2009-06

Structure-based drug design strategies in medicinal chemistry

Current Topics in Medicinal Chemistry,Sharjah : Bentham Science,v. 9, n. 9, p. 771-790, Jun. 2009
http://www.producao.usp.br/handle/BDPI/49757

Structure-Based Drug Design Strategies in Medicinal Chemistry

Adriano D. Andricopulo[1,*], Lívia B. Salum[1] and Donald J. Abraham[2,*]

[1]*Laboratório de Química Medicinal e Computacional, Centro de Biotecnologia Molecular Estrutural, Instituto de Física de São Carlos, Universidade de São Paulo, Av. Trabalhador São-Carlense 400, 13560-970 São Carlos-SP, Brazil,* [2]*Department of Medicinal Chemistry, School of Pharmacy, Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Virginia*

Abstract: A broad variety of medicinal chemistry approaches can be used for the identification of hits, generation of leads, as well as to accelerate the development of high quality drug candidates. Structure-based drug design (SBDD) methods are becoming increasingly powerful, versatile and more widely used. This review summarizes current developments in structure-based virtual screening and receptor-based pharmacophores, highlighting achievements as well as challenges, along with the value of structure-based lead optimization, with emphasis on recent examples of successful applications for the identification of novel active compounds.

Keywords: Structure-based drug design, medicinal chemistry, virtual screening, QSAR, pharmacophores.

STRUCTURE-BASED DRUG DESIGN

The performance of biochemical processes and cell mechanisms are dependent upon complex and multiple non-covalent intermolecular interactions between proteins and small-molecule modulators. The understanding of the structural and chemical binding properties of important drug targets in biologically relevant pathways allows the design of small molecules capable of regulating or modulating specific target functions in the body that are closely linked to human diseases and disorders, through multiple intermolecular interactions within a well-defined binding pocket [1-4]. In general, the identification of promising hits for further optimization is a major challenge faced by the both pharmaceutical and academic laboratories. Although the trial-and-error nature is inherent in drug research, rational concepts and modern computational methods have become widely employed for lead selection and optimization.

The use of three-dimensional (3D) protein structure information in the development of new biologically active molecules, which is termed *Structure-Based Drug Design* (SBDD), is a well-established, successful and highly attarctive strategy used by academic and pharmaceutical research laboratories worldwide [3-8]. As a creative and knowledge-driven approach, an essential requirement for structure-based studies is a substantial understanding of the spatial and energetic aspects that affect the binding affinities of protein-ligand complexes. Considering that the shape and chemical nature of the binding site of a specific target protein are known, and the possible intermolecular interactions between ligands and the protein within its active site have been identified, this qualified information can be directly employed for the identification of new ligands and the optimization of lead compounds. This opens new possibilities to boost the search for lead molecules and to limit the number of compounds that need to be evaluated experimentally.

Hits can be identified through the docking of small-molecule ligands (selected from databases of chemical structures) into protein active sites or by using receptor-based pharmacophore models. Furthermore, drug candidates can be designed *de novo* by improving the complementary binding properties of lead compounds and the respective target proteins (i.e., intermolecular interactions between amino acid residues of the target active site and the chemical groups of the lead candidates). Molecules that mimic the transition state of enzyme catalyzed reactions are interesting examples [3-12]. Early drug discovery steps usually require structural optimization of lead compounds in order to build the highest possible level of potency, selectivity and affinity for the target of interest, as well as appropriate physico-chemical and pharmacokinetic characteristics. Substrates and cofactors of several enzymes have been structurally modified to generate excellent inhibitors using X-ray crystallographic data. Fig. (**1**) shows examples of potent and bioavailable nonpeptide inhibitors of human renin that were developed using structural information concerning proteins and small molecules (receptor-ligand intermolecular interactions) [13-15]. Renin is an aspartyl protease involved in the regulation of blood pressure and its inhibition has been considered a promising strategy for the development of new alternative therapies for the treatment of hypertension. As can be seen in Fig. (**1**), the understanding of the cleavage mechanism of the substrate (angiotensinogen), and the detailed characterization of the enzymatic site (covering the S4 to S2' subsites, and the catalytically central aspartates, Asp32 and Asp215) led to the development of substrate-based inhibitors (peptide analogs of the amino-terminal portion of angiotensinogen). The replacement of the scissile dipeptide moiety based on the transition-state analog concept allowed the development of new peptide-like inhibitors having potent *in vitro* activity,

*Address correspondence to these author at Laboratório de Química Medicinal e Computacional, Centro de Biotecnologia Molecular Estrutural, Instituto de Física de São Carlos, Universidade de São Paulo, Av. Trabalhador São-Carlense 400, 13560-970 São Carlos-SP, Brazil; Tel: + 55 16 3373-8095; E-mail: aandrico@if.sc.usp.br

Department of Medicinal Chemistry, School of Pharmacy, Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University; Tel + 1 804 828-7575; E-mail: dabraham@vcu.edu

1568-0266/09 $55.00+.00

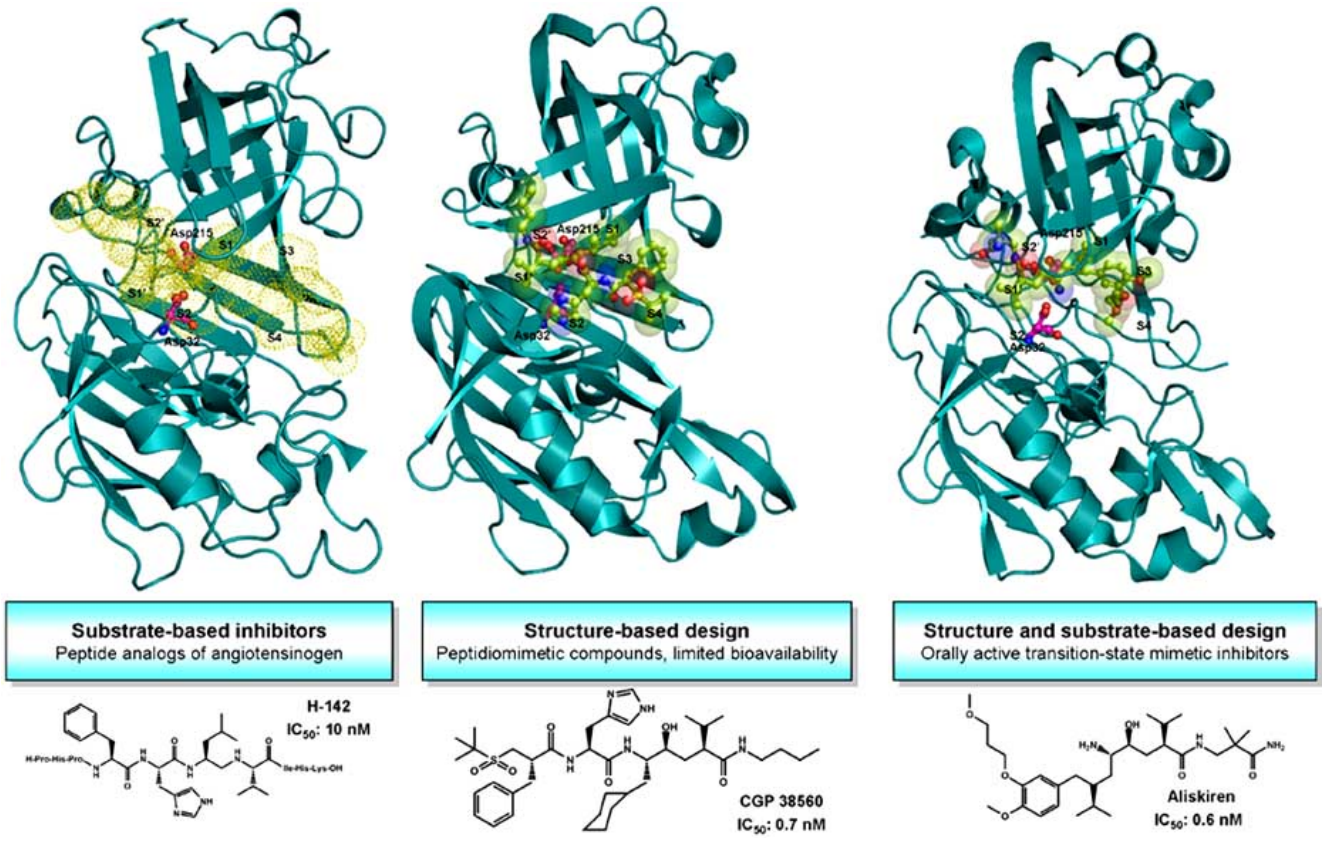

Fig. (1). Structure-based drug design of orally active nonpeptide inhibitors of human renin. Inhibitors are shown in green, while the two catalytic aspartic acid residues in magenta.

but not exhibiting good oral absorption and stability (e.g., CGP 38560, IC_{50} = 0.7 nM). To overcome these hindrances, hydroxyethylene transition-state mimetic inhibitors of lower molecular weight were designed to explore the shape and chemical properties of the large hydrophobic S1/S3 binding cavity of the active site. This approach efficiently generated novel nonpeptide inhibitors with improved oral bioavailability and stability, while retaining high potency. Aliskiren (Tekturna®, Rasilez®, from Novartis and Speedel) was approved by the U.S. Food and Drug Administration in 2007, being the first in this class of drugs called renin inhibitors for primary hypertension [16]. The inhibitor (Fig. **1**, IC_{50} = 0.6 nM) was positioned into the extended binding cleft of renin (S3 to S2' subsites) making interactions with the hydrophobic cavity, which has not been previously explored. It is interestingly to note that pharmacokinetic properties and drug metabolism can also be examined and improved by exploring crystal structures of the human cytochrome P450 isoforms [17,18].

The receptor-based approach is carried out in an iterative manner, proceeding *via* multiple computational and experimental paths until the development of an optimized lead compound having high affinity and selectivity, as well as optimized pharmacokinetic properties, as shown in Fig. (**2**). The developed drug candidate is then appropriate to move into phase one clinical trials.

As a starting point, it is always useful to perform a meticulous analysis of the structural and chemical features of the target binding site (i.e., amino acid residues of the protein pocket: tautomerism, protonation, ionization). Protein structures (apo, ligand-free; or holo, ligand-bound) are experimentally determined by X-ray crystallography and nuclear magnetic resonance (NMR). Alternatively, protein structure homology models can be a valuable alternative [3,5-10,19-23]. Several *in silico* methods can be used in combination with experimental evidences to extract and organize the molecular information in order to assist the understanding of the structural and chemical basis involved in receptor-ligand binding affinity and biological activity. Receptor-based pharmacophore models and molecular docking methods can be employed in early drug discovery stages for hit identification and lead generation (Fig. **2**). High performance computational searches are performed to screen small molecule chemical libraries that vary in size and complexity, and only a very small subset of promising compounds is selected for synthesis, acquisition and *in vitro* biological evaluation. Lead candidates can also be generated using a variety of experimental methods, including (i) high throughput screening (HTS); (ii) small-scale screening of compounds that are structurally related to modulators of a target protein; (iii) SAR studies of biologically interesting molecules; or (iv) fragment-based screening (Fig. **2**).

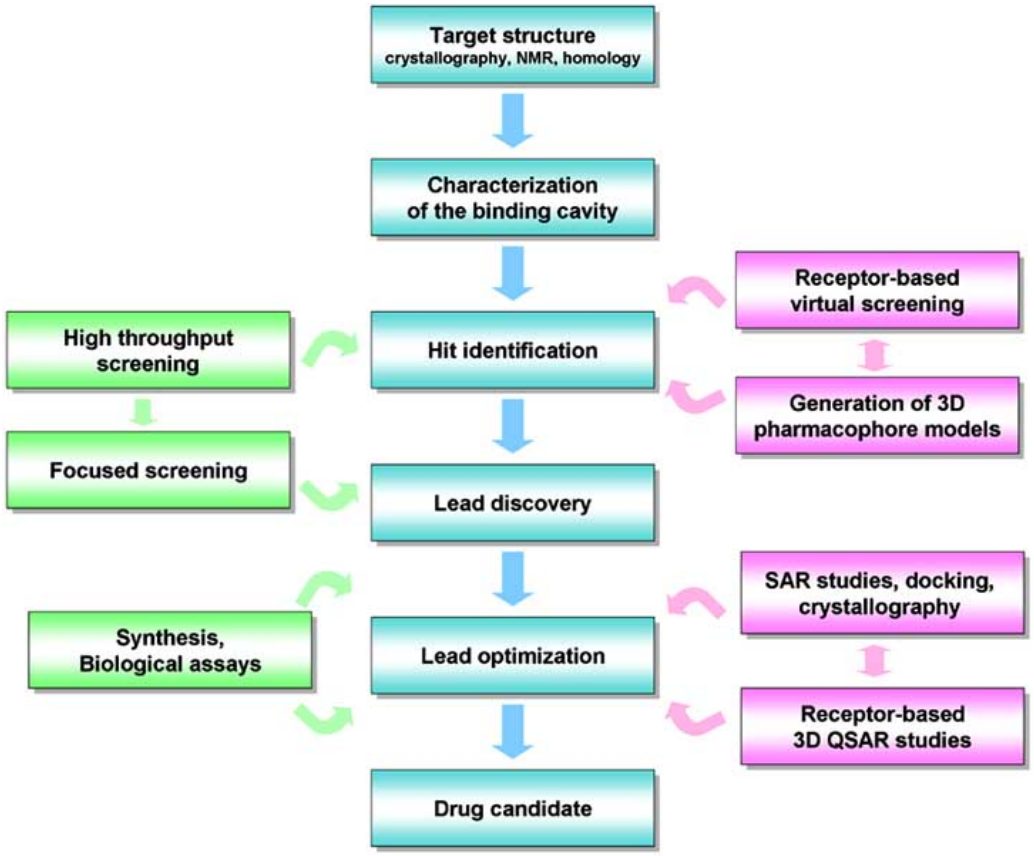

Fig. (2). Description of the workflow of the iterative process of structure-based drug design.

