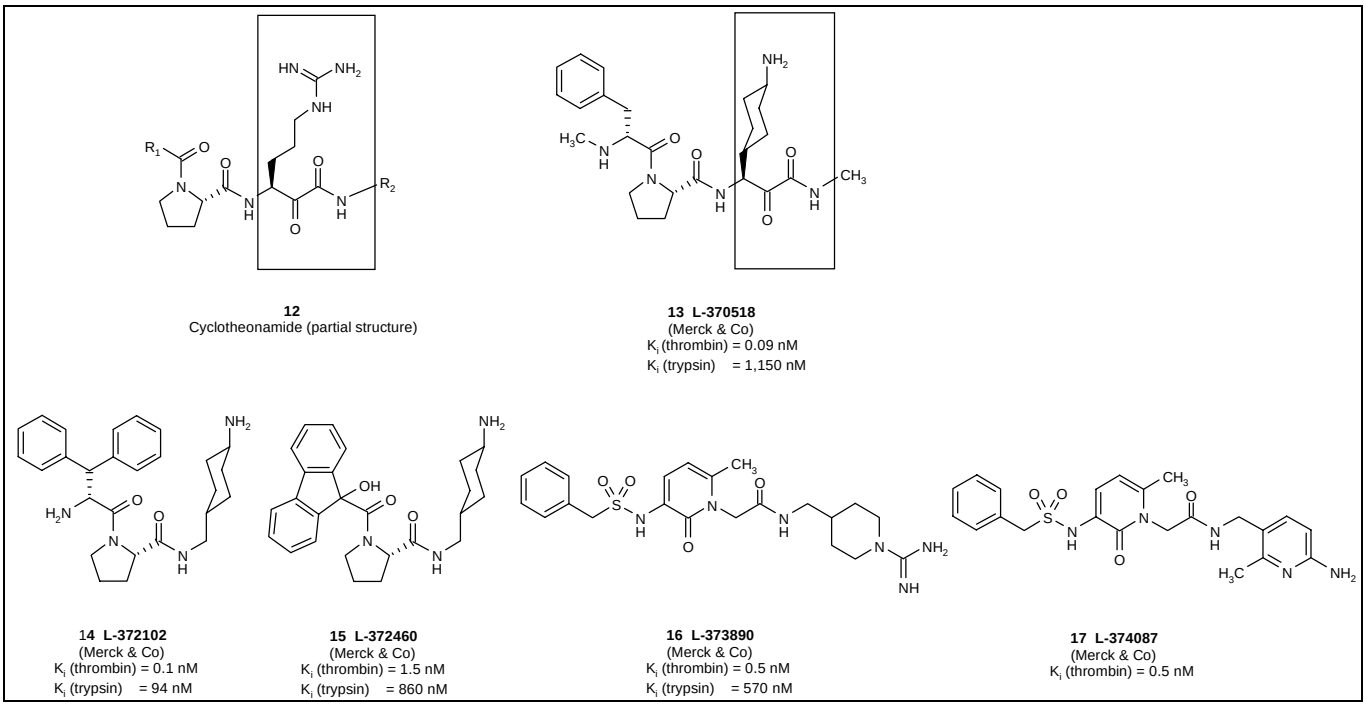


Cyclic urea HIV protease inhibitors resulted from a pharmacophore hypothesis (upper left). A first lead, **7**, was discovered by a 3D database search. The inhibitors, **8**-**10**, where P1, P1', P2 and P2' are different aralkyl groups, were intermediates in the design of the picomolar inhibitor, **11**.

affinity as well as selectivity against trypsin were significantly enhanced [70a,70b]. Correspondingly, the conversion of the amidine, **18**, into the amidrazone, **19**, increases the thrombin affinity by about two orders of magnitude, whereas trypsin affinity is reduced by a factor of 4 to 5 (Figure 4), resulting in a 600-fold increase of thrombin selectivity [70a,70b].

