1
From Traditional Medicine to Modern Drugs: Historical Perspective of Structure-Based Drug Design

1.1
Introduction

The drug design and discovery process of today is a highly interdisciplinary research endeavor [1–3]. Advances in molecular biology, synthetic chemistry, and pharmacology, as well as technological breakthroughs in X-ray crystallography and computational methods have brought dramatic changes to medicinal chemistry practices during the late twentieth century. Drug design efforts based upon the three-dimensional structure of a target enzyme have become the hallmark of modern molecular design strategies. This structure-based design approach has revolutionized the practice of medicinal chemistry and recast the preclinical drug discovery process. Many of the FDA-approved drugs have evolved through structure-based design strategies. By 2012, as many as 35 newly approved drugs have emanated from structure-based design. The post-genomic era holds huge promise for the advancement of structure-based design of drugs for new therapies. Human genome sequencing has now revealed that there are an estimated 20,000–25,000 protein-coding human genes, and each gene can code for one protein. These proteins are responsible for carrying out all the cellular functions in the human body. These proteins can also be involved in disease pathologies, providing unique opportunities and challenges for structure-based design of new drugs. It may be appropriate to review briefly how the first half of the twentieth century was shaped and enriched by a number of seminal discoveries and the advent of new technologies, all of which left an important imprint on today's drug discovery and medicinal chemistry. A number of previous reviews have provided some insight [4,5].

1.2
Drug Discovery During 1928–1980

The history of medicinal chemistry is marked by examples in which the discovery of novel drugs relied upon serendipity and clinical observations. It is interesting to consider the role of chance in unexpected and accidental scientific discoveries. This serendipity is not simply luck. Rather, it is a process of finding significance

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and value in the lucky coincidence. As Pasteur observed, "Chance favors the prepared mind." Without engaging creative thinking and analysis, accidents do not lead to discoveries. The discovery of penicillin is a famous example of serendipity [6,7]. Alexander Fleming departed for a vacation in the summer of 1928. He left a bacterial culture of *Staphylococcus aureus* on his laboratory bench. When he returned a month later, he found that the culture was contaminated by a patch of blue-green mold that caused the lysis of bacteria. Fleming later demonstrated that the mold, *Penicillium notatum*, produced an active ingredient that he called penicillin. The discovery of penicillin was particularly fortunate since the penicillin that landed on Fleming's bacterial culture was not ordinary *Penicillium*! If it were, it would not have produced penicillin in high enough concentrations to cause the lysis of bacteria.

The discovery of penicillin was not mere luck. Much more subsequent investigation was required before it could be used as an antibiotic. More than a decade later in the 1940s, Howard Florey and Ernest Chain, with their Oxford team, unveiled its therapeutic potential. During this time, fermentation methods were developed that allowed the effective application of penicillins (Figure 1.1) for the treatment of bacterial infections in humans. Bactericidal penicillin rapidly replaced the bacteriostatic sulfonamide drugs used until then for the treatment of some bacterial infections.

The discovery of bacteriostatic sulfonamides has its own interesting story of serendipity and intuition [8]. The dye industry was advanced and promoted chemical manufacturing to develop new dyes. German chemists working with azo dyes observed that certain dyes could preferentially stick to and stain bacterial colonies. Could this serve as a way to target bacteria? In 1935, the German biochemist Gerhard Domagk, assisted by a group of chemists, synthesized and tested hundreds of dyes and finally discovered, by a trial-and-error approach, the potent

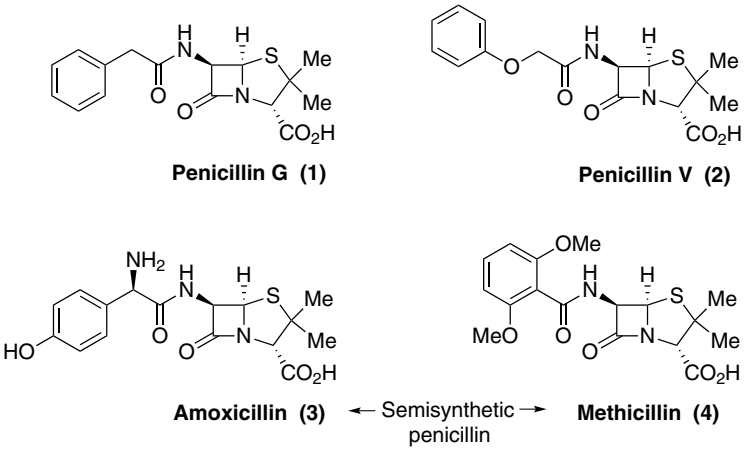


Figure 1.1 Structures of penicillins G and V and semisynthetic penicillins.