Focused libraries can be designed based on new bioactive molecules, through the incorporation of new data sets of compounds sharing a high degree of chemical similarity, as well as interesting structural diversity. The molecular and biological properties of new compounds (structurally related) can be further rationalized and even predicted (in terms of their chemical modifications), as a consequence of their direct interactions with the target receptor. Biochemical, crystallographic and spectroscopic methods are useful in determining the binding properties and mechanism of action of promising ligands.

The knowledge generated (chemical and biological) from these steps is a key component in medicinal chemistry, and can be used in the iterative design of new ligands with improved properties characteristics (lead optimization) [3-11,19-27]. For this purpose, 3D quantitative structure-activity relationships (3D QSAR) methods are among the most important strategies that can be applied for the successful optimization of leads (Fig. **2**). In this context, 3D QSAR models are generated to explain the relationships between the intermolecular interactions related to the 3D conformations of a set of structurally related molecules and their experimental activity (e.g., IC_{50}, K_i), therefore, providing a rational basis for the development of new promising compounds [24,28,29].

Structure-based approaches have increasingly demonstrated their value in drug design. The impact of these technologies on early discovery and lead optimization is significant. Although there is a multiplicity of different approaches being employed in early stages of drug discovery, SBDD is one of the most powerful techniques, and has been used quite frequently by scientists in the pharmaceutical industry as well as in academic laboratories over the past forty years. SBDD approaches continue to drive important advances in drug design, integrating traditional and modern technologies from the fields of medicinal chemistry, computational chemistry, informatics, biology, biochemistry, and structural biology. Structure-based methods bring the 3D structures of proteins to light, and thereby greatly enable many drug discovery efforts to identifying novel, small molecule drug candidates that selectively and potently modulates the right biological target. The ability to make knowledge-based decisions during the early phases of drug discovery is the key to decreasing hit-to-lead and lead optimization cycle times. The significant advances in structural capabilities (e.g., protein generation and purification techniques, high throughput crystallography, virtual screening, SAR by NMR) combined with robust and more efficient computational tools (faster and cheaper) have improved molecular modeling tools to evaluate ligand-protein interactions. The evolution of medicinal chemistry has resulted in an increase in the number of successful applications of structure-based approaches. The importance of these approaches in exploring the chemical space of biologically active compounds is well established, considering that these powerful strategies have significantly contributed to the discovery and introduction of several NCEs into clinical trials for a wide variety of therapeutic applications. Some successful examples include inhibitors of HIV-

protease, neuraminidase, renin, carbonic anhydrase, tyrosine phosphatase, β-lactamase and DNA gyrase, among others [15,22,30-39]. As depicted in Fig. (**2**), pharmacophore analyses, molecular docking and 3D QSAR are among the medicinal chemistry methods of utmost importance in modern rational drug design. Some case studies will be presented in this short review to explore the value and potential of each of these techniques involved in the state-of-the-art drug research, highlighting the identification of novel, potent and selective receptor modulators with drug like properties.

STRUCTURE-BASED VIRTUAL SCREENING

Genomic and proteomic approaches have provided novel insights into the identification of new targets for drug intervention, presenting attractive opportunities for the discovery of new agents with important therapeutic properties. Several hundred molecular targets have been cloned and are currently evaluated as drug targets. These mainly include G-protein coupled receptors (GPCRs), ligand-gated ion channels (LGICs), nuclear receptors (NRs), cytokines, and reuptake/transport proteins. Every week, new potential therapeutic approaches for the treatment of important diseases are suggested as a result of the exponential proliferation of novel biological targets. The sheer volume of genetic information produced in the last decade has shifted the emphasis from the generation of novel DNA sequences to the determination of which of the many potential targets offer unique opportunities for drug research. Therefore, target selection and validation are current bottlenecks in drug discovery and will continue to be so in the future [19-24]. Solving the protein folding process is paramount for this excellent approach to be successful.

The identification of promising hits and the generation of high quality leads are crucial steps in the early stages of any drug discovery project. Recent advances in medicinal chemistry at the interface of chemistry and biology have created an important foundation in the search for new drug candidates possessing a combination of optimized pharmacodynamic and pharmacokinetic properties. Despite the impact of the recent technological and scientific advances, drug discovery has become more expensive and time consuming over the same period of time [24,29]. The widespread use of combinatorial chemistry and HTS for the discovery of lead compounds has created a large demand for small organic molecules that act on specific drug targets. These technologies focus on the generation of a huge number of molecules integrated with the biological screening of a very large number of samples. However, due to the ever increasing pressure to reduce drug development time and costs, there is a clear paradigm shift from the random screening of collections of compounds to a more rational process, which would directly affect the success rate of NCE generation. One of the most important challenges for the pharmaceutical industry is the identification of innovative NCEs from an incredibly large reservoir of real and virtual possible compounds. Several steps of the drug discovery process (e.g., hit identification, lead optimization, pharmacokinetic profile) can be improved in a rational way with the application of computational methods.

The search for new biologically active molecules from large compound databases by means of computer-assisted methods is a process known as virtual screening. VS methods have rapidly become an essential component of the modern drug discovery process [24-27]. Structure-based virtual screening (SBVS) approaches explore information about the target protein structure in order to select molecules that are likely to favorably interact. These molecules can then be selected for *in vitro* biological tests. In SBVS approaches, the chemical space is broadly explored using databases of commercially available compounds for virtual screening [3-11,19,20,23-27]. High-performance hardware and specialized software, combined with advanced knowledge of 3D protein structure and small-molecule binding modes, have made this technology a useful complement, and in some cases, a reasonable alternative to HTS [3,5,6,-20,24,27]. Recently, several examples of success stories have been described for the use of this approach in the discovery of novel lead compounds, including cases where HTS was not effective or failed. Moreover, SBVS approaches showed a unique ability to significantly enhance hit-rates for obtaining lead compounds when compared with HTS approaches [6,36,39,40].

The combined use of both HTS and SBVS is an important strategy in medicinal chemistry that has allowed the identification of inhibitors of the protein tyrosine phosphatase 1B (PTP1B) (Fig. **3**), which plays an important role in metabolism and has been identified as a target for obesity and type II diabetes. A corporate library of 400,000 compounds was biologically screened against PTP1B, with 543 hits being identified in a single-point assay at 300 μM concentration of compound. From these initial hits, 85 had their potency determined with IC_{50} values ranging from 1 to 100 μM (hit rate of 0.021%), where the most active compound had an IC_{50} value of 4.2 μM. On the other hand, SBVS was used to screen more than 230,000 compounds against PTP1B, using the X-ray crystallographic structure PDB ID 1PTY. The docking top-scoring 1,000 molecules were considered for further evaluation, from which 365 were selected based on the occupancy and complementary interactions with the two tyrosine sites that are related to high affinity and specificity. From the compounds selected for the biological evaluation, 127 (34.8%) inhibited the target enzyme PTP1B, with IC_{50} values less than 100 μM. The most potent compound shows an IC_{50} of 1.7 μM. As shown in Fig. (**3**), the SBVS approach presented a hit rate much higher than that of HTS (about 1,700-fold, with molecules having drug-like properties) [36]. The selected docking hits incorporated a range of functional characteristics, including molecules with negative groups (compound **8**, Fig. **3**) capable to interact into the phosphate binding-site of the catalytic cavity, as well as neutral molecules (compound **3**, Fig. **3**) presenting extensive shape complementarity to the enzyme surface. The characteristic carboxylic acid-bearing molecule compound **8** (IC_{50} = 21.6 μM) was predicted to interact with the catalytic site residues Arg221 and Cys215. In contrast, the docking positions of compound **3** (IC_{50} = 8.6 μM) revealed that this larger molecule actually occupies both tyrosine sites to achieve a more favorable steric complementarity.

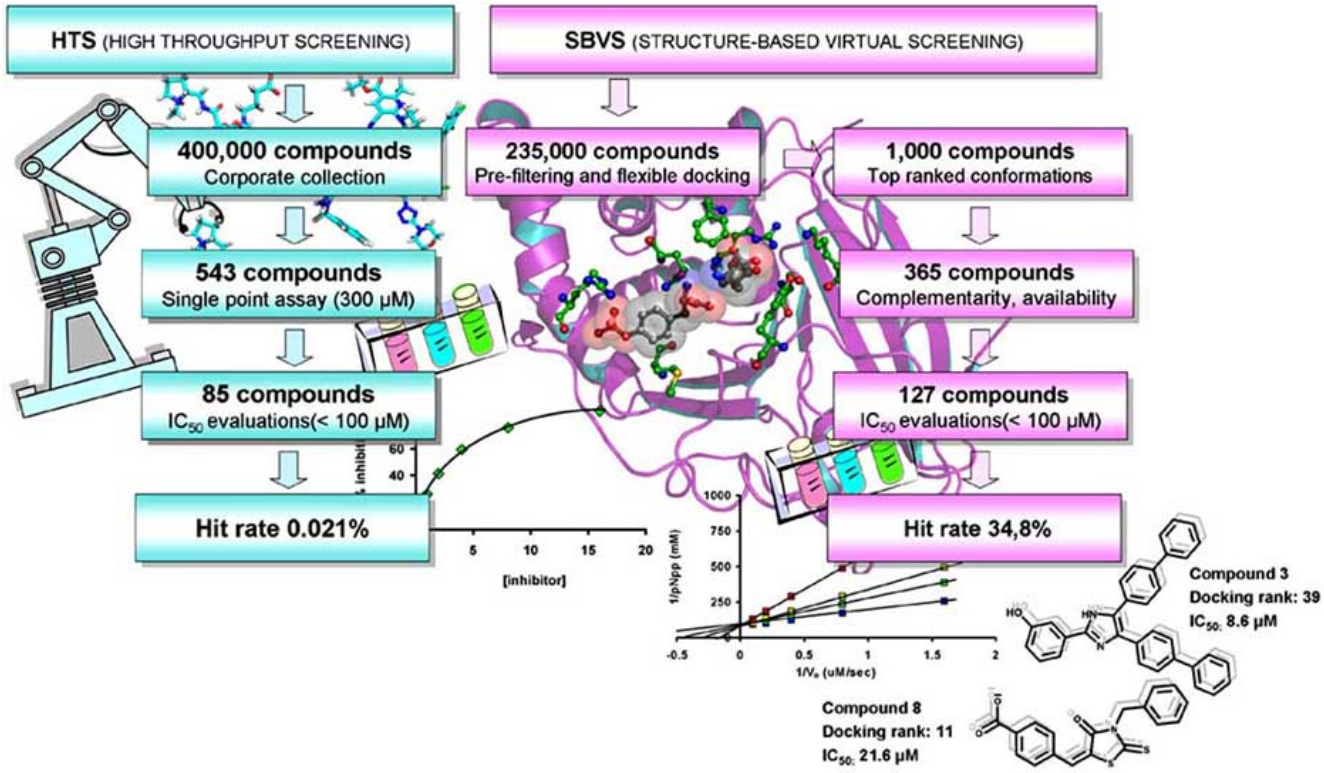

Fig. (3). Workflow of HTS and SBVS strategies in the identification of new leads for tyrosine phosphatase, highlighting differences in hit rates and the impact of the two screening approaches.

SBVS typically encompasses a sequence of crucial computational steps, including target preparation, chemical database selection, docking, scoring, post-docking analysis, ranking, visual inspection and prioritization of compounds for testing, as schematically shown in Fig. (**4**) to briefly illustrates previous studies for human carbonic anhydrase II (hCAII) inhibitors [34,35]. The knowledge-based SBVS approach is strongly affected by the quantity and quality of the information about the systems under investigation. The process of selection and preparation of the macromolecular target involves essential issues, such as druggability of the target receptor, selection of the most relevant geometry, flexibility, assignment of charges, protonation and tautomeric states, ionization, and the inclusion of conserved water molecules in the binding cavity. Some relevant molecular characteristics such as partial charges, stereochemistry, ionization and tautomeric states must also be correctly assigned to the small molecule compounds [7,9,10,19,20, 24,41].

Regardless the source of the small-molecule database (e.g., private collections, virtual libraries of synthetically accessible compounds, databases of commercially available compounds, *in-house* libraries of natural and synthetic compounds, and so on), screening libraries generally contain a large number of molecules with broad chemical diversity. In the important process of library design, several molecular filters can be used to reduce the number of compounds to be screened. Common filtering methods are variations of Lipinski's rule of five that include physicochemical and pharmacokinetic parameters in order to guide the selection of compounds with lead-like, fragment-like, and drug-like properties. The chemical space can also be reduced by taking into account molecular properties presented by series of modulators with known biological activity, or through the identification of specific features required for ligand binding. Additional filters are often applied to remove specific chemical structures associated with chemical instability and toxicity [19,42,43]. All of these computational (virtual) screening filters are useful to improve the quality of small-molecule libraries and are crucial to the application of SBVS methods.

