The impact of structure-guided drug design on clinical agents

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Structure-based or structure-guided drug design methods have had a significant impact on the creation of high-value compounds entering the market as drugs, or at least entering clinical trials. This report provides an update on the utility of structure-guided methods for creating compounds that have reached human testing. Seven such compounds are now approved and marketed drugs.

For several decades, academic and industrial scientists have used the 3D atomic structures of proteins, derived mostly from macromolecular crystallographic studies, as design templates for small molecule ligands. In some cases, a targeted protein structure is used directly. In other cases, the template is a 'homology model,' based upon an experimental study of a structural homolog of the actual target. Here we look at the impact of structure-guided drug design (SGDD) on drug discovery and development.

Utility of structure-guided methods

Table 1 lists 40-plus compounds, discovered with the aid of structure-guided methods, that have entered clinical trials. These drugs and drug candidates are directed against two-dozen different molecular targets, in a wide range of therapeutic areas, although over half of the compounds are used for oncology or viral infections. There may even be additional compounds that we have overlooked in our survey of information in the public domain. At the time of writing (August 2003), seven of these compounds have become approved and marketed drugs. Captopril was the first drug whose discovery relied upon the explicit use of X-ray structural information as a guide for small molecule design. This occurred over two decades ago. Although the template used for discovery of captopril was a homology model (based on the X-ray structure of bovine carboxypeptidase A), the recognition of the 'heuristic' value of such a model was a landmark. A similar conceptual model of acetylcholinesterase

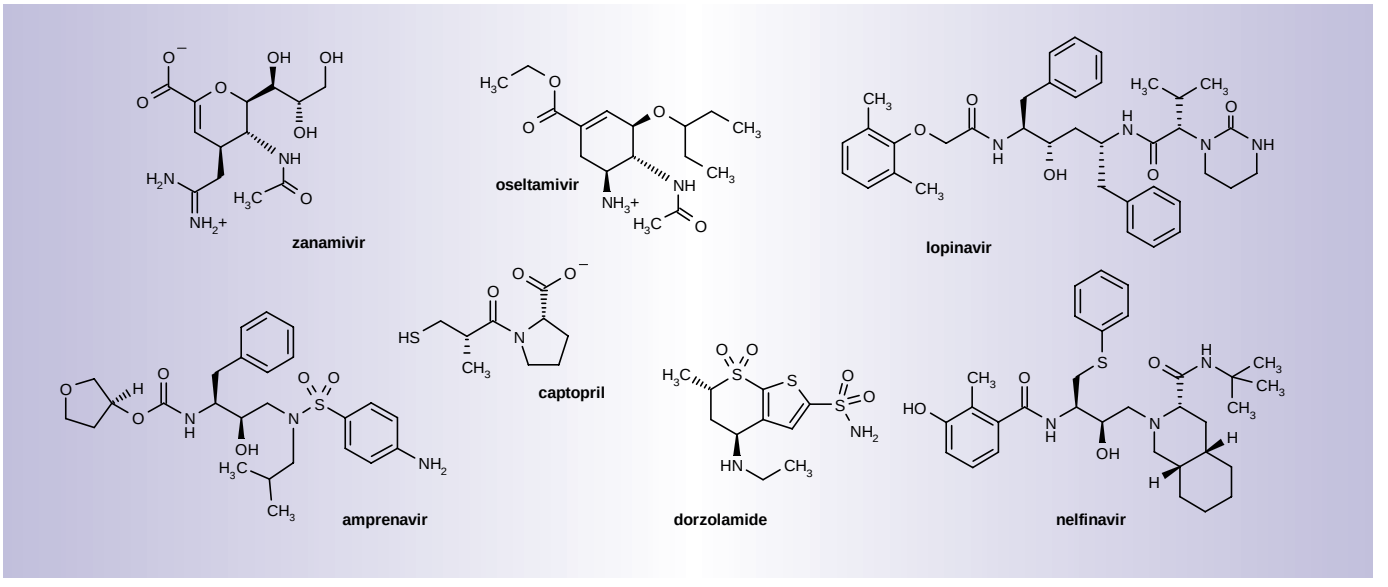

Figure 1. Approved and marketed drugs whose discovery has been aided by structure-guided design methods.

(based on the experimental X-ray structure of chymotrypsin) was used for the development of the agent zanapezil.

Two of the drugs (captopril and dorzolamide; see Figure 1) have now become generic products. The largest fraction of approved drugs developed by structure-guided methods is the set of HIV protease inhibitors, which were pivotal during the late 1990s in converting HIV infection from a death sentence into a chronic disease.