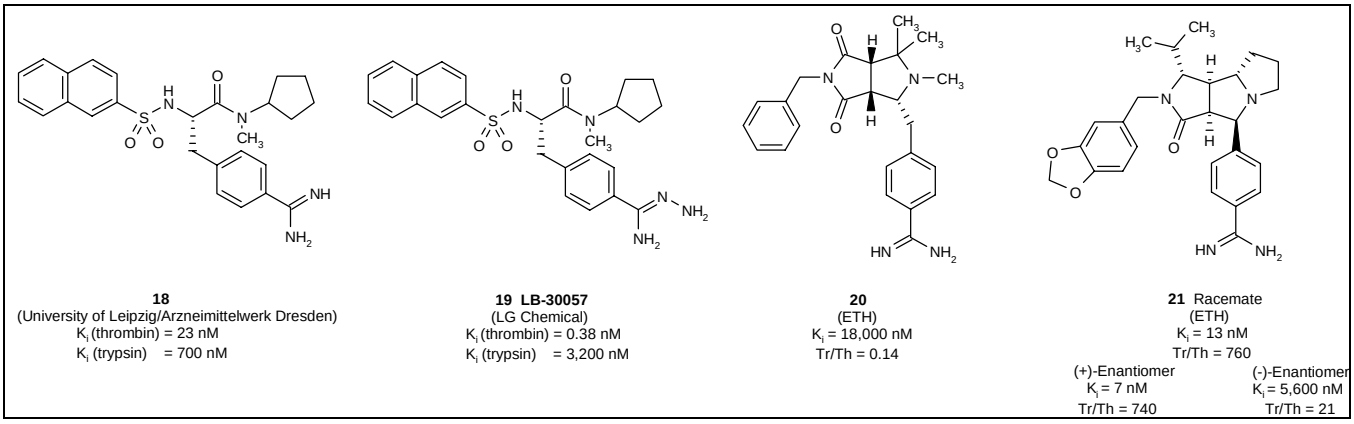
A very interesting *de novo* design of thrombin inhibitors was realized by Ulrike Obst and Francois Diederich at the ETH in Zurich, Switzerland. They began with a rigid bicyclic core structure, accessible via 1,3-dipolar cyclo addition. The first lead structure, **20** (Figure 4), showed micromolar affinity to thrombin but insufficient selectivity. In the next design cycle, an enantiomeric phenyl-substituted analog, instead of the benzyl-substituted compound, showed even better inhibitory activity. This serendipitous discovery resulted from two synthetic shortcuts: firstly, the phenyl analog was planned as a more easily accessible reference compound and secondly, the compounds were synthesized and tested as racemates. A third design cycle yielded the nanomolar, highly selective thrombin inhibitor, **21** (Figure 4) [71••].

Figure 3.



The partial structure **12** of cyclotheonamide served as a starting point for the stepwise development of the thrombin inhibitors, **13**-**17**. Whereas **13** still possesses most of the structural features of this partial structure, **14** and **15** lack the α-keto carbonamide part. The final optimization products, compound **16** and the orally active inhibitor **17**, are no longer related to the original lead structure.

Figure 4.



The conversion of the TAPAP-type thrombin inhibitor, **18**, into its amidrazone analog, **19**, significantly increased the thrombin affinity and the selectivity versus trypsin. The bicyclic thrombin inhibitor, **20**, resulted from a *de novo* design; optimization in two design cycles yielded the nanomolar inhibitor, **21**.