The fundamental goal of SBVS is to identify molecules with the proper shape, hydrogen bonding, electrostatic and hydrophobic interactions that are complementary to the target receptor. Therefore, reliable methods for prediction of ligand orientation and conformation into the binding cavity of the macromolecular target (docking) are required. Afterwards, it is also necessary to have a solid evaluation of the quality of the fit or the calculated binding affinity, associated to each predicted binding mode. However, there are still many challenges in developing fully satisfactory docking algorithms and scoring functions for VS approaches. Exploiting ligand conformation and protein flexibility, treating desolvation, incorporating water molecules and calculating ligand-receptor binding energies are among the major difficulties [3,4,7,9,19,20,24-26]. Although certain key points and limitations have to be considered, the potential of SBVS lies in its ability to generate robust hypotheses that can be tested in iterative cycles. In addition, several strategies have been successfully used to increase hit rates

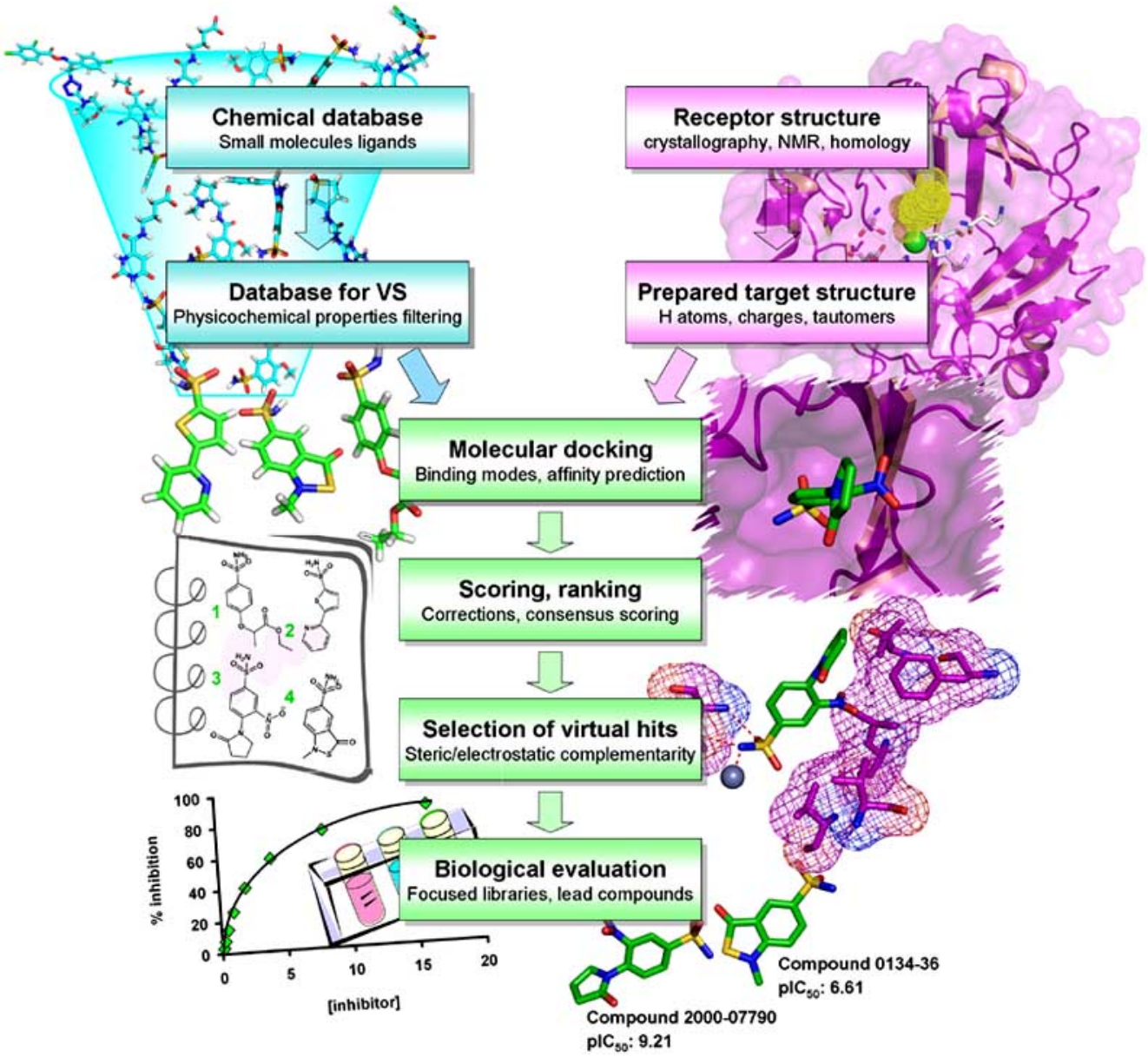

Fig. (4). Computational steps in SBVS, illustrated by the identification of a novel class of carbonic anhydrase inhibitors.

(enrichment) in SBVS [3,4,7,20,27,44]. The *in silico* docking and scoring tools are constantly being optimized and redesigned to improve their performance. It is estimated that there are about 30 individual docking programs available (e.g., Dock [45], Autodock [46], FlexX [47], FlexE [48], Gold [49], Glide [50]), which follow different concepts and approaches, and are thus more appropriate for specific proteins and molecular systems. In this way, the selection of a docking procedure suitable to describe the relevant molecular properties of the system under investigation is one of the crucial steps in the early stages of SBVS [10,24,26]. The several possible post-docking analyses are important to minimize the number of compounds selected for biological evaluation, and to reduce the false positives rate. Multiple scoring functions, consensus scoring, more complex and time-consuming functions or parameters to include flexibility, solvent effects and water molecules, and datasets of known bioactive compounds or decoys to calibrate the methodologies are among the most used strategies. Geometric analyses and visual inspection of molecular surface complementarity (3D analysis of receptor-ligand interactions) are also useful approaches to balance deficiencies associated with docking, scoring and ranking functions [4,6-9,19,25, 51-53].

In the SBVS strategy used in the search for novel hCAII inhibitors (Fig. **4**), atomic partial charges and protonation states were carefully assigned for 90,000 ligand molecules, which were submitted to a series of hierarchical filters [34,35]. While coordination to zinc was thought to be important for hCAII binding, the database molecules were analyzed to include zinc-binding anchor groups, such as amides, sulfonamides, hydroxyacetamides and carboxylic and phosphonic acids. Following the selection of 2D func-

tional groups and the application of Lipinski's rules, around 6,000 compounds were screened using a hot-spot based pharmacophore model. For the pharmacophore generation, special attention was given to the hydrogen acceptor groups near the residues Thr199NH and Gln92, as well as to the hydrogen donor groups near the zinc and Thr199OH. In fact, these interactions are involved in the molecular recognition of the sulfonamide group of dorzolamide, a potent hCAII inhibitor (Fig. **4**). Favorable hydrophobic interactions were found in a long extended region near the residues Leu198, Val121, Val143, Phe131 and Ile91. Approximately 3300 virtual hits that satisfied the pharmacophore query were ranked according to molecular physicochemical similarities with reference ligands. Finally, the 100 best scoring candidates were flexibly docked into the enzyme binding pocket, which was sterically restricted by four conserved water molecules. The predicted binding affinities were examined based on their surface complementarity with the metalloproteinase site, the number of rotatable bonds, the quality of the overall binding conformation and the formation of hydrogen bonds to Glu92 and Thr200, which compensates for desolvation of these residues in the binding pocket. Accordingly, 13 compounds were selected for experimental evaluation. Of these, 11 sulfonamides were active against hCAII, with potencies ranging from nanomolar to micromolar. Crystal structures of two sulfonamide inhibitors (shown in Fig. **4**) revealed that the predicted binding modes were rather correct. The sulfonamide group establishes a network of hydrogen bonds with the enzyme binding site, with the deprotonated terminal nitrogen group coordinated to zinc. Furthermore, the remaining skeleton of the ligands is oriented into the previously identified hydrophobic pockets [34,35].

The ultimate measure of success of any SBVS campaign would be increase hit rates while reducing the number of compounds for *in vitro* biochemical evaluation. Over the past few years, a high number of case studies has been reported using a variety of SBVS methods, demonstrating its broad applicability and the level of interest in this drug design strategy. Some important applications include, besides the examples abovementioned, the identification of antagonists of the thyroid hormone receptor, the discovery of inhibitors of tyrosine kinase p56 Lck, the selection of epidermal growth factor receptor (EGFR) inhibitors, as well as the discovery of inhibitors of the Bcl-2 protein [54-57]. The strategy for identification of ellagic acid, a natural compound, as nanomolar competitive inhibitor of casein kinase 2 (CK2), is depicted in Fig. (**5**) [58]. This enzyme is a ubiquitous, essential, and highly pleiotropic protein kinase whose abnormally high constitutive activity is related to neoplasia and other infectious diseases. In the SBVS approach for the identification of ligands of the ATP binding site of CK2, an in-house molecular database containing 2,000 naturally-occurring compounds (including polyphenols as flavones, flavonols, isoflavones, catechins, anthraquinones, coumarins, and tannic acid derivatives, Fig. **5**) was generated and a combination of different docking protocols and scoring functions was used. Firstly, rigid body orientations for each compound were evaluated according to their ability to fit into the ATP binding cavity. Afterwards, the compounds predicted to bind into the kinase site were submitted to a second step of flexible ligand-docking using four different programs and five scoring functions. Interestingly, a naturally occurring tannic acid derivative, ellagic acid, was classified among the top 5% compounds ranked by all possible combinations of flexible-docking/scoring functions, independently from the nature of the scoring function. This compound was then characterized as a potent and selective competitive inhibitor of CK2 (K_i = 20 nM) with respect to the substrate ATP (the inhibition of other kinases was in the micromolar range).

RECEPTOR-BASED PHARMACOPHORES

The SBVS steps of docking, scoring and ranking are powerful tools for the selection of compounds from large libraries on the base of desired biological properties, and steric and electrostatic complementarity between macromolecular sites and ligands. Applying receptor-based constraints in SBVS protocols can significantly improve the docking results and lead to better hit lists [3,4,9,59]. Thus, only the molecules sharing a series of particular 3D steric and electrostatic features, that is, characteristics to satisfy pharmacophore requirements in a way to ensure optimal supramolecular interactions with the biological target structure, would be selected [59-62]. In order to explore these specific structural characteristics, the chemical landscape of the binding cavity can be used to define functional-group maps or hot-spots for protein ligand interactions, leading to the generation of binding-site pharmacophore models. A number of different methods that accurately probe and map ligand binding pockets has been reported either using information from ligand-protein complexes or knowledge of the sole protein structure (e.g. GRID [63], SuperStar [64], Drug-Score [65], LigandScout [66], Pocket [67], GBPM [68], LUDI [69], Cerius [70]). These strategies allowed a remarkable improvement of the quality of pharmacophore models.

Pharmacophore models derived from receptor mapping, as represented in Fig. (**6**) for the estrogen receptor β (ERβ), are an attractive approach for SBDD [71]. Structure-based pharmacophore models can be developed from the information gathered by the superposition of X-ray crystallographic structures through the identification of important regions of molecular interactions (Fig. **6a-d**). In this example, the ligand-binding domains of the ERβ structures in complex with three different modulators were superposed (genistein, WAY-244 and ERB-041, genistein is displayd in green, Fig. **6A**) and a box encompassing the binding cavity was selected. Then, hydrophobic (yellow) and hydrophilic (cyan) GRID probes were selected to map the binding cavities of the ERβ structures (Fig. **6B**), and the conserved essential regions for ligand binding (e.g., hydrogen bonds with the Asp305/Arg394 and His475 ends, and the central planar hydrophobic core) were identified (Fig. **6C**). A pharmacophore model was thus generated (Fig. **6D**) based on hydrogen bond acceptors (cyan) and hydrophobic groups (yellow). It is worth noting that this approach has also been applied to study selectivity between the ER α- and β-subtypes (Fig. **6E**). The receptor-based information was exploited with the aim to highlight the most relevant 3D structural features involved in ERβ subtype selectivity [71].