Potency

Potency (high affinity) for a molecular target is the sine qua non for the development of a new drug. The ideal of using a high-resolution structure of a target protein to design the perfect ligand is a challenging goal.

It is still very difficult to accurately predict relative binding affinities for closely related compounds based upon computational analysis of structural interactions alone, although useful correlations are attainable over a large order of magnitude. The structural plasticity of proteins is partially responsible. The discovery of the inhibitor of which oseltamivir is a prodrug exemplifies the recognition and exploitation of such unpredictable flexibility; in this example, viral neuraminidase was the flexible target. Accurate calculation of interaction energies also suffers from the complex electronic structures of both proteins and small molecules, and from their difficult-to-model solvation in aqueous solutions. The power of SGDD lies in its ability to rapidly generate robust hypotheses that can be tested in iterative cycles, with experimentally determined co-crystal structures and physiologically relevant bioassays, using small sets of new compounds provided by clever synthetic chemists. The structures are more powerful indicators of which part of an existing molecule should NOT be altered, rather than what should be made, in the pursuit of improved potency. But co-crystal structures also empower medicinal chemists with direct visualization of new potential interactions that may be explored synthetically.

For almost all of the drugs and clinical candidates listed in Table 1, the application of the SGDD approach began with a small molecule that already had some affinity for the target protein. Completely de novo design of a lead molecule that produced a clinical candidate is clearly documented for only one compound in Table 1 (AG-331, entry 41). Thus, SGDD has had its greatest impact in optimization, rather than in hit or lead generation. This may change if and when the predictive accuracy of computational methods increases.

X-ray crystallography

Almost all of the structural information for these drugs and drug candidates was derived from X-ray crystallography. Although NMR-based approaches have become more powerful and more widely used in drug discovery in the past several years, they have had minimal impact on currently existing drugs and drug candidates. An exception to this generalization is provided by the fibrinogen receptor antagonist elarofiban (entry 8). The lead molecules that led to elarofiban were designed to mimic the conformation of a portion of fibrinogen when bound to its receptor. Those conformations were derived from NMR studies of fibrinogen peptides, not structural studies of the receptor itself. Hence, the structural information cannot explicitly guide which atoms in the molecular scaffold should not be substituted during optimization to preserve interactions with the target (receptor). This distinction between crystallographic and NMR approaches need not be generally the case, since NMR can now directly probe target protein - small molecule interactions in favorable cases. However, the impact of such NMR methods has been delayed because of the long intrinsic time required for protein structures to be determined via NMR.

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Selectivity

A rule-of-thumb in medicinal chemistry is that the more potent an inhibitor or agonist is, the greater its selectivity. This rule is based on the supposition that each therapeutic target differs in structural details at the atomic level, so that the more perfectly a ligand fits one target, the less well it will fit any other. This rule may have decreased relevance, however, when a therapeutic target belongs to a protein family whose members all contain a highly conserved active site (eg, protein kinases). However, the success of Gleevec has shown that it is possible for kinase inhibitors that bind near the ATP binding site to possess sufficient selectivity to be useful drugs. Despite this success, the close structural similarity between the active sites of multiple family members remains a serious concern for drug discovery efforts targeting protein kinases and for similar efforts that target other such protein families.

A possible road to selectivity is to use high-throughput approaches to solve the structures of as many family members as possible, so that the desired selectivity can be designed 'in' by designing 'out' affinity for counter-targets. The plasticity of protein active sites and the difficulty in making accurate predictions of exact affinities makes this questionable. Moreover, it is not necessary to have the X-ray structures of all the counter-targets that must be avoided for SGDD to increase the likely selectivity of a compound. Structural 'prospecting' studies at Boehringer Ingelheim led to early recognition that a screening hit against p38 MAP kinase had a novel mode of binding to an inactive conformation of the kinase. The compound also had weak cell-based activity and a novel structure. The fact that binding of the hit compound required a pronounced conformational change suggested that this binding mode might be target-specific.

Table 1. Drugs and drug candidates developed using SGDD methods.