Neuraminidase inhibitors

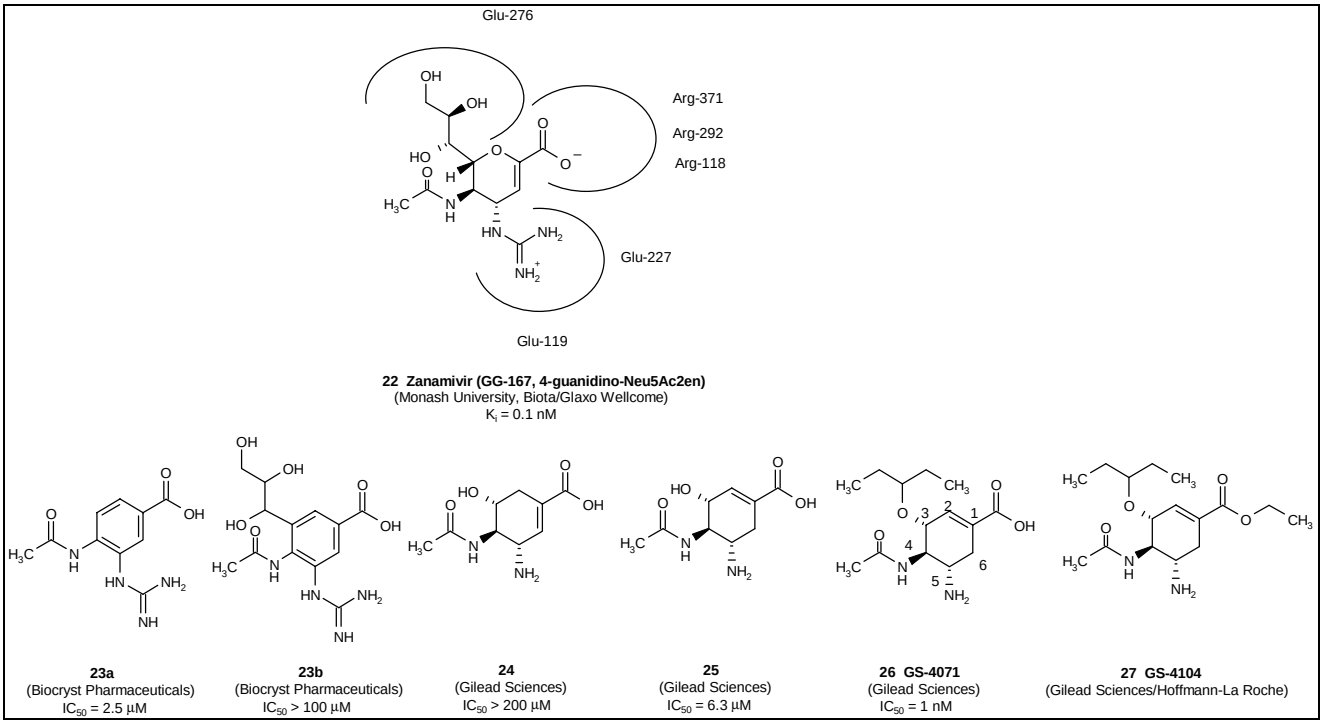
In contrast to the common cold, influenza is a serious, potentially deadly disease. Between 1918 and 1919, the 'Spanish flu' killed 22 million people, ie, twice as many as the number of victims of the First World War. Even nowadays, influenza is one of the ten most common causes of death in the US, killing about 20,000 persons per year. To date, no efficient protection or treatment against new strains of the influenza virus are available. Thus, there is always the latent danger of a new pandemic.

Neuraminidase, which is also known as sialidase, is an essential influenza viral coat enzyme. It cleaves sialic acid from the carbohydrate side chains at the surface of the cells, thus enabling the virus to penetrate the polar outer cell surfaces of the respiratory tract. In 1983, the determination of the 3D structure of neuraminidase provided, for the first time, a target for the structure-based design of active anti-influenza agents. In an elegant study, Mark von Itzstein (Monash University, Australia) investigated the binding site

of the protein and determined interaction energies with different probes [72••] using the computer program, GRID. He predicted that the introduction of basic groups, such as $-NH_2$ or $-C(=NH)NH_2$, into the relatively weak inhibitor, Neu5Ac2en, should significantly improve inhibitory activities. This is indeed the case: the neuraminidase inhibitor, **22**, is about five orders of magnitude more active than its 4-unsubstituted analog, Neu5Ac2en [35,36•,37, 38•,72••,73]. The binding mode of **22** (Figure 5) shows interactions between the new amidinium group and two glutamate side chains. Zanamivir (**22**) is orally inactive but can be applied as a nasal spray. It is now under broad clinical investigation and the first clinical results look promising, despite a relatively short duration of action and the evolution of resistant strains after *in vitro* selection. Of greatest importance is the need for relatively early application of the drug, preferably as soon as possible after the first symptoms of illness are observed.