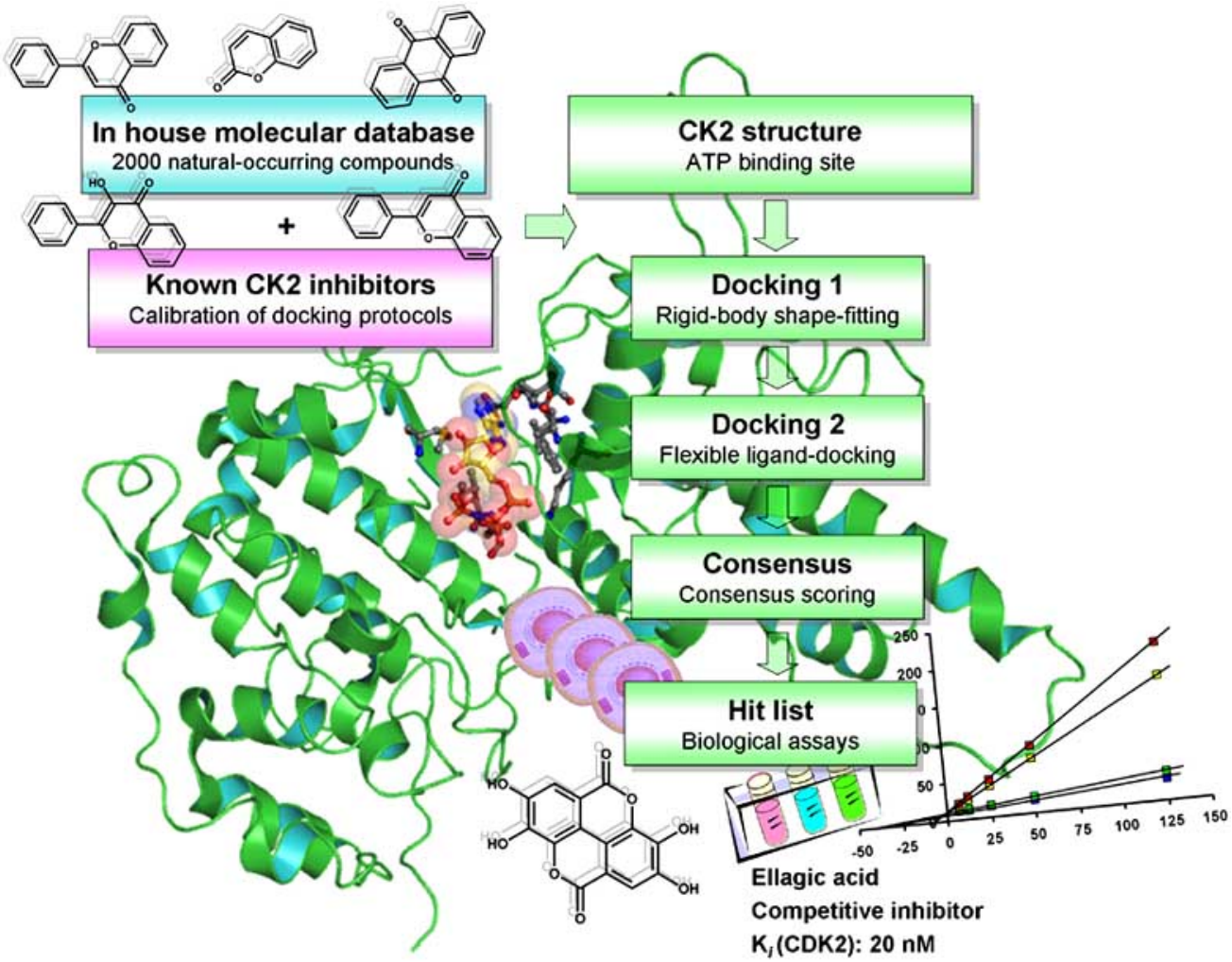

Fig. (5). Flowchart of the high-throughput consensus docking strategy for the identification of ellagic acid as a potent competitive inhibitor of CK2.

Another example of 3D pharmacophore generation from macromolecule-ligand complexes is illustrated in Fig. (**7**) [66]. Three relevant structures of Abelson tyrosine kinase (Abl) were retrieved from the PDB (1FPU, 1IEP, and 1OPJ) and used for pharmacophore modeling. The inadvertent activation of this kinase causes chronic myelogenous leukemia (CML), and the inhibition of this enzyme by small-molecule ligands has been recognized as an attractive strategy for the treatment of CML. The study of the biochemical mechanism and binding mode of STI-517 (Imatinib, Fig. **7**) has revealed that this potent and selective ligand binds and stabilizes the inactive form of Abl, preventing its activation [72,73]. Therefore, a pharmacophore model was derived from the Abl-bond conformations of the STI-517 analogs. The structural information on the inhibitors was extracted from the PDB structures and interpreted in terms of their chemical characteristics (e.g., molecular topology and geometry, hybridization state, binding characteristics). Subsequently, six different preliminary pharmacophore models were derived from both the small-molecule ligands and respective surrounding amino acid residues, based on a set of possible intermolecular interactions, (e.g., hydrogen bond acceptors and donors, charge-transfer interactions, hydrophobic regions, volume constraints). The models were refined to merge together into one single 3D pharmacophore, which was substantially robust to describe the selective binding mode of the ligands into the Abl binding cavity [66]. The resulting pharmacophore model (Fig. **7**) contained four lipophilic aromatic regions, two acceptors and eight excluded volume spheres.

As can be seen, a pharmacophore model is a versatile 3D arrangement of important features that can incorporate restrictions on the size and shape of specific regions of the ligand binding pocket of the receptor, in addition to hydrogen bonding, electrostatic and hydrophobic interactions within the limits of the geometric constraints.

Pharmacophore models are useful tools for lead identification and optimization, and one of the strengths of this type of tool is that it takes into account the complex chemical representation of diverse structural scaffolds that can express similar chemical functions [62]. The models allow medicinal chemists to search 3D databases of compounds

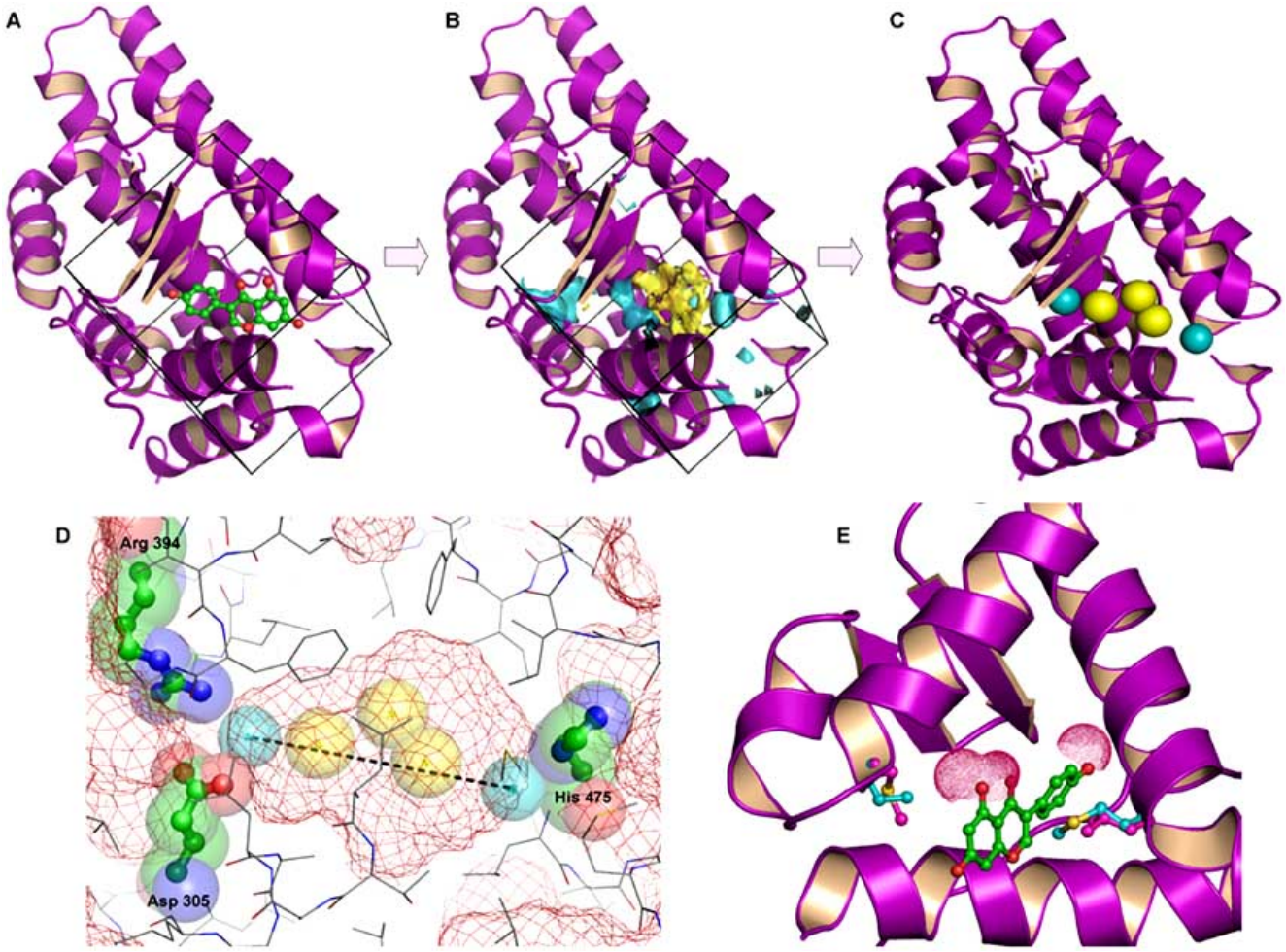

Fig. (6). Schematic representation of a receptor-based pharmacophore model for ER. **A**) Ligand-binding domain of ERβ structure in complex with genistein (green). **B**) Hydrophobic (yellow) and hydrophilic (cyan) GRID probes used to map the binding cavities of the ERβ structures. **C**) Important ligand binding regions translated into a pharmacophore query (terminal hydrogen bond acceptors in cyan, and a central hydrophobic part in yellow). **D**) View of the proposed pharmacophore model, with some important residues displayed in green. **E**) Selectivity model for ER.

using appropriate software, such as UNITY [74], Catalyst [75] and FlexX-Pharm [76]. Structure-based pharmacophore design and database searching enable the investigation of the intermolecular interactions and binding mode in the active site region, leading to the identification of compounds that can display good affinity and selectivity for the target receptor, as schematically shown in Fig. (**8**). Moreover, VS procedures using pharmacophore models represent a useful tool to assess the docking results and the rank order of the compounds [24,34,59,77-79].

As shown in Fig. (**9**) for a series of indazole derivatives as DNA-gyrase inhibitors, pharmacophore models can be used to guide the synthesis of advanced molecules for hit-to-lead and lead optimization programs [39,80]. DNA gyrase is a well-established therapeutic target for antimicrobial agents, since it is an essential prokaryotic type II topoisomerase with no direct mammalian counterpart. As HTS has not provided suitable hits, a rational approach was applied in the search for novel inhibitors based on a combination of computational and experimental techniques. Firstly, detailed structural information obtained both from SAR studies for cyclothialidines and from complexes of the ATP binding site with ADPNP, cyclothialidine A and novobiocin, a clinical agent used against multi-resistant *Staphylococcus aureus*, was used to generate pharmacophore models. Although these ligands interact in different subregions of the same binding pocket, a common binding motif was identified, as shown in Fig. (**9**): they were found to donate a hydrogen bond to Asp73 and to accept a hydrogen bond from a conserved water molecule. Additionally, a hydrophobic part, which is complementary to the enzyme pocket, was recognized. The final pharmacophore model (Fig. **9**) was used in VS experiments leading to the discovery of novel DNA gyrase inhibitors. In this context, a data set of 350,000 compounds was virtually screened to identify molecules that could fulfill the pharmacophore model. Subsequently, the molecules identified and their corresponding analogs (MW < 300) were clustered, generating representative potential low molecular weight

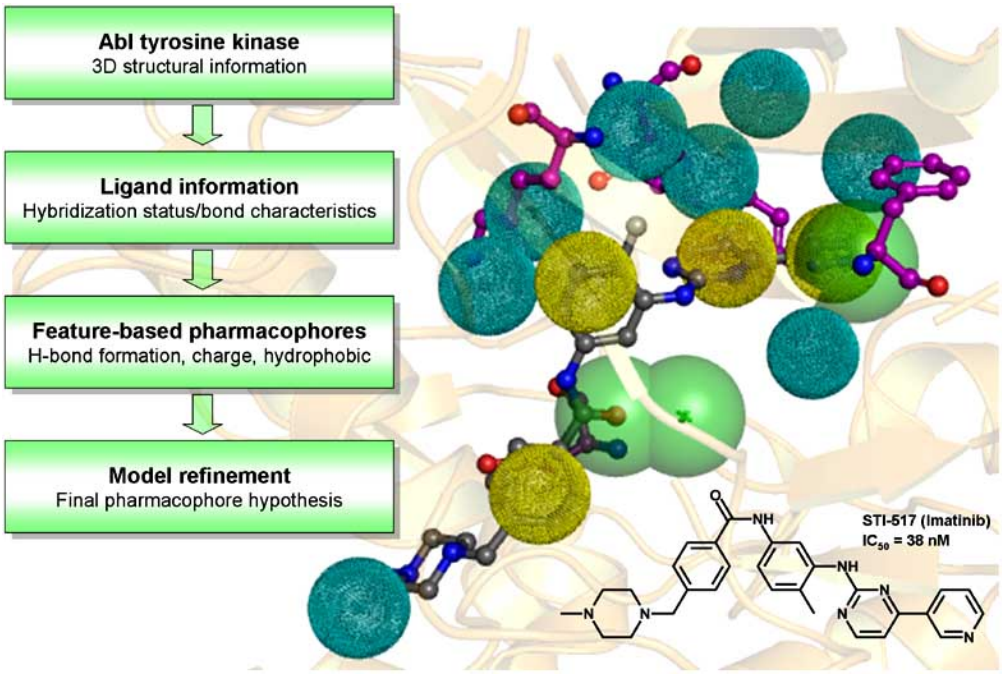

Fig. (7). Key steps for the generation of a 3D pharmacophore model based on the Bcr-Abl tyrosine kinase crystal structures. The pharmacophore model consists of four hydrophobic aromatic groups (yellow) and two hydrogen acceptors (green) handled by vectorized representations. Spheres for excluded volume are shown in blue.