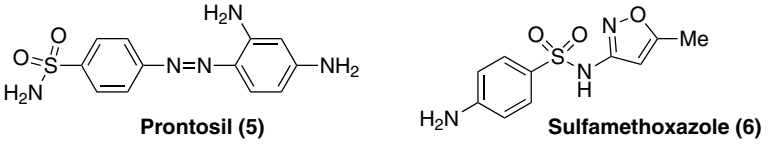


Figure 1.2 Structures of prontosil and its derivative.

antibacterial activity of Prontosil rubrum (Figure 1.2). Subsequent studies revealed that the active moiety of the compound was the 4-aminobenzenesulfonamide moiety. Introduction of substituents at both the *p*-aniline and the sulfonamide groups led to the development of new sulfanilide derivatives with broad-spectrum activity, improved pharmacokinetic properties, and lowered therapeutic side effects. The synthesis of new derivatives became less important due to the discovery and introduction of penicillin and subsequently discovered antibiotics.

Research on sulfonamide derivatives, however, continued. Close observation of side effects led to the development of new uses and expansion of this class of compounds. The clinical observation that sulfa drugs induced hypoglycemia was followed by studies aimed at maximizing this side effect and dissociating it from the bacteriostatic activity. This led to the advent of oral hypoglycemia drugs for the treatment of diabetes. In 1940, Mann and Keilin discovered the inhibitory activity of sulfanilamide against carbonic anhydrase. This key discovery paved the way for the subsequent development of diuretic sulfonamides [9].

The discovery of the antidepressant agent iproniazid is also due to the clinical observation of a "side effect" [10]. Both isoniazid (Figure 1.3) and its isopropyl-substituted derivative iproniazid were originally developed as tuberculostatic drugs. However, it was observed that in contrast to isoniazid, patients treated with

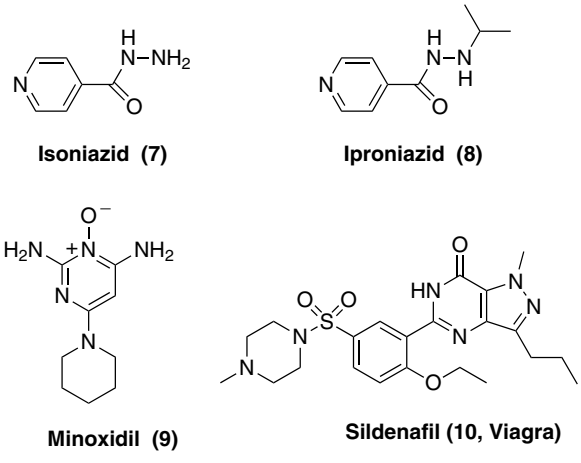


Figure 1.3 Structures of isoniazids, minoxidil, and sildenafil.

iproniazid experienced elevation of mood. Subsequent studies clarified that the antidepressant activities of iproniazid were due to the inhibition of the centrally active enzyme monoamine oxidase (MAO). Iproniazid was approved in 1958 for the treatment of depression. There are also more recent examples of clinical observations leading to the discovery of new drugs. Sildenafil or Viagra, a drug used for the treatment of erectile dysfunction, was originally developed for the treatment of angina [11,12]. Minoxidil [13,14], originally developed as an antihypertensive agent, was later approved for the treatment of hair loss.