Entry	Target	Compound	Therapeutic utility	Status[1]	Company
1	Acetylcholinesterase	Zanapezil (TAK-147)	Alzheimer's disease	Phase III ⇨ discontinued	Takeda
2	Aldose reductase	Lidorestat (IDD-676)	Diabetic neuropathy	Phase II ⇨ discontinued	Institute for Diabetes Discovery
3	Angiotensin converting enzyme	Capoten (captopril; Q-14225)	Hypertension	Approved & marketed, 1981	Bristol-Myers Squibb
4	Carbonic anhydrase	Trusopt (dorzolamide; MK-507; L-671, L-152)	Glaucoma	Approved & marketed, 1995	Merck
5	Caspase-1	Pralnacasan (VX-740)	Rheumatoid & osteo arthritis	Phase II	Vertex
6	Cyclin-dependent kinase	BMS-387032	Cancer	Phase I	Bristol-Myers Squibb
7	erbB (EGF receptor) tyrosine kinase	Canertinib (CI-1033)	Cancer	Phase II	Pfizer
8	Fibrinogen receptor	Elarofiban (RWJ-53308)	Thrombosis	Phase IIa ⇨ discontinued	Johnson & Johnson
9	Glycinamide ribonucleotide formyl-transferase	AG-2034	Cancer	Phase I ⇨ discontinued	Agouron ⇨ Pfizer
10	Glycinamide ribonucleotide formyl-transferase	AG-2037	Cancer	Phase I	Agouron ⇨ Pfizer
11	Hemaglobin	Efaproxiral (RSR-13)	Radiosensitizer	Filed in US	Allos Therapeutics
12	HIV reverse transcriptase	TMC-125 (R-165335)	HIV	Phase I/II	Janssen
13	HIV protease	Viracept (nelfinavir; AG-1343)	HIV	Approved & marketed, 1999	Agouron and Lilly ⇨ Pfizer
14	HIV protease	Agenerase (amprenavir)	HIV	Approved & marketed, 1999	Vertex
15	HIV protease	Aluviran (ABT-378, lopinavir)	HIV	Approved & marketed, 2000	Abbott
16	HIV protease	tipranavir	HIV	Phase IIb/III	Pharmacia ⇨ Boehringer Ingelheim
17	HIV protease	Mozenavir (DMP-450)	HIV	Phase I/II ⇨discontinued	DuPont Merck ⇨ Gilead
18	IMP dehydrogenase	Merimepodib (VX-497)	HCV	Phase II	Vertex
19	IMP dehydrogenase	VX-148	Psoriasis	Phase II	Vertex
20	Influenza	Relenza; neuraminidase Flunet (zanamivir)	Influenza	Approved & marketed, 1999	Monash University ⇨ Glaxo Wellcome
21	Influenza neuraminidase	Tamiflu (oseltamivir; GS-4104; prodrug of GS-4071)	Influenza	Approved & marketed, 1999	Gilead ⇨ Roche
22	Influenza neuraminidase	Peramivir (BCX-1812; RWJ-270201)	Influenza	Phase III ⇨ discontinued	BioCryst
23	Matrix metallo-proteinase	Prinomastat (AG-3340)	Cancer	Phase III	Agouron ⇨ Pfizer
24	P38α MAP kinase	Doramapimod (BIRB-796)	Rheumatoid arthritis	Phase IIb/III	Boehringer Ingelheim
25	P38α MAP kinase	VX-745	Rheumatoid arthritis	Phase II ⇨ discontinued	Vertex
26	P38α MAP kinase	VX-702	Acute coronary syndromes	Phase IIa	Vertex
27	Peroxisome proliferator-activated receptor-γ	GW-501516	Hyperlipidemia	Phase I	GlaxoSmithKline

Table 1. Continued.

Entry	Target	Compound	Therapeutic utility	Status[1]	Company
28	Phospholipase (non-pancreatic)	LY-315920	Severe sepsis	Phase IIb ⇨ discontinued	Lilly
29	Picornavirus coat proteins	Picovir (pleconaril; WIN-63843)	Picornavirus infections	Phase III ⇨ NDA not approved[3]	Winthrop ⇨ ViroPharma
30	Picornavirus coat proteins	WIN-54954	Picornavirus infections	Phase II ⇨ discontinued	Winthrop
31	Picornavirus coat proteins	Disoxaril (WIN-51711)	Picornavirus infections	Phase I ⇨ discontinued	Winthrop
32	Purine nucleoside phosphorylase	Peldesine (BCX-34)	Cancer	Phase III ⇨ discontinued	Biocryst
33	Purine nucleoside phosphorylase	BCX-1777	Cancer	Phase I/II	Biocryst
34	Receptor protein tyrosine kinases	SU-6668	Cancer	Phase II	Sugen ⇨ Pfizer
35	Renin	Aliskiren	Hypertension	Phase II	Novartis
36	Rhinovirus-3C protease	AG-7088	Rhinovirus infection	Phase IIb/III	Aguoron ⇨ Pfizer
37	Thrombin	BIBR-1048 (prodrug of BIBR- 953)	Thrombosis	Phase IIb/III	Boehringer Ingelheim
38	Thrombin	aminochloropyrazinone, pyridine N-oxide (no generic name released)	Thrombosis	Phase I	Merck
39	Thrombin	aminochloropyrazinone (no generic name released)	Thrombosis	Phase I	Merck
40	Thymidylate synthase	Thymitaq (nolatrexed; AG-337)	Cancer	Phase III	Agouron ⇨ Eximias
41	Thymidylate synthase	AG-331	Cancer	Phase I ⇨ discontinued	Agouron ⇨ Pfizer
42	Thymidylate synthase	AG-85	Psoriasis	Phase I ⇨ discontinued (1993)	Agouron