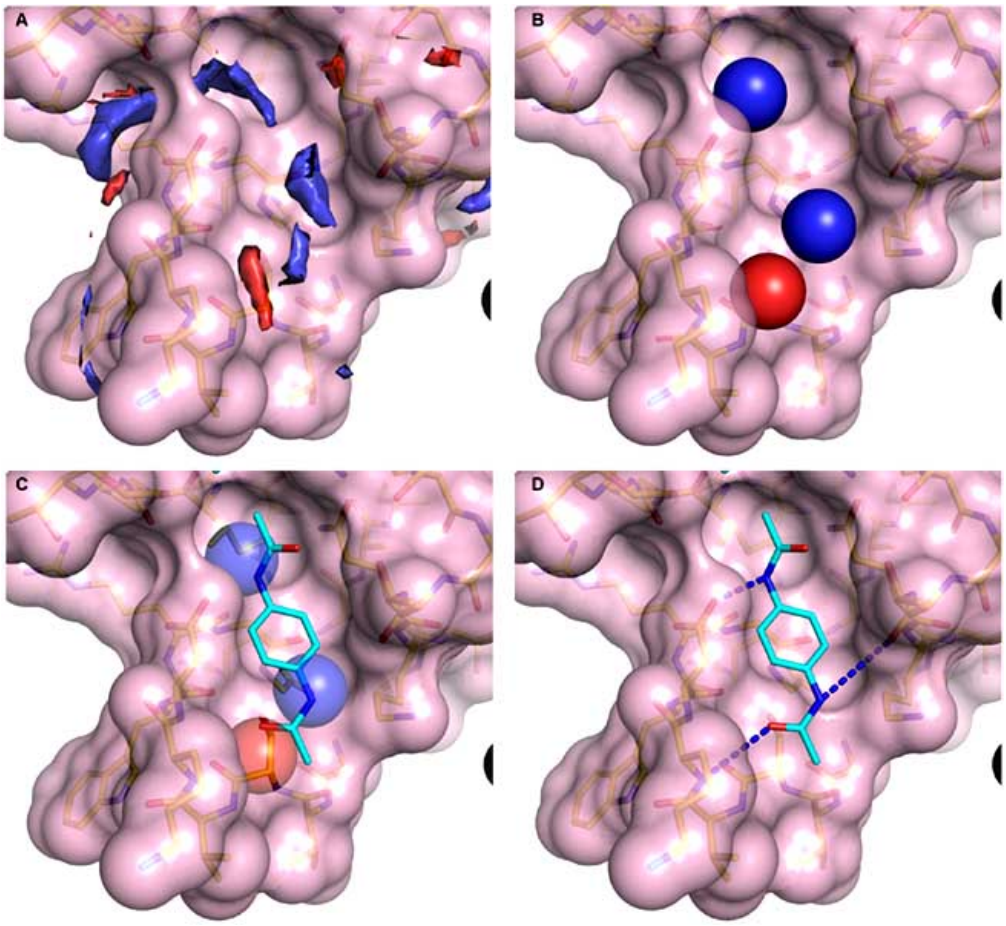

Fig. (8). **A**) Hot spots mapping the active site of a protein structure. Properties of putative hydrogen bond donors are colored blue while acceptors are colored red. **B**) Target-based pharmacophore points based on MIF. Hydrogen bond acceptor groups are displayed in blue and donors in red. **C**) Fit of a ligand over its corresponding target-based pharmacophore points. **D**) Polar interactions of the proposed ligand.

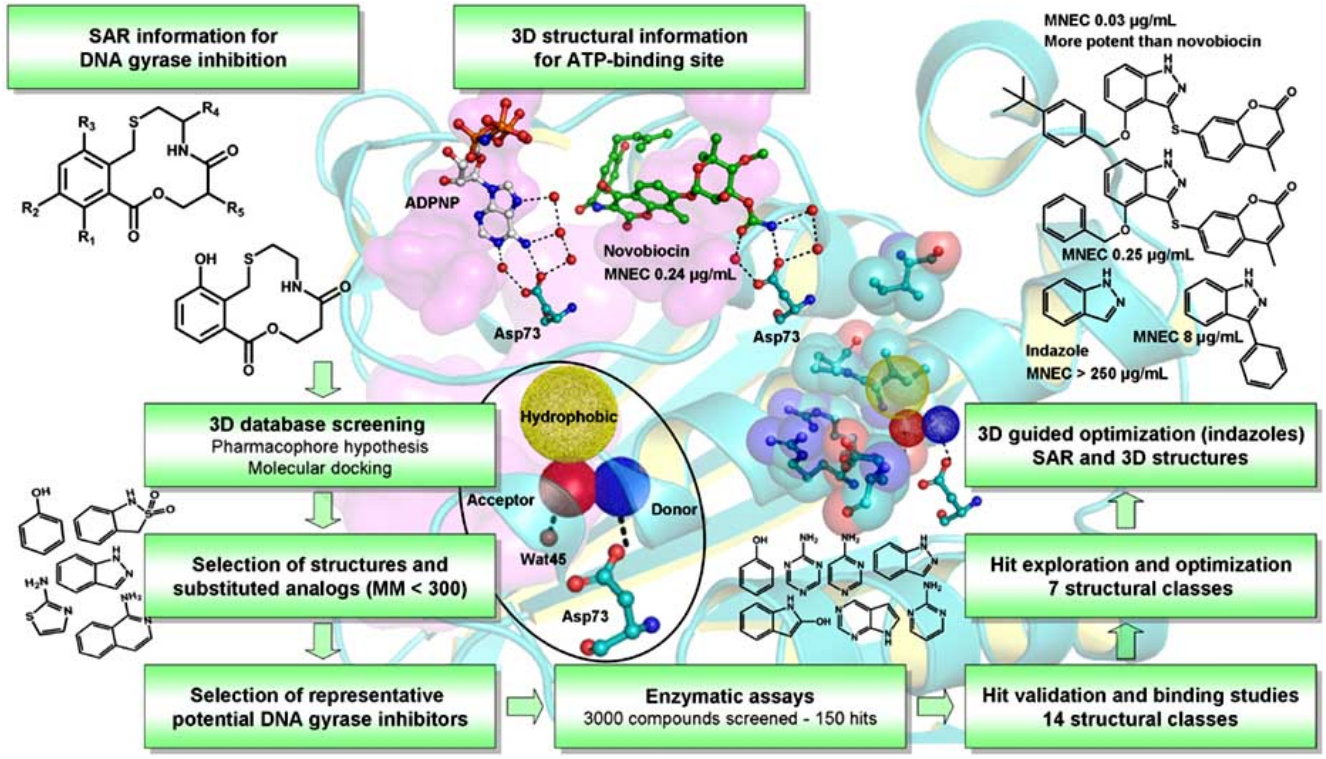

Fig. (9). Identification and optimization of a series of indazole derivatives as DNA gyrase inhibitors.

inhibitors. A total of 3,000 molecules were screened in enzymatic assays providing 150 hits, but most of them exhibited moderate to low inhibition (maximal noneffective concentration, MNEC, in the micromolar range). Results from biophysical and binding studies indicated that these hits are specific modulators of the DNA gyrase ATP binding site. Seven chemical classes were then validated as novel DNA gyrase inhibitors, including: indazoles, phenols, 2-amino-triazines, 4-amino-pyrimidines, 2-amino-pyrimidines, pyrrolopyrimidines, and 2-hydroxymethyl-indoles. The indazoles were then selected for 3D receptor-based optimization, resulting in highly potent inhibitors, such as 3,4-disubstituted indazole (Fig. **9**), about 10-fold more potent than the aminocoumarin novobiocin.

The complexity of the structural 3D requirements can progressively be increased, thus restricting the features presented by the molecules during the process of optimization. This strategy is especially useful to reduce the number of compounds to be considered or tested in subsequent phases. Furthermore, pharmacophores can be used to align molecules in order to develop 3D QSAR models [81]. Recently, it has also been proposed that pharmacophore models could be applied to study the potential biological properties of molecules for different targets, considering their biological and chemical properties [82]. Interestingly, effective strategies have been developed to account for protein flexibility, including the creation of dynamic pharmacophore models [41,83-85].

STRUCTURE-BASED LEAD OPTIMIZATION

The identification of a very limited number of promising hits and the generation of high quality leads are crucial steps in the early stages of drug discovery (Fig. **2**). Regardless of the strategy adopted (e.g., HTS, fragment-based, VS) for the identification of hits, these are generally in the micromolar range of activity. Hence, potency and affinity are parameters that must be improved by iterative cycles of ligand-receptor optimization and biological evaluation. Moreover, additional pharmacodynamic and pharmacokinetic properties, such as target selectivity, *in vivo* efficacy and bioavailability, have to be progressively adjusted and optimized in order to provide NCEs for clinical development (i.e., drug candidates) [4,7,-10,27,86-88]. Successful lead optimization requires a better understanding of the complex factors responsible for binding affinity and specificity. Structural information, biological data, and advanced medicinal chemistry approaches have been applied to this context and have resulted in the identification of numerous late-stage development compounds. Detailed knowledge and understanding of the SAR is a cornerstone for the progress of lead optimization projects. The rational design of experiments for the generation of standard large-scale biological data is a critical step, making possible comparisons of predictions and experimental results. The broad variety of hits and leads that need to be further optimized highlights the importance of SAR studies in drug design, and as a consequence, allows the generation of a wide range of data sets [29]. As schematically shown in Fig. (**10**), structure-based lead optimization involves the synthesis and biological evaluation of several compounds sharing a high degree of chemical similarity, but still possessing interesting structural diversity. Its success and effectiveness will depend to a large extent on the existence of a well-defined and controlled integration of medicinal chemistry efforts, including molecular modeling,

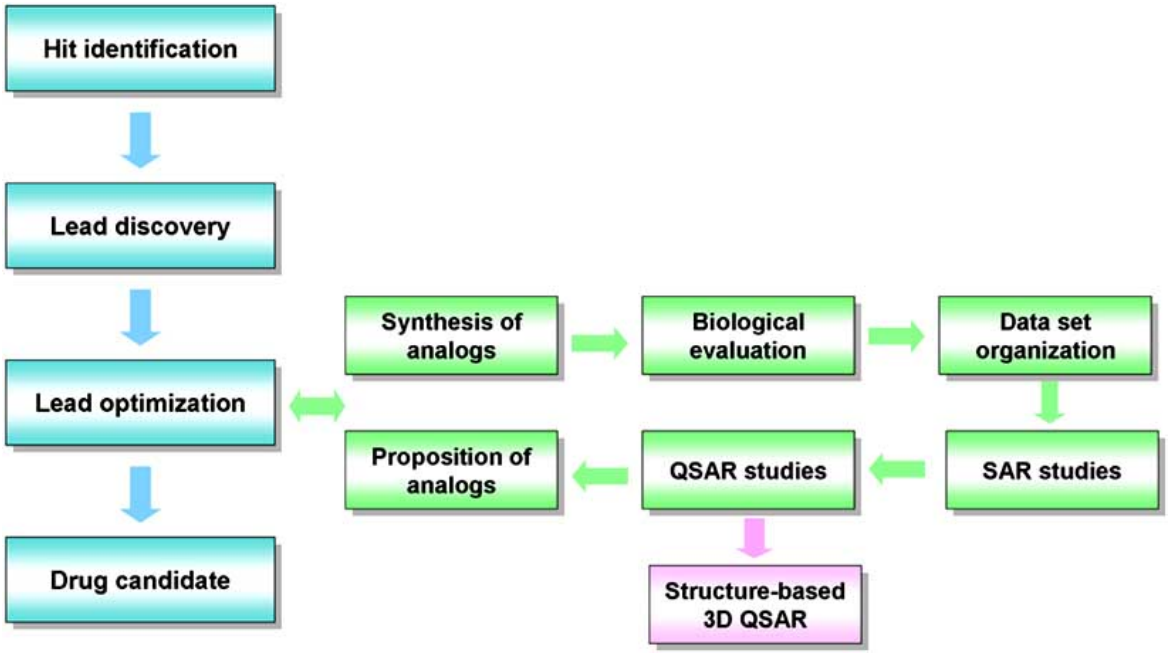

Fig. (10). Process of hit-to-lead optimization and drug candidate selection. SAR and QSAR studies are essential elements of this complex paradigm.

design, synthesis and biological evaluation [10,15,25,39,89-97].

An example of fragment-based lead discovery and optimization is shown in Fig. (**11**) for the development of cyclin dependent kinase 2 (CDK2) inhibitors with low nanomolar affinity, improved cellular activity and good pharmacokinetic profile [91]. The inhibition of CDK2, which is a serine-threonine kinase involved in cell cycle regulation, has been demonstrated to be an effective method for controlling tumor growth, and hence is an attractive approach for cancer chemotherapy. Initially, the application of fragment-based screening techniques to CDK2 identified multiple fragments that bind to the ATP binding site (e.g., chloropyrazinamine, indazole and pyrazolopyrimidine). Although the hits showed only moderate to low potency (40 μM to 1 mM), they were highly efficient binders (ligand efficiency, LE, between 0.35 to 0.6) due to their low molecular weight (<225) and limited functionality, and were then considered suitable targets for further optimization. A detailed analysis of the ATP binding cavity, as well as of the binding mode of known CDK2 ligands (throughout the optimization process of pyrazolopyrimidines) led to the identification of important molecular regions associated with enhanced activity, including (i) the essential hydrogen bonds anchoring the ligand to the hinge region (Glu81 and Leu83); (ii) a water mediated hydrogen bond between the benzamide group and the catalytic Asp145 of the DFG motif; (iii) stabilization of the twisted benzamide conformation by introduction of ortho-substituents; (iv) introduction of the solubilizing aminopiperidine amide group to improve physicochemical and pharmacokinetic properties, further resulting in the occupancy of a hydrophobic pocket near the solvent accessible area, which is related to improved cellular activity and selectivity over non-CDK kinases [98]. The iterative cycle of optimization of potency, cellular activity and pharmacokinetic properties, guided by SAR and structure-based studies, resulted in the development of AT7519, which is currently being evaluated in clinical trials for the treatment of human cancers (Fig. **11**) [91]. The process of lead optimization is typically a major challenge which requires substantial research and the development of innovative approaches integrating science and technology in order to advance to the next level of improved affinity or potency by several orders of magnitude.