Serendipity also played a role in the discovery of Librium, the first antianxiety benzodiazepine, but it did not happen by accident [15,16]. In 1954, Dr. Leo Sternbach was actively involved in the development of new tranquilizers in the New Jersey laboratories of Hoffmann-La Roche. He decided to explore the chemistry of benzheptoxdiazines, a class of compounds he had synthesized 20 years ago in search of new dyes but whose biological activity was unknown. His research group synthesized 40 new derivatives and determined that they were six-membered ring compounds such as **11** and **12** (Figure 1.4) rather than seven-membered ring compounds **13** and **14**, as was originally thought. Pharmacological testing showed these compounds were inactive. As their project on tranquilizers was coming to an end, during their laboratory cleanup work, they realized two of their earlier crystalline derivatives had never been submitted for pharmacological evaluation. They decided to send them for biological testing. One of the compounds that resulted from the reaction of a quinazoline derivative **15** with methylamine showed potent sedative and hypnotic effects. This compound was superior to phenobarbital. Subsequent structural work on the compound led to its characterization as benzodiazepine derivative chlordiazepoxide (**17**, Figure 1.5) known as Librium. This resulted from a rearrangement of the original benzo-fused six-membered heterocycle to afford a benzo-fused seven-membered

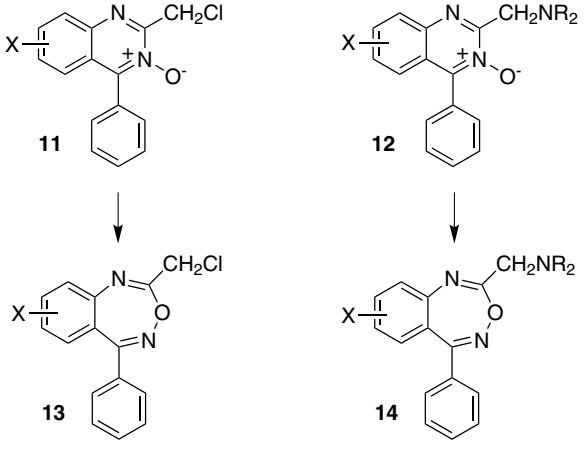


Figure 1.4 Structures of benzheptoxdiazines and quinazoline-3-oxides.

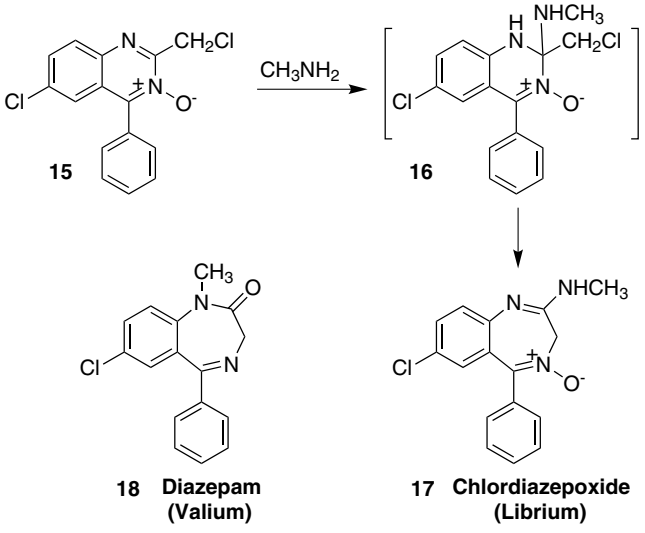


Figure 1.5 Structures of benzodiazepine derivatives, Librium and Valium.

heterocycle **17**. This discovery led to the subsequent development of a host of benzodiazepines, including diazepam (**18**, Valium).

Natural products have long served as a key source for the development of numerous new drugs. Biological screening of natural products has proven to be extremely useful. The anticancer agent Taxol was discovered in the 1970s as a result of a project implemented in 1960 by the American National Cancer Institute consisting of the biological screening of extracts arising from various natural sources [17]. One of the extracts showed promising anticancer activity against a wide range of tumors in mice. After the initial discovery, the active compound was isolated from the *Taxus brevifolia* and in 1972 its chemical structure (**19**, Figure 1.6) was fully characterized [18]. Another important anticancer treatment resulting from the screening of natural products was camptothecin (**20**), which was isolated from *Camptotheca acuminata* [19]. A number of camptothecin derivatives have been approved and are used in cancer chemotherapy.