Usually, one should expect that a drug such as zanamivir could not be improved upon. Research by Gilead Sciences, however, has proved otherwise. The scientists there started from the observation that the typical glycerol side chain does not contribute to affinity in the simple aromatic analogs, **23a** and **23b**; its introduction even destroys biological activity [74]. From modeling results, it was expected that the synthetically easily-accessible carbocyclic analog, **24**, should be more active than its isomer, **25**; however, the opposite result was obtained. Another surprising observation was the fact that 3-alkoxy substitution produced highly active analogs. Starting with small alkyl groups, an optimal inhibitory activity was observed for the branched pent-3-yl analog, **26**, GS-4071 [75••]. As compared to zanamivir, GS-4071 has an identical binding mode, but the pockets which accommodate the glycerol side chain of **22** and the pentyl group of **26** look very different. Whereas the carboxylate group of Glu-276 forms two hydrogen bonds to the glycerol hydroxyl groups of **22**, it is forced to orient this pocket outwards in the neuraminidase complex of **26**; side chain methylene groups of Arg-224 and Glu-276, as well as the side chains of Ile-222 and Ala-246, form a perfect hydrophobic pocket - a serendipitous discovery and a gift of mother nature to kill influenza viruses!. The ethyl ester prodrug, **27**, GS-4104, has good oral bioavailability and is in clinical development in collaboration with Hoffmann-La Roche. The speed of the development of this new, promising drug is remarkable: Gilead commenced the rational design in 1994, with first leads obtained in early 1995, and the inhibitor, GS-4071, in late 1995; GS-4104 was developed in March 1996. During preclinical investigations, the cooperation contract with Hoffmann-La Roche was signed and clinical investigations began in mid-1997.

Figure 5.



The introduction of a guanidino group into the weakly active neuraminidase inhibitor, Neu5Ac2en, led to an increase of inhibitory activity by a factor of 10,000; zanamivir (**22**), originated at Monash University and later developed at Biota, is in clinical development with Glaxo Wellcome. Aromatic analogs, **23a** and **23b**, gave the first hint that a removal or replacement of the glycerol side chain could produce active analogs. The carbocyclic Neu5Ac2en analogs, **24** and **25**, show significantly different biological activities. Optimization of the alkoxy residue produced a nanomolar inhibitor, **26**, of neuraminidase; its prodrug, **27**, is in clinical development.

Drug design based on ligand 3D structures

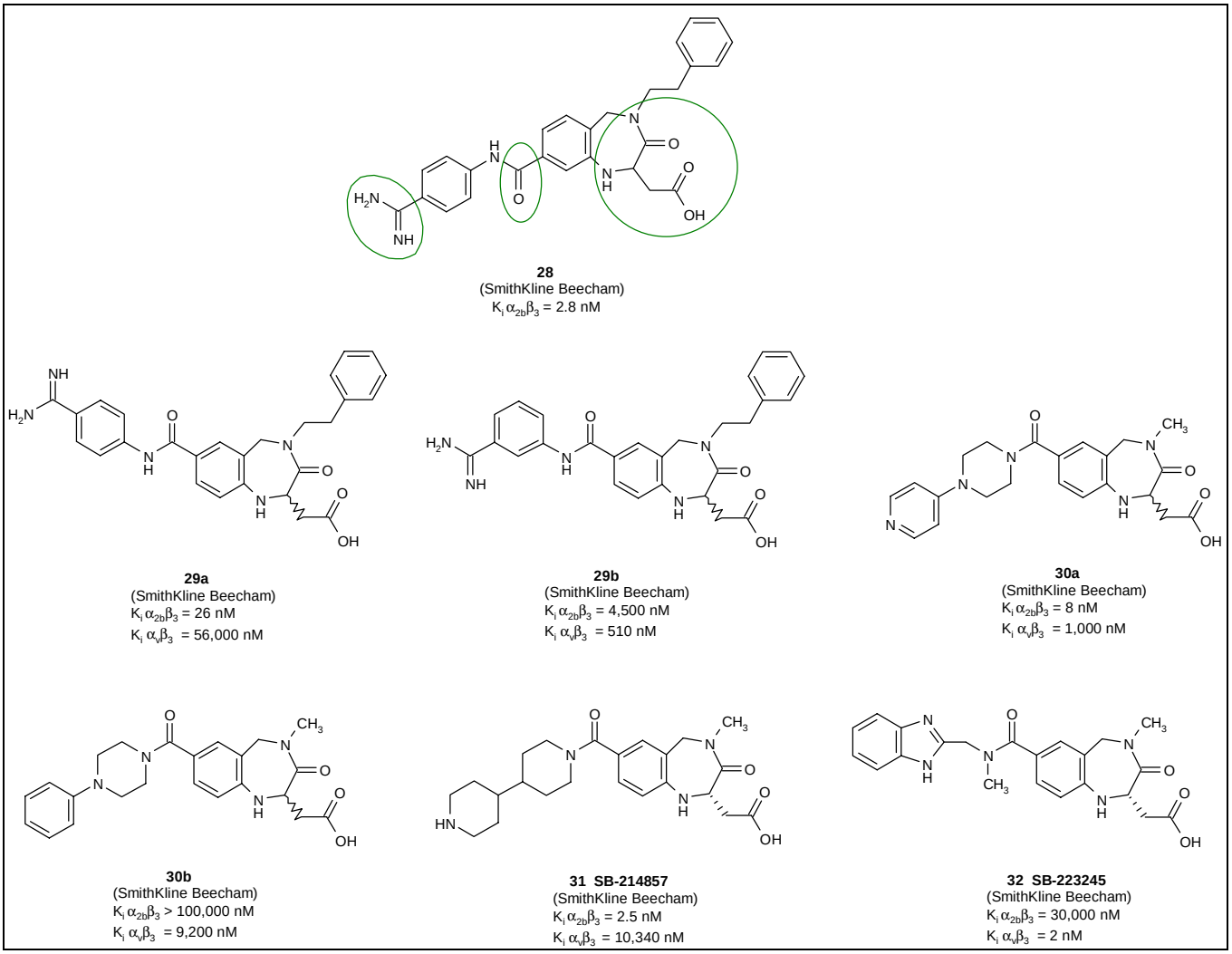
Sometimes, only the 3D structures of enzymes or receptor ligands are known, especially in the case of agonists or antagonists of membrane-embedded receptors. It is beyond the scope of this review to discuss the different modeling approaches which use 2D or 3D structures of ligands to design new analogs with improved properties [2••,10••,76••]. In certain cases, a straightforward design process starts from conformationally restricted natural receptor ligands, such as from polypeptides or proteins. Under such fortunate circumstances, the success rate is comparable to that of 'real' structure-based design, as is demonstrated by the example given below.