When the relationship between the chemical structures of the ligands and their corresponding biological activity can be quantitatively measured, expressed and compared (i.e., SARs within data sets of structurally related compounds), they become quantitative SARs (or QSARs). QSAR is a methodology that applies statistical analysis of relationships between descriptors based on molecular structures and biological activity in a quantitative and mechanism-oriented manner. QSAR has a long history in the drug discovery field, and achieved a remarkable impact in the optimization of promising leads that act on specific targets. QSAR approaches are widely used for improving and optimizing the performance of the several rounds required for lead optimization (e.g., design, synthesis, biological evaluation) [29, 99-105]. This technology plays a vital role in drug design and has been employed, and continue to be developed and employed, both to correlate information in data sets and as a tool to facilitate, for example, the discovery of enzyme inhibitors, agonist or antagonists of important drug targets (Fig. **10**) [24,71,106-113].

The availability of advanced molecular modeling techniques and several 2D and 3D QSAR methods has attracted the attention of many scientists around the world for the integration of computational drug design tools [24]. Many useful QSAR models have been developed in conjunction with improved knowledge of the structure and function of the target receptor, thus providing useful opportunities to capture and incorporate important information for the design of promising small-molecule drug candidates. An interesting

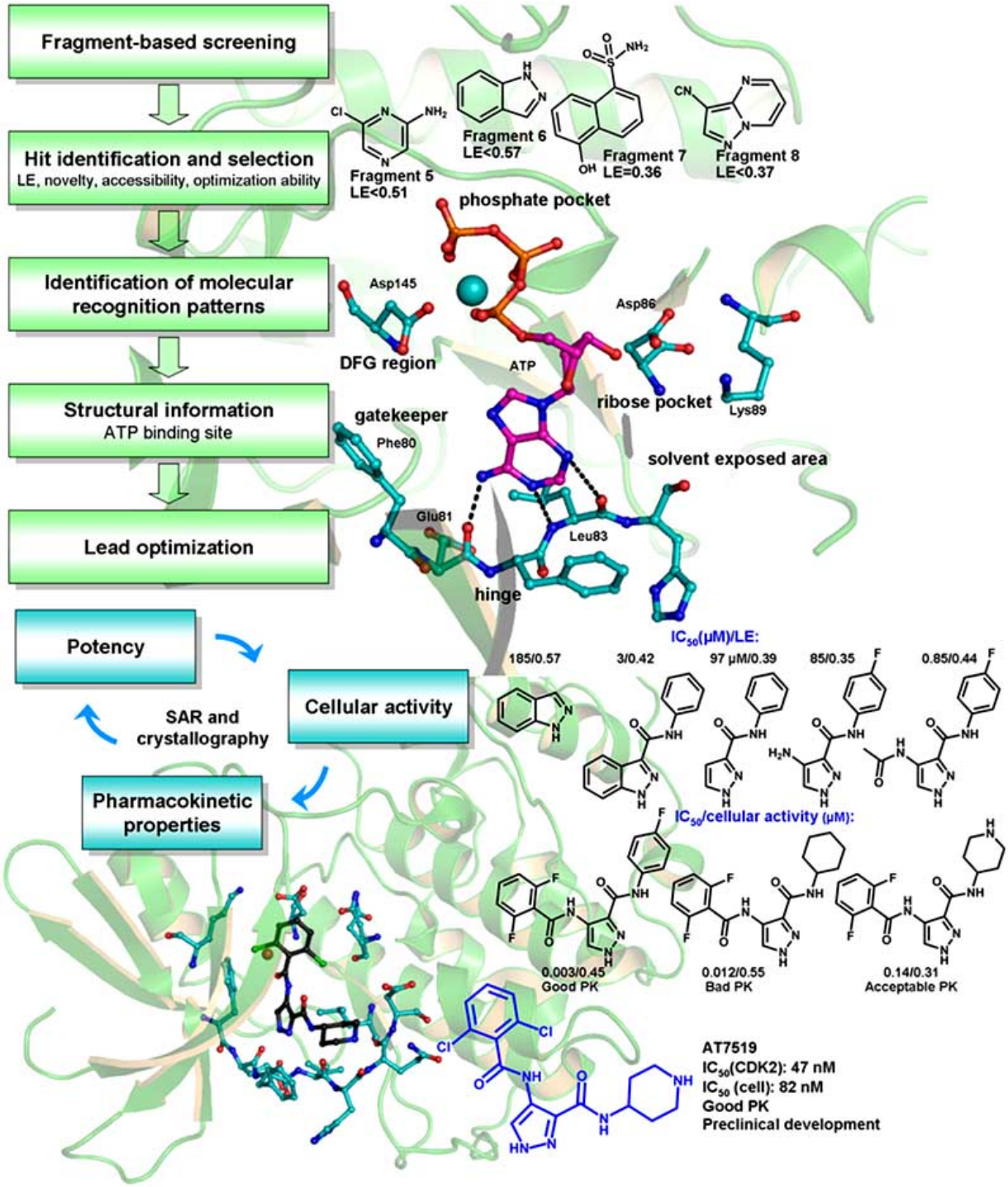

Fig. (11). Fragment-based identification and lead optimization strategies in the developing CDK2 inhibitors with high affinity, cellular activity and good pharmacokinetic properties.

example of integration of QSAR and SBDD in the area of cancer research is showed in Fig. (**12**) [29,110]. In this study, in the absence of tubulin-bound discodermolide crystal structures, a powerful fragment-based 2D QSAR method, hologram QSAR (HQSAR), was used to generate molecular recognition patterns that were then integrated with molecular modeling studies as a step for the understanding of essential discodermolide-tubulin interactions associated with its high antiproliferative activity. The conformation-independent HQSAR method was especially adequate, since the discodermolide system is very flexible and complex. As shown in Fig. (**12**), the most important 2D fragments associated with biological activity (positive contributions) were selected and used for the study of the main intermolecular interactions within the β-tubulin system.

The receptor binding affinity and respective biological activity of a small-molecule modulator are directly related to

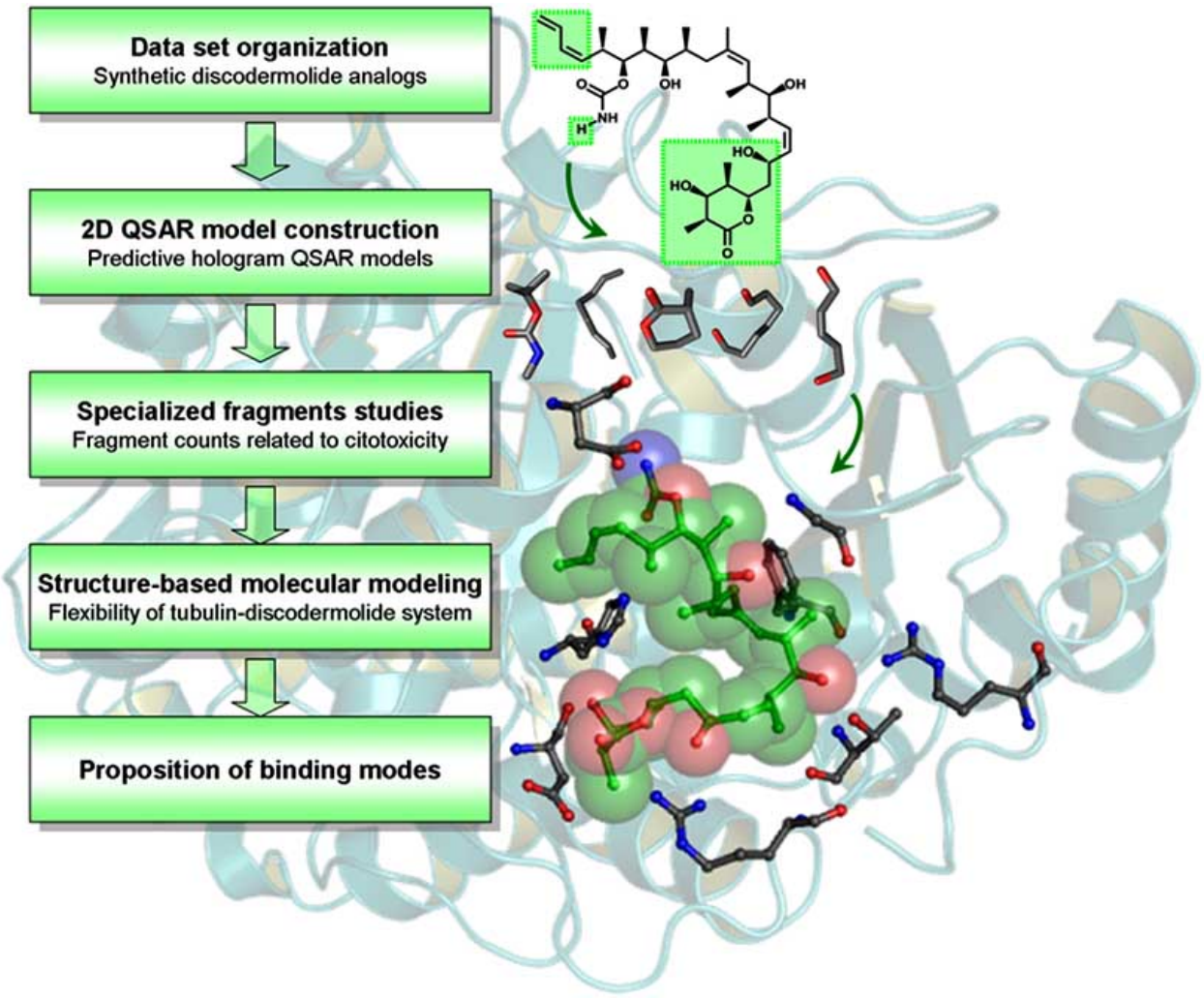

Fig. (12). Integration of 2D QSAR and molecular modeling studies in the generation of 3D models of interaction between discodermolide and the β-tubulin cavity, employing the most important HQSAR molecular fragments related to the antiproliferative activity of the discodermolide analogs.

multiple and complex intermolecular interactions and forces of non-covalent nature (e.g., hydrogen bonds, electrostatic interactions, steric and hydrophobic effects). Therefore, molecular properties (descriptors) derived from the optimized 3D structures of biologically active compounds are especially useful in describing the reversible and specific receptor-ligand interactions [29]. In this context, structure-based 3D QSAR approaches are able to explore in detail both spatial and electrostatic properties that play a vital role in the formation of high affinity receptor-ligand complexes. This is of fundamental importance in the understanding of the molecular aspects that may reflect changes in the activities of series of structurally related compounds (which target the same receptor), and consequently, these studies are a positive effort to improve the connections between the chemical and biological space of data sets of compounds.

The comparative molecular field analysis (CoMFA) is the most widely used 3D QSAR approach for the prediction of biological activity and ligand-binding properties of ligands within data sets of high quality. In this method, quantitative relationships may be derived by sampling the steric and electrostatic fields surrounding a set of ligands, and the calculated fields are examined and related to the biological activity on a specific system. A major challenge in this approach is the selection of an optimized 3D conformation of each ligand or a common spatial orientation with respect to the other ligands (the entire data set). The molecular alignment rule is the most important adjustable parameter and strongly affects the outcome of the 3D statistical analysis. The ideal alignment should represent the ligand-binding conformations adopted in the receptor binding site. For this reason, SBDD approaches (e.g., docking, pharmacophore models) are commonly used in CoMFA studies to generate 3D structural alignments of distinct data sets, incorporating important structural elements for the development and interpretation of the 3D QSAR models in terms of their chemical and biological significance (e.g., molecular mechanisms underlying the biological effects). In fact, several studies have shown the successful use of 3D QSAR and SBDD methods in a complementary way [71,106-113]. The integration of these technologies represents an efficient approach for drug discovery and lead optimization.

As an illustrative example of CoMFA guided drug design, a large series of flavanoids, dihydrobenzoxathiins and dihydrobenzodithiins as estrogen receptor (ER) modulators was employed to generated 3D QSAR models possessing high internal quality and significant predictive power [100]. In this study, structural and chemical features related to pharmacological properties, such as improved binding affinity and potency (inhibition of MCF-7 cell growth) were investigated. It is important to note that in addition to predicting accurately the property values (IC_{50}) of untested compounds that are mostly structurally-related to the training set molecules, robust QSAR models can provide important information about the relevance of the applied descriptors for the property of interest being studied. This is the case as the statistical results often return weights that indicate the sign and magnitude of the steric and electrostatic fields in the modulation of the dependent variable. This information provides insights into the mechanism of action of series of active molecules and guides the synthesis pathway toward particular structures with improved properties.