The antimalarial drug artemisinin (**21**, Figure 1.7) [20] is also the result of a screening campaign. After a heavy outbreak of malaria in the 1950s, the spread of

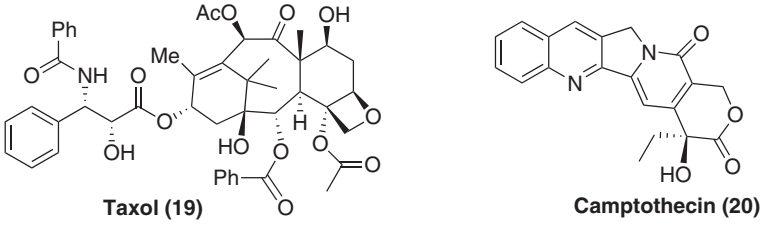
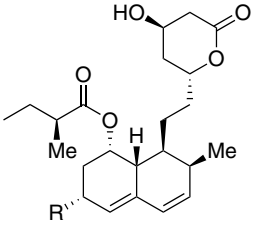


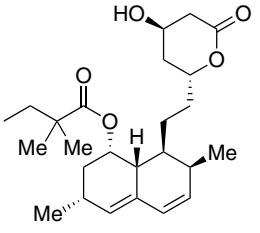
Figure 1.6 Structures of Taxol and camptothecin.

Artemisinin (21)

Artemether (22)



Compactin (R = H, 23)
Mevinolin (R = Me, 24)



Zocor (25)

Figure 1.7 Structures of artemisinin and mevinolin and their derivatives.

drug-resistant malaria strains raised huge treatment concerns. A Chinese national project implemented a campaign aimed at discovering, isolating, and characterizing natural products as potential antimalarial leads. Phytochemist Tu Youyou and his colleagues found that the extract of the traditional Chinese herbal remedy *Artemisia annua* was effective in a mouse model against malaria. Later, the sesquiterpene lactone artemisinin was characterized as the active ingredient and its semisynthetic derivatives, such as artemether (**22**), are used for the treatment of multidrug-resistant malaria.

During the 1970s, a number of other important natural products were also introduced as new drugs. Natural products compactin and mevinolin were isolated from *Penicillium citrinum* and *Aspergillus terreus*, respectively. Both these natural products showed very potent inhibitory activity of HMG-CoA reductase, responsible for biosynthesis of cholesterol in human liver. Mevinolin (lovastatin) and derivatives of mevinolin (Zocor) were introduced for the treatment of atherosclerosis by lowering cholesterol levels and inhibiting the enzyme HMG-CoA reductase [21,22].

1.3
The Beginning of Structure-Based Drug Design

During the late 1970s, rational design evolved into a strategy for the discovery and development of new drugs. With the knowledge of the three-dimensional structure of drug targets and their site of interaction with a prototype drug molecule or

ligand, logical molecular design based upon target–ligand interactions began to take shape. Early during this practice, X-ray structural information was limited. The X-ray structural data of related enzymes were used to model the target enzyme. Advances in technology and molecular biology greatly enhanced the promise of structure-based design. During the 1980s, rapid progress in protein expression, purification, and protein crystallography provided detailed structural knowledge of disease-relevant target proteins. The progress of chemical synthesis was timely as well. New and efficient reagents, protecting groups, catalytic transformations, and multistep chemical synthetic strategies provided the power of creative design capabilities for structure-based design. Drug design through structure-based approaches rapidly revolutionized the field of medicinal chemistry and changed the approach toward the identification and optimization of novel drugs.

In structure-based design, the shape and the electronic features for the binding site of a specific target protein are generated early on. Also, the crystal structures of protein and ligand complexes are determined to obtain information on intermolecular interactions within the protein active site. This molecular insight often provides the bioactive conformation of the ligand for molecular design [23,24]. Starting from this key information, structure-based design strategies allow the optimization of ligand–protein interactions to improve potency, affinity, and selectivity, while at the same time preserving and optimizing selected drug-like properties.

An early example of rational design utilizing structural information of an enzyme–inhibitor interaction can be traced to the discovery of the angiotensin-converting enzyme (ACE) inhibitor, captopril [25]. Although the X-ray structure of the actual ACE was unknown at that time, the structure of a similar enzyme, carboxypeptidase A, had already been determined. Both carboxypeptidase A and ACE have a number of common features, including the presence of a zinc ion in the protease active site. Based on this structural knowledge and a number of peptidic lead ACE inhibitors from snake venoms, investigators at Bristol-Myers Squibb (BMS) modeled the active site of ACE and rationally designed captopril, the first FDA-approved ACE inhibitor for the treatment of hypertension in 1981.