The design of selective integrin receptor ligands

Besides the G protein-coupled receptors, which are the biological targets of neurotransmitters and several non-peptide and most peptide hormones, there are the integrin receptors, which are another large and therapeutically significant group of membrane-embedded receptors. They mediate the aggregation of cells, such as thrombocytes [53,57], or the adherence of cells to the extracellular matrix [54,55,56•]. All integrin receptors are made up of an α-chain and a β-chain. Due to several different α- and β-chains, a multitude of combinations result. Some of these receptors, namely the GPIIb/IIIa ($\alpha_{2b}\beta_3$) receptor and the vitronectin ($\alpha_v\beta_3$) receptor, already play an important role in the search for new drugs.

Whereas 3D structures of these receptors are still unavailable, the binding motifs of the natural ligands are well known. They all contain an RGD motif, ie, an Arg-Gly-Asp sequence in a certain 3D conformation. The NMR-spectroscopic investigation of diastereomeric cyclic pentapeptides showed significant differences in their

Figure 6.



The peptidomimetic $\alpha_{2b}\beta_3$ receptor antagonist, **28**, 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 position of the amidine group in **29a** and **29b** and the presence or absence of the nitrogen atom in **30a** and **30b** produce significantly different receptor selectivities; the $\alpha_{2b}\beta_3$ -selective receptor antagonist, **31**, and the $\alpha_v\beta_3$-selective receptor antagonist, **32**, were derived from these observations. The analogs, **31** and **32**, differ in their selectivity by nearly eight orders of magnitude.

binding affinities [56•]. Whereas cyclo-(Arg-Gly-Asp-Phe-D-Val), RGDFv (v = D-Val), is a high-affinity ligand of the GPIIb/IIIa receptor (K_i = 2 nM), its isomer, RGDfV (f = D-Phe), binds with high specificity to the $\alpha_v\beta_3$ receptor (K_i $\alpha_{2b}\beta_3$ = 42,000 nM; K_i $\alpha_v\beta_3$ = 10 nM) [56 ,77]. NMR studies and biological results indicated that an extended conformation of the RGD motif produces $\alpha_{2b}\beta_3$ selectivity, whereas a turn around the Gly, ie, a slightly bent conformation, is responsible for $\alpha_v\beta_3$ selectivity. The benzodiazepine, **28** (Figure 6), was derived from modeling studies, comparing cyclic peptides with peptidomimetics. It contains the essential structural features of the RGD motif in an extended conformation; correspondingly, it is a high-affinity ligand for the GPIIb/IIIa receptor.