The CoMFA results were highly compatible with the 3D environment of the ER binding site as demonstrated in Fig. (**13**) for the generated molecular contour fields. The CoMFA electrostatic contour maps for affinity (Fig. **13A**) and potency (Fig. **13 B**) show blue contours surrounding the protonated nitrogen atom in the pyrrolidine ring, representing a region where electropositive environment is related to increasing binding affinity and potency. The electrostatic fields are, however, more important to explain the differences of potency than the corresponding variations in the binding affinity values [106]. Therefore, it indicates that the protonated nitrogen of the pyrrolidine ring is important not only for binding affinity, but also for the antagonist biocharacter, which is in agreement with the chemical environment of the protein, and also with previous studies of the electrostatic interactions between the N of the heterocyclic ring and Asp351 (responsible for the antiestrogenic activity). The CoMFA steric fields are similar in both 3D contour maps as the yellow contour near the benzoxathiin reveals that less steric bulky substituents attached to this ring are positively related to affinity and potency. On the other hand, bulky *para*- or *metha*-substituents in the phenyl ring are related to enhanced affinity, but not with increased potency as indicated by the green region encompassing the phenyl ring. These results are in agreement with the 3D target environment, as depicted in Fig. (**13**) and suggest that these models should be useful for the design of novel structurally related ER antagonists presenting optimized binding affinity and potency properties.

Other 3D QSAR approaches commonly used in drug design studies include GRID/GOLPE (generating optimal linear PLS estimations) [63,114], CoMSIA (comparative molecular similarity index analysis) [98], and EVA (QSAR by eigenvalue analysis) [115]. Variations of well know methods can also be found, such as CoMMA (comparative molecular moment analysis) [116], AFMoC (adaptation of fields for molecular comparison) [117], and HASL (hypothetical active site lattice) [118].

The strategy of combining 3D contour maps with the structural environment of the protein (target binding site) has proven useful to investigate important parameters such as affinity and potency, but can also be used to study other pharmacodymanic and pharmacokinetic properties, including selectivity, reactivity and metabolism [71,107,119-127]. Furthermore, considering that the QSAR models may provide useful insights into structural and chemical features related to the property of interest (biological activity parameter), it is possible to envisage that the same general approach can be applied in SBVS for the identification of hits [128-131]. For QSAR model development, different weights are associated with each selected independent variable (physicochemical parameters). These weights can be interpreted as a measure of the importance of the different intermolecular interactions for the target biological activity. Therefore, rigorously validated QSAR models can be used as a filter to select and rank molecules in the process of SBVS [128, 131-134]. An interesting example of the integration of 3D QSAR and pharmacophore models in the search for new compounds targeting the 16S RNA A site of the bacterial ribosome is shown in Fig. (**14**), revealing the potential of this strategy in overcoming difficulties in docking and scoring of RNA-ligand complexes [134,135]. The increasing bacterial resistance to existing antibiotics highlights the need for the development of novel therapeutic agents. One of the main targets for designing selective antibacterial drugs is the prokaryotic ribosome, the target of a clinical relevant class of broad spectrum antibiotics, the aminoglycosides. As shown in Fig. (**14**), aminoglycosides bind to the 16S RNA of ribosome at the double helix of aminoacyl-tRNA decoding site (A site). The ring I of the neamine core is inserted into the helix, where it stacks over G1491 and forms a pseudobase pair with A1408. Amino groups linked to the ring II (2-deoxystreptamine) are involved in hydrogen bonds with a conserved U1406.U1495 pair as well as G1494 and a phosphate group of A1493. Additional contacts, formed by the remaining rings as well as water molecules in different positions, are responsible for the diversity of ligands that can be accommodated. The binding of aminoglycosides reduces the ability of the ribosome to discrimate between tRNAs, leading to death of the bacterial cell.

SBDD strategies for RNA-binders remain a major challenge, because most of the methods were developed for studying protein-ligand complexes. Therefore, in the search for novel 16S rRNA ligands containing a neamine motif, an integrated strategy was used. Pharmacophore models were derived based on features of the 16S RNA bound to paromomycin (PDB code 1J7T). In this way were selected hydrogen bonds with C1407 (acceptor) and G1491 (donor), positive charges near G1405 and steric constraints (Fig. **14**). A rigid 3D search resulted in promising compounds, which were positioned and minimized into the binding site. Predictive 3D QSAR models for aminoglycoside derivatives were used to evaluate and score the proposed binding modes of selected compounds and fragments. The most interesting compounds were further prioritized for biological tests [134].

CONCLUSION

The explosion of genomic, proteomic, and structural information has provided hundreds of new targets and opportunities for drug discovery. The modern drug discovery process is increasingly becoming more information driven.

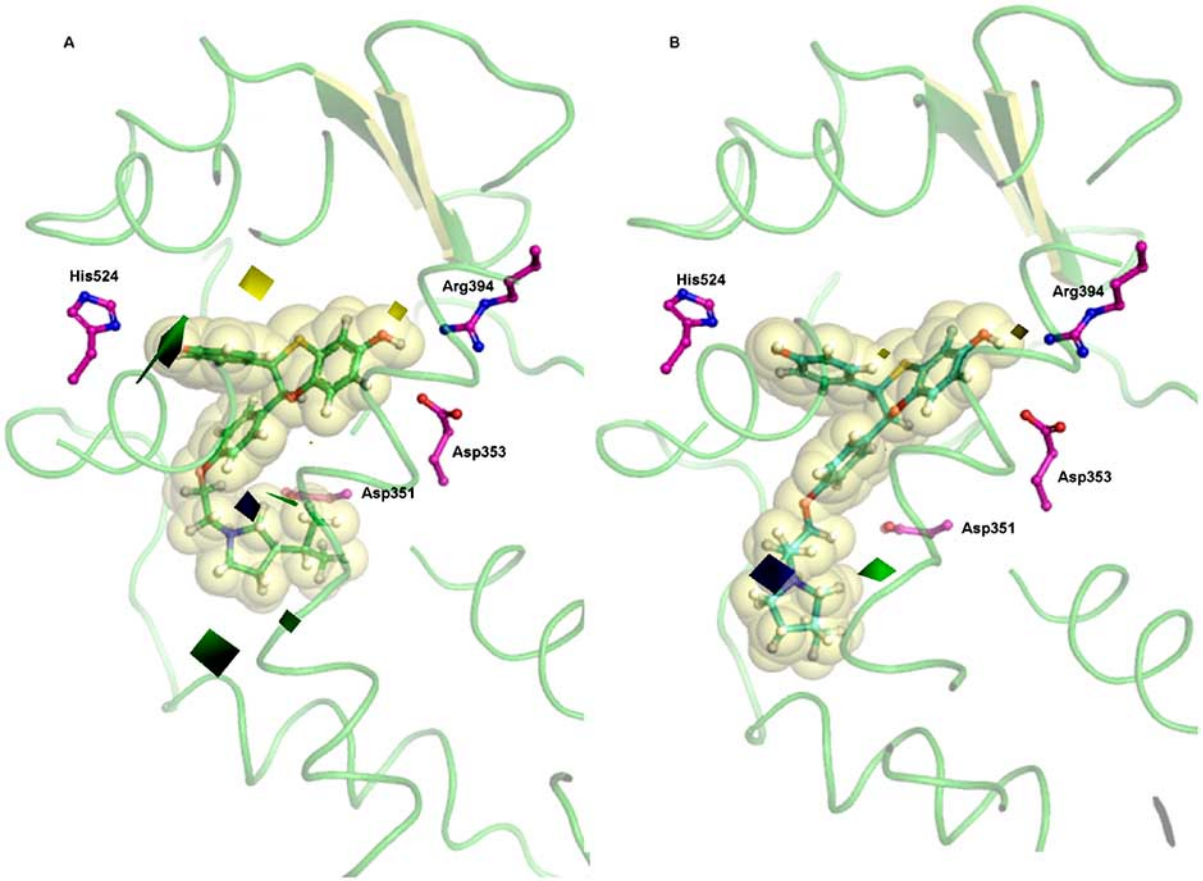

Fig. (13). Structure-based 3D QSAR CoMFA studies in the design of improved ligands of ER (PDB ID 1XP1). A. CoMFA contour steric and electrostatic maps for binding affinity. The highest binding affinity of the ER modulators (IC_{50}=0.3 nM) is shown as background reference. B. CoMFA contour steric and electrostatic maps for potency. The most potent antagonist (IC_{50}=0.03 nM) is displayed in the background for reference.

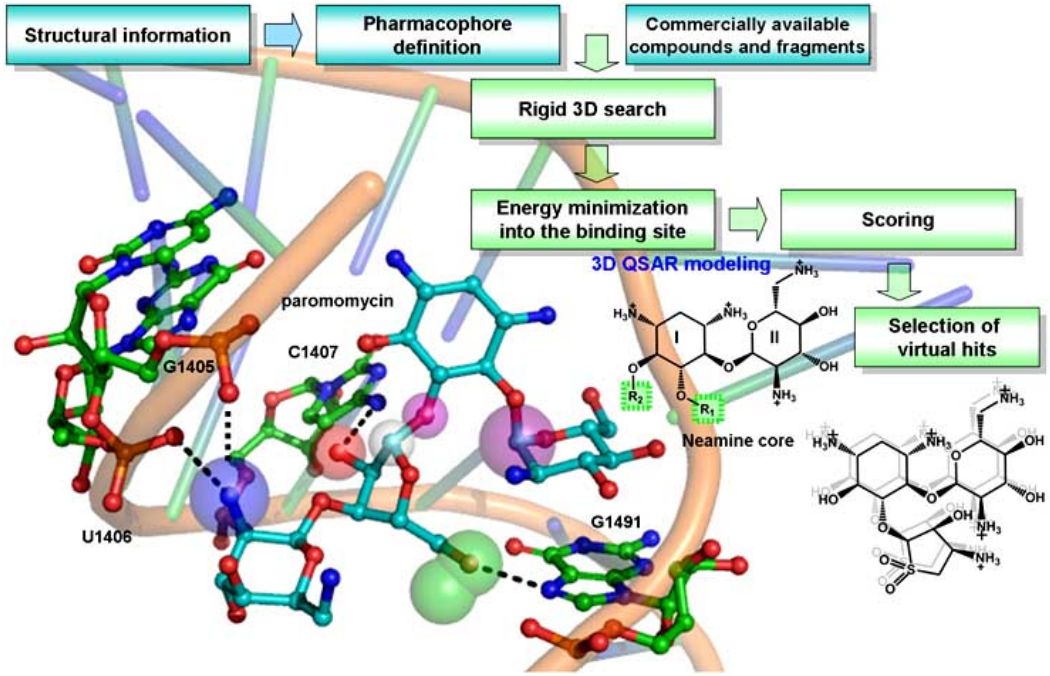

Fig. (14). Integration of 3D QSAR and pharmacophore models in SBVS for 16 S rRNA ligands. Red spheres: hydrogen bond acceptors; green spheres: hydrogen bond donors; blue spheres: positive nitrogen atom; violet spheres: steric features corresponding to receptor atoms. Small gray and violet spheres denote positions of those atoms necessary to form bonds with oxygen atoms O5 or O6.

Recent years have seen a tremendous increase in new technologies and methods for the design of NCEs. Virtual screening and pharmacophore modeling are state of the art knowledge-based approaches that use structural information from both targets and ligands. They are useful tools to find novel molecules with similar biological activity, or to improve the potency, affinity or selectivity of active compounds of interest. The use of these drug design strategies has increased enormously in recent years because of the availability of databases with millions of commercially available compounds, as well as 3D structures of several target proteins. Structure-based drug design has a long and rich history and continues to expand and evolve in response to scientific and technological developments, and hopefully will have a long and interesting future in the identification and optimization of promising leads having high potential for generating new therapeutic agents.

ACKNOWLEDGMENTS

We gratefully acknowledge financial support from the National Council for Scientific and Technological Development (CNPq, Conselho Nacional Desenvolvimento Científico e Technológico) and the State of São Paulo Research Foundation (FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo), Brazil.

REFERENCES

[1] Hopkins, A. L.; Groom, C. R. The Druggable Genome. *Nat. Rev. Drug Discov.* **2002**, *1*, 727-730.

[2] Hajduk, P. J.; Huth, J. R.; Tse, C. Predicting Protein Druggability. *Drug Discov. Today* **2005**, *10*, 1675-1682.

[3] Cavasotto, C. N.; Orry, A. J. Ligand Docking and Structure-Based Virtual Screening in Drug Discovery. *Curr. Top. Med. Chem.* **2007**, *7*, 1006-1014.

[4] Cardoso, C. L.; Lima, V. V.; Zottis, A.; Oliva, G.; Andricopulo, A. D.; Wainer, I. W.; Moaddel, R.; Cass, Q. B. Development and characterization of an immobilized enzyme reactor (IMER) based on human glyceraldehyde-3-phosphate dehydrogenase for on-line enzymatic studies. *J. Chromatogr. A*, **2006**, *1120*, 151-157.

[5] Bajorath, J. Integration of Virtual and High-Throughput Screening. *Nat. Rev. Drug Discov.* **2002**, *1*, 882-894.

[6] Jorgensen, W. L. The Many Roles of Computation in Drug Discovery. *Science* **2004**, *303*, 1813-1818.