The clinical success of ACE inhibitors fueled a great deal of interest in the development of inhibitor drugs against renin, an aspartic acid protease [26]. Renin is responsible for the regulation of blood pressure, and therapeutic inhibition of renin was considered a promising strategy for the development of novel therapies for the treatment of hypertension. It was presumed that a successful renin inhibitor would possess fewer side effects than ACE inhibitors due to the exquisite selectivity of renin for a single physiological substrate. Key information in the development of renin inhibitors was the understanding of the substrate cleavage mechanism and the characterization of the endogenous peptide binding site [27–29]. The X-ray structure of renin was not known; however, a model structure of renin was created based upon the X-ray structure of related aspartic acid proteases such as *Rhizopus chinensis* carboxyl proteinase, endothiapepsin, and other aspartic acid proteases. The X-ray structural studies with peptide inhibitors also provided the details of molecular interactions. Based upon this knowledge,

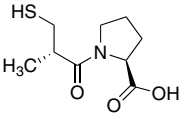
substrate-based inhibitors were developed to mimic the N-terminal portion of angiotensinogen, in which the scissile peptide bond was modified based on the transition-state mimetic concept. Subsequent structure-based optimization of the early renin inhibitors led to the successful modification of inhibitors with improved drug-like properties, resulting in the discovery of aliskiren in 2007, the first FDA-approved renin inhibitor for the treatment of hypertension [30,31].

In the late 1980s, the power of structure-based design was unveiled in the context of the structure-based design and synthesis of HIV protease inhibitors for the treatment of HIV infection and AIDS. The discovery of the key role of HIV protease in the viral life cycle and the documentation that inhibition of the viral HIV-1 protease resulted in noninfectious virions brought hope and urgency to the therapeutic inhibition of HIV protease [32,33]. Knowledge and expertise gained in the design of renin inhibitors, and the determination of the X-ray structure of HIV-1 protease at the early stages of inhibitor design, led to rapid progress in structure-based design capabilities [34]. Within a decade, hundreds of X-ray structures of HIV protease, inhibitor-bound HIV-1 protease, and mutant proteases aided in the design of conceptually novel inhibitors. In this context, numerous tools and concepts have emerged for the design of novel inhibitors and for addressing issues of drug resistance [35,36]. The first HIV-1 protease inhibitor, saquinavir, received FDA approval in 1996. Structure-based drug discovery efforts expanded rapidly in many other areas. As can be seen in Table 1.1, structure-based approaches contributed to the approval of 34 new drugs for the treatment of hypertension, HIV/AIDS chemotherapy, various cancers, and other human diseases through 2012 [37–70].

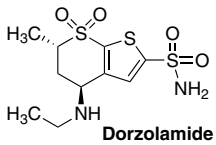
Structure-based drug design approaches have been widely utilized in the design and development of inhibitors of protein kinases for the treatment of a range of human carcinomas [71–73]. Imatinib was the first example of an anticancer drug specifically directed at inhibiting a drug target Bcr-Abl fusion protein involved in the pathogenesis of chronic myelogenous leukemia. Detailed structural studies of imatinib and Abl kinase complexes provided much molecular insight into imatinib resistance. The lead compound for imatinib was discovered through high-throughput screening (HTS). Lead optimization to improve potency, selectivity, and pharmacokinetic properties led to the discovery of imatinib. The structural studies paved the way for development of other kinase inhibitors. Once protein kinases were recognized as important drug targets for the development of anticancer therapies, much effort was directed toward obtaining structural insight into the binding sites of various protein kinases. X-ray crystallography was central to the understanding of binding mode of various classes of inhibitors. This molecular insight was extensively utilized in the structure-based design of various kinase inhibitor drugs.

The full potential of structure-based design has yet to be realized. Progress in structure-based design of ligands for G-protein-coupled receptors (GPCRs) has been growing steadily [74–77]. The recent evolution of techniques for X-ray crystallography resulted in the determination of novel GPCR structures at a rapid pace. Many high-resolution X-ray structures of ligand-bound GPCRs provided important understanding of the molecular determinants of ligand binding and receptor

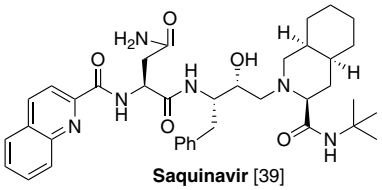
Table 1.1 Drugs derived from structure-based design approaches.