In accordance to the different conformations of the model peptides, cyclo-RGDFv and cyclo-RGDfV, the isomers, **29a** and **29b** (*meta*- and *para*-amidine groups) and **30a** and **30b** (pyridyl and phenyl substituents), show significantly different receptor specificities [77]. Further structural variation produced the $\alpha_{2b}\beta_3$-selective receptor antagonist, **31** [78], and the $\alpha_v\beta_3$-selective receptor antagonist, **32** [77]; the selectivities of these two analogs differ by nearly eight orders of magnitude (Figure 6).

Conclusions

In rational drug design, several basic assumptions are made. First of all, the analogs within a series are supposed to act via the same biological mechanism, a precondition which sometimes is not fulfilled. Isosteric replacement of atoms or groups is performed with the expectation that the resulting effects are more or less obvious. The isosteric replacement of atoms may have a significant but hardly predictable effect on the biological activities. In some cases, conformational restrictions may stabilize the bioactive conformation, while for other structures some additional energy may be required to adopt such a conformation. For ligands causing an allosteric effect, such as receptor agonists, biological activity cannot be expected to be a simple function of the binding affinity. Finally, the overall affinity of a drug is by no means only a function of its enthalpic interactions. Entropy plays an additional, important role.

Pharmacological testing of compounds has shifted from animal to *in vitro* models. Whilst there are unquestionable advantages caused by this development, some major problems can also arise. Many diseases have multifactorial causes which cannot be tested in a simple *in vitro* system. Absorption, distribution, metabolism and excretion of drug candidates are investigated in only a few compounds and thus, structural optimization often neglects these factors. Some side-effects of drugs can only be observed in animals but not in *in vitro* models.

Ligand design is not drug design! Many companies have learned a painful lesson in this respect. Poor bioavailability resulting from peptide-like structures with too many polar groups, or from too many hydrophobic groups in the molecule, killed off many drug candidates which were highly active *in vitro* but inactive in cell systems and *in vivo*. To avoid such problems, increasing efforts are now being made to consider ADME (absorption, distribution, metabolism, excretion) parameters in the early phases of lead optimization. Simple rules are applied, as well as screening tests for bioavailability, eg, cell culture models for intestinal absorption and blood-brain barrier permeation. Microsomal and liver cell preparations are used to predict drug metabolism in different species, and short term toxicity models to extrapolate toxic side-effects.

In recent years, the paradigms of drug discovery have changed significantly. Due to its interdisciplinary character, involving chemistry, molecular biology, biochemistry, pharmacology, toxicology, and medicine, drug research is almost exclusively performed in industry. Even small venture capital companies, who give evidence that drug research and development can be done in a university-like environment, either grow to become larger companies, such as Agouron and Vertex, or they are absorbed by major competitors. Today, the pharmaceutical industry reacts rapidly to new developments. All companies, worldwide, have already shifted, or are going to shift, a larger part of their capacities from classical chemistry to automated syntheses of combinatorial libraries, from classical design to structure-based and computer-assisted methods, from *in vivo* and small-scale *in vitro* screening to faster, fully automated, high-throughput screening. Cooperations and mergers of companies have led to a concentration in drug research which will continue in the future.

Despite the enormous efforts of drug companies, there has been a steady decline in the number of drugs introduced into human therapy, from approximately 60 to 70 new chemical entities (NCE) per year, in the decade between 1970 and 1979, to about 50 NCEs per year, between 1980 and 1989, and 38 to 44 in the years between 1990 and 1995; with a slight increase observed very recently, ie, 52 in 1996 and 56 in 1997 [79•]. In parallel, the costs of drug research and development increased to about 300-350 million US$ per new drug. Every additional year of drug development is a waste of resources, with money being spent and revenue lost due to late marketing and patent expiry. Thus, time becomes an important factor in drug development. As the first clinical trials determine the potential of a new drug, most companies attempt to arrive at this stage faster than before. Several drug candidates are developed in parallel, in an effort to avoid the failure of a whole program if a single compound gives a negative result in its first application to humans. Phase II trials are now more carefully planned, to avoid serious problems in phase III, the most time- and cost-consuming phase in drug development.

In the future, the success of drug companies will depend on their size, on the skill and motivation of their coworkers and on the flexibility of their organization. Those companies, which adapt their strategies of research in an appropriate manner, will have a better chance to discover and develop new, valuable drugs.

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