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[1] Most advanced stage attained and current status, if available. [2] For entries that have no publication cited, see the first paper listed in the futher reading box for references to the relevant primary literature. The chemical structures of all the compounds listed are available in a PDF file at *www.aurigene.com/newsroompress.asp*. [3] In 2002, the US FDA did not approve the ViroPharma new drug application for oral dosing of Picovir for treatment of the 'common cold'. ViroPharma is pursuing the utility of the compound for more narrow indications.

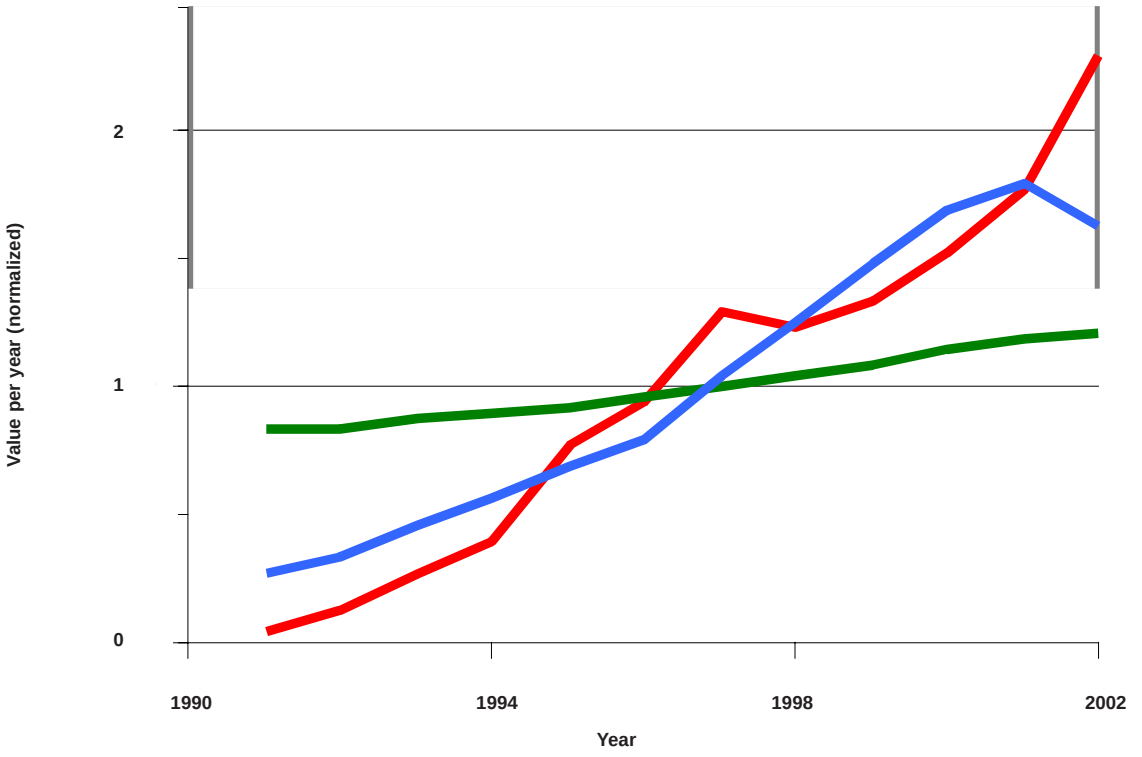

Figure 2. The plots show the number of publications within the PubMed database that used the term 'structure-based design' (in red) or 'therapeutic' (in green), and the number of X-ray structures (deposited within the indicated year) in the Protein Data Bank (in blue). The values were normalized by dividing the number of citations (structures) in each year by the mean annual value over the 12 years analyzed.

This idea was confirmed when that hit was converted by iterative SGDD into one of the most selective protein kinase inhibitors ever designed (entry 24 in Table 1).

Physicochemical and PK properties

Structural studies of target protein-lead molecule interactions cannot per se improve the low aqueous solubility or the metabolic instability of problem compounds. However, co-crystal structures reveal which target interactions are crucial to preserve potency, and which parts of the lead molecule structure can be altered to improve solubility, metabolic stability, or other ADME-relevant properties. This structural knowledge, used in parallel with direct assessments of how those properties change, allows much more rapid generation of new lead molecules with improved pharmacokinetic behavior than would be possible at random. Again, the structures guide the synthetic chemistry.