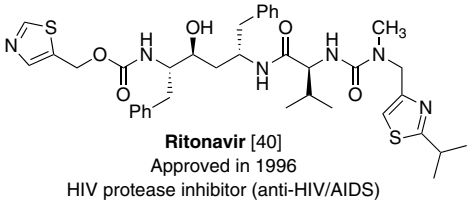
Captopril [37]
Approved in 1981
ACE inhibitor (antihypertensive)



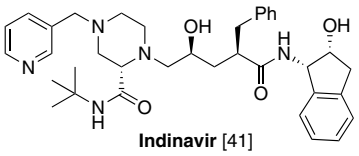
Dorzolamide [38]
Approved in 1995
Carbonic anhydrase inhibitor (antiglaucoma)



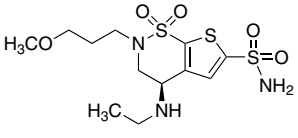
Saquinavir [39]
Approved in 1995
HIV protease inhibitor (anti-HIV/AIDS)



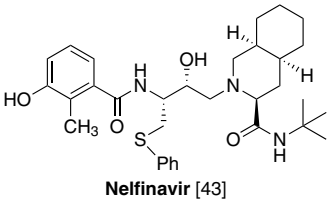
Ritonavir [40]
Approved in 1996
HIV protease inhibitor (anti-HIV/AIDS)



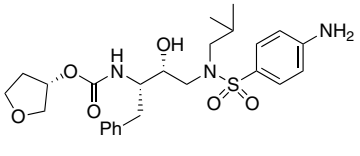
Indinavir [41]
Approved in 1996
HIV protease inhibitor (anti-HIV/AIDS)



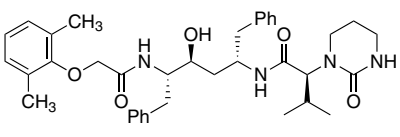
Brinzolamide [42]
Approved in 1999
Carbonic anhydrase inhibitor (antiglaucoma)



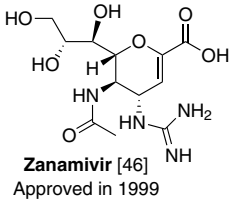
Nelfinavir [43]
Approved in 1999
HIV protease inhibitor (anti-HIV/AIDS)



Amprenavir [44]
Approved in 1999
HIV protease inhibitor (anti-HIV/AIDS)



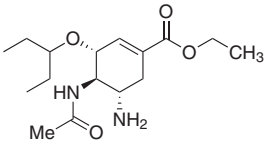
Lopinavir [45]
Approved in 1999
HIV protease inhibitor (anti-HIV/AIDS)



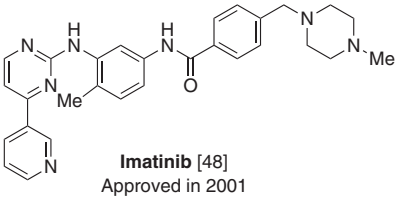
Zanamivir [46]
Approved in 1999
Neuraminidase inhibitor (anti-influenza)

(*continued*)

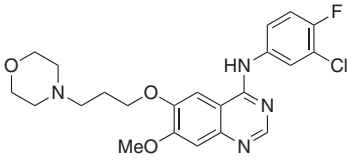
Table 1.1 (*Continued*)



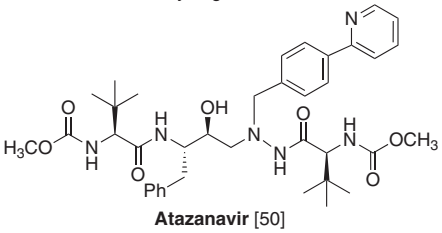
Oseltamivir [47]
Approved in 1999
Neuraminidase inhibitor (anti-influenza)



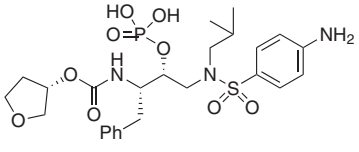
Imatinib [48]
Approved in 2001
Chronic myelogenous leukemia



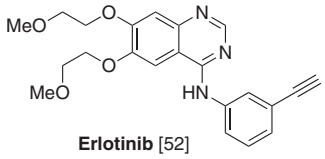
Gefitinib [49]
Approved in 2003
EGFR inhibitor (anticancer)



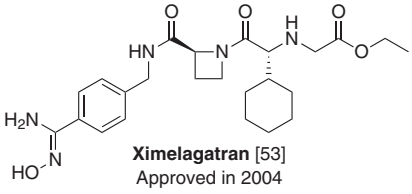
Atazanavir [50]
Approved in 2003
HIV protease inhibitor (anti-HIV/AIDS)



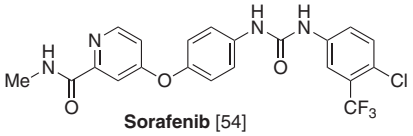
Fosamprenavir [51]
Approved in 2003
HIV protease inhibitor (anti-HIV/AIDS)



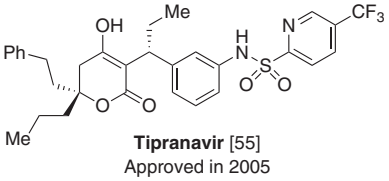
Erlotinib [52]
Approved in 2004
EGFR inhibitor (anticancer)



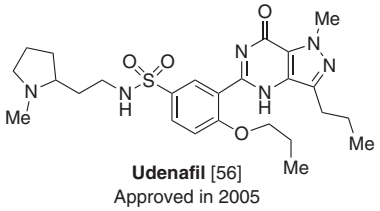
Ximelagatran [53]
Approved in 2004
Thrombin inhibitor (anticoagulant)



Sorafenib [54]
Approved in 2005
VEGFR inhibitor (anticancer)



Tipranavir [55]
Approved in 2005
HIV protease inhibitor (anti-HIV/AIDS)



Udenafil [56]
Approved in 2005
PDE-5 inhibitor (erectile dysfunction)

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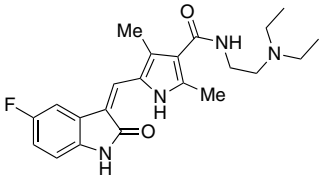
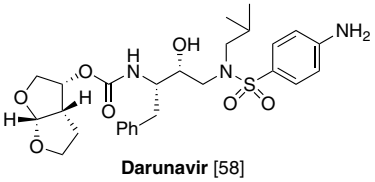
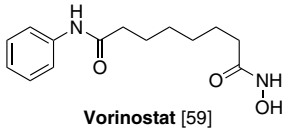


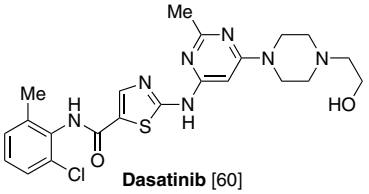
Sunitinib [57]
Approved in 2006
Multikinase inhibitor (anticancer)



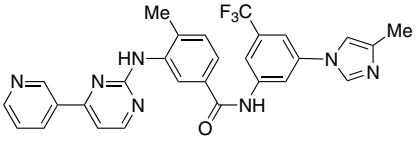
Darunavir [58]
Approved in 2006
HIV protease inhibitor (anti-HIV/AIDS)



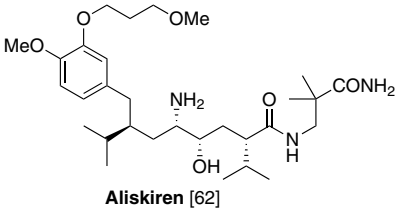
Vorinostat [59]
Approved in 2006
Histone deacetylase inhibitor (anticancer)



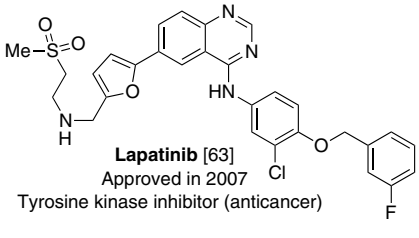
Dasatinib [60]
Approved in 2006
Tyrosine kinase inhibitor (antileukemia)



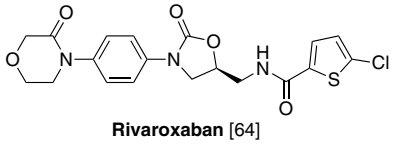
Nilotinib [61]
Approved in 2006
BCR-ABL kinase inhibitor (antileukemia)



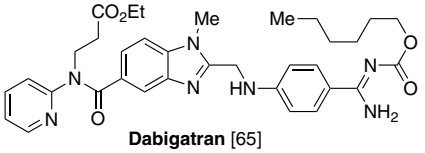
Aliskiren [62]
Approved in 2007
Renin inhibitor (antihypertensive)