Driving PK

An early example of this process in action was in the design of the carbonic anhydrase inhibitor, dorzolamide, for topical treatment of glaucoma. Other carbonic anhydrase inhibitors were administered orally for many years to reduce intraocular pressure, but produced undesirable systemic side effects. Development of a more amphiphilic inhibitor, which was more lipid soluble but retained high potency and water solubility, was aided by the availability of carbonic anhydrase-inhibitor co-crystal structures. The enhanced lipid solubility increased membrane penetration enough to allow topical application. The co-crystal structures guided the manipulation of ligand stereochemistry, allowing the optimization of both physicochemical properties and target potency, and ultimately yielding the chiral drug, dorzolamide.

"Cleverly applied, SGDD can be used to address a variety of DMPK issues, as well as attacking the problem of drug resistance. The utilization and impact of SGDD will surely continue to increase."

The first designed inhibitor of viral neuraminidase to become a drug, zanamivir, is not orally bioavailable, and must be inhaled as an aerosol. One of the efforts to make more stable neuraminidase inhibitors produced puzzling SAR. Conformational flexibility of the target, as noted above, was the culprit. This was uncovered by X-ray structural studies, and led to the sytheses of derivatives that allowed the development of oseltamivir at Gilead and of peramavir at Biocryst. Both agents are orally active against influenza. Crystal structures were also used to guide the metabolism-directed optimization of several thrombin inhibitors (entries 38 and 39) recently developed at Merck.

Resistance

The efficacy of HIV protease inhibitors and of non-nucleoside reverse transcriptase inhibitors have both been compromised by the rapid emergence of drug-resistant mutants of HIV. In both cases, the mutations have decreased the affinities of the drugs for their targets by altering the drug-binding site. SGDD has been used in this case to design new compounds whose affinities do not depend upon interaction with target residues that are altered in the mutant viruses. This approach has yielded both lopinavir, an HIV protease inhibitor approved for worldwide use just 5 years after its discovery, and TMC-125, an HIV reverse transcriptase inhibitor in phase II trials. These compounds are effective against strains of HIV that are resistant to previous drugs. Lopinavir is dosed with another HIV protease inhibitor, ritonavir, to slow its metabolic clearance and the combination is marketed as Kaletra. Extensive clinical studies of Kaletra have documented significantly slower emergence of drug resistance than was found with first-generation HIV protease inhibitors.

Technology acquisition

A metric for the value of a new technology is the degree to which it is adopted throughout an industry. A pioneering company in creating and applying SGDD methods was Agouron Pharmaceuticals. Agouron, established in 1984, was acquired in 1999 by Warner Lambert, and hence became part of Pfizer in 2000. In the same year, Vertex agreed to provide access to its SGDD platform to Novartis Pharmaceuticals in a deal that may be worth up to $800 million for Vertex.

Another company that developed a strong technology platform for SGDD, 3D Pharmaceuticals, was recently acquired by Johnson and Johnson. Other pharmaceutical companies have built structural biology capabilities internally. Most of the top 20 (and all of the top 10) global pharmaceutical companies (as ranked by Contract Pharma based upon product revenues for 2001) have structural biology groups that are actively generating structures of therapeutic target proteins by NMR and/or X-ray diffraction methods.

At Boehringer Pharmaceuticals, for example, a third of the phase IIb/III candidates in the development pipeline in 2003 are derived from structure-guided approaches. These facts clearly indicate the importance of SGDD approaches for drug discovery within the pharmaceutical industry.

Another indicator of the impact of structure-based design approaches (see Figure 2) is the frequency of publications in the PubMed database with abstracts that contain the term 'structure-based design'.

Clever use of SGDD

High-resolution determination of atomic structures of drug target proteins is a well-proven method to speed drug discovery. Integration of SGDD approaches into the discovery process has allowed the creation of a wide variety of drugs and drug candidates. However, SGDD requires more than structure determinations. To be most effective, it must be used within a broad medicinal chemistry framework. Cleverly applied, SGDD can be used to address a variety of DMPK issues, as well as attacking the problem of drug resistance. The utilization and impact of SGDD will surely continue to increase.

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FURTHER READING

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FURTHER INFORMATION

For excellent presentations on SGDD *home.t-online.de/home/kubinyi/lectures.html*

Information on drugs in clinical trials *www.clinicaltrials.gov/ct/gui*
www.phrma.org/newmedicines