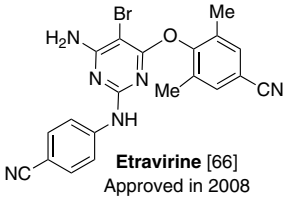
Lapatinib [63]
Approved in 2007
Tyrosine kinase inhibitor (anticancer)



Rivaroxaban [64]
Approved in 2008
Factor Xa inhibitor (anticoagulant)



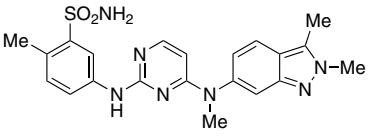
Dabigatran [65]
Approved in 2008
Thrombin inhibitor (anticoagulant)



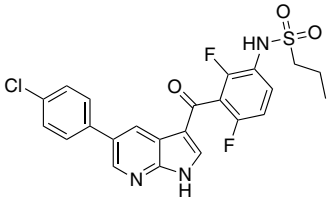
Etravirine [66]
Approved in 2008
NNRT inhibitor (anti-HIV/AIDS)

(*continued*)

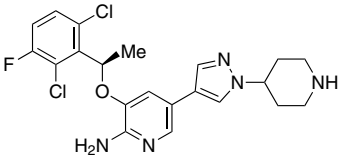
Table 1.1 (*Continued*)



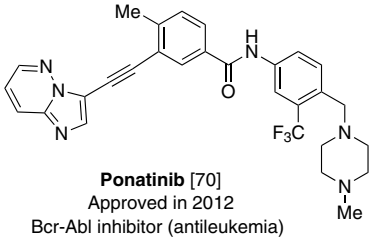
Pazopanib [67]
Approved in 2009
Multikinase inhibitor (anticancer)



Vemurafenib [68]
Approved in 2011
B-Raf kinase inhibitor (anticancer)



Crizotinib [69]
Approved in 2011
c-MET and ALK inhibitor (anticancer)



Ponatinib [70]
Approved in 2012
Bcr-Abl inhibitor (antileukemia)

activation, a critical step for designing agonists or antagonists. Recent studies have been aimed at understanding if this structural information can be effectively employed for the structure-based design of novel, potent, and selective GPCR ligands. Structure-based design of novel ligands for GPCRs has become an exciting area of drug development.

1.4 Conclusions

Serendipity and natural product screening may continue to have an important role in drug design, but it is clear that structure-based design strategies are making a significant impact on the drug discovery process. The success of this approach is already evident in 34 FDA-approved drugs on the market through 2012. Numerous other drugs developed using this approach are undergoing clinical trials. No doubt, the success of structure-based design strategies rests heavily on the structural knowledge of disease-relevant target enzymes and their families. The notable success of drug development in the areas of HIV-1 protease, protein kinase, NS3/4A serine protease, and β-secretase greatly empowered the application of these strategies in other areas of drug development. With continual advances in technology and increasing knowledge of disease mechanisms and protein structures, structure-based design strategies will find wide applications in drug discovery endeavors.

In the post-genomic era, many new and important drug targets are emerging, and structure-based design is expected to offer new opportunities for drug development. The drug discovery efforts in the area of GPCRs have witnessed significant breakthroughs with the availability of high-resolution structures of drug-relevant GPCRs. Structure-based design efforts have greatly benefited from rapid progress in lead generation and validation strategies. Fragment-based screening is providing early structural knowledge of small-molecule leads. Also, virtual screening has witnessed major improvements with the sophistication of computational infrastructure, data sets, and analysis tools. Virtual screening is very important as traditional HTS is often expensive and time consuming and selected compound libraries may not have enough diversity.

Despite the successful trends of structure-based design strategies, it is important to note that the lead optimization and drug design process are driven by medicinal chemistry efforts. It is this ingenuity and innovation of experienced medicinal chemists that will fuel the drug discovery of the future. The ever-increasing knowledge of molecular and structural biology will likely reveal new exciting drug targets. However, for innovative molecular design and synthesis, the role of chemical synthesis will be vital for tomorrow's new treatments. Structure-based design has not yet reached its full potential and these strategies will undoubtedly play a major role in the drug discovery endeavors in the rest of the twenty-first century.

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