[7] Kitchen, D. B.; Decornez, H.; Furr, J. R.; Bajorath, J. Docking and Scoring in Virtual Screening for Drug Discovery: Methods and Applications. *Nat. Rev. Drug Discov.* **2004**, *3*, 935-949.

[8] Henry, C. M. Structure-Based Drug Design. *Chem. Eng. News* **2001**, *79*, 69-74.

[9] Anderson, A. C. The Process of Structure-Based Drug Design. *Chem. Biol.* **2003**, *10*, 787-797.

[10] Klebe, G. Recent Developments in Structure-Based Drug Design. *J. Mol. Med.* **2000**, *78*, 269-281.

[11] Kubinyi, H. Drug Research: Myths, Hype and Reality. *Nat. Rev. Drug Discov.* **2003**, *2*, 665-668.

[12] Andricopulo, A. D.; Akoachere, M. B.; Krogh, R.; Nickel, C.; McLeish, M. J.; Kenyon, G. L.; Arscott, L. D.; Willians, C. H.; Davioud-Chavert, E.; Becker, K. Specific Inhibitors of *Plasmodium falciparum* Thioredoxin Reductase as Potential Antimalarial Agents. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2283-2292.

[13] Rahuel, J.; Priestle, J. P.; Grutter, M. G. The Crystal Structures of Recombinant Glycosylated Human Renin Alone and in Complex with a Transition State Analog Inhibitor. *J. Struct. Biol.* **1991**, *107*, 227-236.

[14] Wood, J. M.; Criscione, L.; de Gasparo, M.; Bühlmayer, P.; Rüeger, H.; Stanton, J. L.; Jupp, R. A.; Kay, J. CGP 38 560: Orally Active, Low-Molecular-Weight Renin Inhibitor with High Potency and Specificity. *J. Cardiovasc. Pharmacol.* **1989**, *14*, 221-226.

[15] Rahuel, J.; Rasetti, V.; Maibaum, J.; Rueger, H.; Goschke, R.; Cohen, N.C.; Stutz, S.; Cumin, F.; Fuhrer, W.; Wood, J. M.; Grutter, M. G. Structure-Based Drug Design: The Discovery of Novel Nonpeptide Orally Active Inhibitors of Human Renin. *Chem. Biol.*, **2000**, *7*, 493-504.

[16] Jensen, C.; Herold, P.; Brunner, H. R. Aliskiren: The First Renin Inhibitor for Clinical Treatment. *Nat. Rev. Drug Discov.* **2008**, *7*, 399-410.

[17] Vasanthanathan, P.; Hritz, J.; Taboureau, O.; Olsen, L.; Jørgensen, F. S.; Vermeulen, N. P.; Oostenbrink, C. Virtual Screening and Prediction of Site of Metabolism for Cytochrome P450 1A2 Ligands. *J. Chem. Inf. Model.* **2009**, *49*, 43-52.

[18] Venhorst, J.; ter Laak, A. M.; Commandeur, J. N.; Funae, Y.; Hiroi, T.; Vermeulen, N. P. Homology Modeling of Rat and Human Cytochrome P450 2D (CYP2D) Isoforms and Computational Rationalization of Experimental Ligand-binding Specificities. *J. Med. Chem.* **2003**, *46*, 74-86.

[19] Lyne, P. D. Structure-Based Virtual Screening: An Overview. *Drug Discov. Today* **2002**, *7*, 1047-1055.

[20] Klebe, G. Virtual Ligand Screening: Strategies, Perspectives and Limitations. *Drug Discov. Today* **2006**, *11*, 580-594.

[21] Whittle, P. J.; Blundell, T. L. Protein Structure-Based Drug Design. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 349-375.

[22] Davis, A. M.; Teague, S. J.; Kleywegt, G. J. Application and Limitations of X-Ray Crystallographic Data in Structure-Based Ligand and Drug Design. *Angew. Chem. Int. Ed.* **2003**, *42*, 2718-2736.

[23] Ghosh, S.; Nie, A.; An, J.; Huang, Z. Structure-Based Virtual Screening of Chemical Libraries for Drug Discovery. *Curr. Opin. Chem. Biol.* **2006**, *10*, 194-202.

[24] Guido, R. V. C.; Oliva, G.; Andricopulo, A. D. Virtual Screening and Its Integration with Modern Drug Design Technologies. *Curr. Med. Chem.* **2008**, 15, 37-46.

[25] Joseph-McCarthy, D.; Baber, J. C.; Feyfant, E.; Thompson, D. C.; Humblet, C. Lead Optimization *Via* High-Throughput Molecular Docking. *Curr. Opin. Drug. Discov. Dev.* **2007**, *10*, 264-274.

[26] Leach, A. R.; Shoichet, B. K.; Peishoff, C. E. Prediction of Protein-Ligand Interactions. Docking and Scoring: Successes and Gaps. *J. Med. Chem.* **2006**, *49*, 5851-5855.

[27] Shoichet, B. K.; McGovern, S. L.; Wei, B.; Irwin, J. J. Lead Discovery Using Molecular Docking. *Curr. Opin. Chem. Biol.* **2002**, *6*, 439-446.

[28] Livingstone, D. J. The Characterization of Chemical Structures Using Molecular Properties. A Survey. *J. Chem. Inf. Comput. Sci.* **2000**, *40*, 195-209.

[29] Salum, L. B.; Andricopulo, A. D. Fragment-Based QSAR: Perspectives in Drug Design. *Mol. Divers.* **2009**, *13*, 277-285.

[30] Kuhn, P.; Wilson, K.; Patch, M. G.; Stevens, R. C. The Genesis of High-Throughput Structure-Based Drug Discovery Using Protein Crystallography. *Curr. Opin. Chem. Biol.* **2002**, *6*, 704-710.

[31] Kaldor, S. W.; Kalish, V. J.; Davies, J. F. II; Shetty, B. V.; Fritz, J. E.; Appelt, K.; Burgess, J. A.; Campanale, K. M.; Chirgadze, N. Y.; Clawson, D. K.; Dressman, B. A.; Hatch, S. D.; Khalil, D. A.; Kosa, M. B.; Lubbehusen, P. P.; Muesing, M. A.; Patick, A. K.; Reich, S. H.; Su, K. S.; Tatlock, J. H. Viracept (Nelfinavir Mesylate, AG1343): A Potent, Orally Bioavailable Inhibitor of HIV-1 Protease. *J. Med. Chem.* **1997**, *40*, 3979-3985.

[32] Wlodawer, A.; Vondrasek, J. Inhibitors of HIV-1 Protease: A Major Success of Structure-Assisted Drug Design. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 249-284.

[33] Babu, Y. S.; Chand, P.; Bantia, S.; Kotian, P.; Dehghani, A.; El-Kattan, Y.; Lin, T. H.; Hutchison, T. L.; Elliott, A. J.; Parker, C. D.; Ananth, S. L.; Horn, L. L.; Laver, G. W.; Montgomery, J. A. BCX-1812 (RWJ-270201): Discovery of a Novel, Highly Potent, Orally Active, and Selective Influenza Neuraminidase Inhibitor Through Structure-Based Drug Design. *J. Med. Chem.* **2000**, *43*, 3482-3486.

[34] Grüneberg, S.; Stubbs, M. T.; Klebe, G. Successful Virtual Screening for Novel Inhibitors of Human Carbonic Anhydrase: Strategy and Experimental Confirmation. *J. Med. Chem.* **2002**, *45*, 3588-3602.

[35] Grüneberg, S.; Wendt, B.; Klebe, G. Subnanomolar Inhibitors from Computer Screening: A Model Study Using Human Carbonic Anhydrase II. *Angew. Chem. Int. Ed.* **2001**, *40*, 389-393.

[36] Doman, T. N.; McGovern, S. L.; Witherbee, B. J.; Kasten, T. P.; Kurumbail, R.; Stallings, W. C.; Connolly, D. T.; Shoichet, B. K.

Molecular Docking and High-Throughput Screening for Novel Inhibitors of Protein Tyrosine Phosphatase-1B. *J. Med. Chem.* **2002**, *45*, 2213-2221.

[37] Powers, R. A.; Morandi, F.; Shoichet, B. K. Structure-Based Discovery of a Novel, Noncovalent Inhibitor of AmpC Beta-Lactamase. *Structure* **2002**, *10*, 1013-1023.

[38] Tondi, D.; Morandi, F.; Bonnet, R.; Costi, M. P.; Shoichet, B. K. Structure-Based Optimization of a Non-Beta-Lactam Lead Results in Inhibitors That Do Not Up-Regulate Beta-Lactamase Expression in Cell Culture. *J. Am. Chem. Soc.* **2005**, *127*, 4632-4639.

[39] Boehm, H. J.; Boehringer, M.; Bur, D.; Gmuender, H.; Huber, W.; Klaus, W.; Kostrewa, D.; Kuehne, H.; Luebbers, T.; Meunier-Keller, N.; Mueller, F. 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.* **2000**, *43*, 2664-2674.

[40] Irwin, J. J.; Shoichet, B. K. ZINC - A Free Database of Commercially Available Compounds for Virtual Screening. *J. Chem. Inf. Model.* **2005**, *45*, 177-182.

[41] Cozzini, P.; Kellogg, G. E.; Spyrakis, F.; Abraham, D. J.; Costantino, G.; Emerson, A.; Fanelli, F.; Gohlke, H.; Kuhn, L. A.; Morris, G. M.; Orozco, M.; Pertinhez, T. A.; Rizzi, M.; Sotriffer, C. A. Target Flexibility: An Emerging Consideration in Drug Discovery and Design. *J. Med. Chem.* **2008**, *51*, 6237-6255.

[42] Walters, W. P.; Stahl, M. T.; Murcko, M. A. Virtual Screening - An Overview. *Drug. Discov. Today* **1998**, *3*, 160-178.

[43] Walters, W. P.; Namchuk, M. Designing Screens: How to Make Your Hits a Hit. *Nat. Rev. Drug Discov.* **2003**, *2*, 259-266.

[44] Hardy, L. W.; Malikayil, A. The Impact of Structure-Guided Drug Design on Clinical Agents. *Curr. Drug Discov.* **2003**, 15-20.

[45] Shoichet, B. K.; Kuntz, I. D. Protein Docking and Complementarity. *J. Mol. Biol.* **1991**, *221*, 327-346.

[46] Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated Docking Using a Lamarckian Genetic Algorithm and an Empirical Free Energy Function. *J. Comput. Chem.* **1998**, *19*, 1639-1662.

[47] Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. A Fast Flexible Docking Method Using an Incremental Construction Algorithm. *J. Mol. Biol.* **1996**, *261*, 470-489.

[48] Claussen, H.; Buning, C.; Rarey, M.; Lengauer, T. FlexE: Efficient Molecular Docking Considering Protein Structure Variations. *J. Mol. Biol.* ***2001***, *308*, 377-395.

[49] Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and Validation of a Genetic Algorithm for Flexible Docking. *J. Mol. Biol.* **1997**, *267*, 727–748.

[50] Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy. *J. Med. Chem.* **2004**, *47*, 1739-1749.

[51] Kellogg, G. E.; Abraham, D. J. Hydrophobicity: Is LogP(o/w) More Than the Sum of Its Parts? *Eur. J. Med. Chem.* **2000**, *35*, 651-661.

[52] Fornabaio, M.; Spyrakis, F.; Mozzarelli, A.; Cozzini, P.; Abraham, D. J.; Kellogg, G. E. Simple, Intuitive Calculations of Free Energy of Binding For Protein-Ligand Complexes. 3. The Free Energy Contribution of Structural Water Molecules in HIV-1 Protease Complexes. *J. Med. Chem.* **2004**, *47*, 4507-4516.

[53] Kellogg, G. E.; Fornabaio, M.; Spyrakis, F.; Lodola, A.; Cozzini, P.; Mozzarelli, A.; Abraham, D. J. Getting It Right: Modeling of pH, Solvent and "Nearly" Everything Else in Virtual Screening of Biological Targets. *J. Mol. Graph. Model.* **2004**, *22*, 479-486.

[54] Schapira, M.; Raaka, B. M.; Das, S.; Fan, L.; Totrov, M.; Zhou, Z.; Wilson, S. R.; Abagyan, R.; Samuels, H. H. Discovery of Diverse Thyroid Hormone Receptor Antagonists by High-Throughput Docking. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 7354-7359.

[55] Huang, N.; Nagarsekar, A.; Xia, G.; Hayashi, J.; MacKerell, A. D Jr. Identification of Non-Phosphate-Containing Small Molecular Weight Inhibitors of the Tyrosine Kinase p56 Lck SH2 Domain *Via* in Silico Screening Against the pY + 3 Binding Site. *J. Med. Chem.* **2004**, *47*, 3502-3511.

[56] Cavasotto, C. N.; Ortiz, M. A.; Abagyan, R. A.; Piedrafita, F. J. In Silico Identification of Novel EGFR Inhibitors with Antiproliferative Activity Against Cancer Cells. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1969-1974.

[57] Wang, J. L.; Liu, D.; Zhang, Z. J.; Shan, S.; Han, X.; Srinivasula, S. M.; Croce, C. M.; Alnemri, E. S.; Huang, Z. Structure-Based Discovery of an Organic Compound that Binds Bcl-2 Protein and Induces Apoptosis of Tumor Cells. *Proc. Natl. Acad. Sci USA* **2000**, *97*, 7124-7129.

[58] Cozza, G.; Bonvini, P.; Zorzi, E.; Poletto, G.; Pagano, M. A.; Sarno, S.; Donella-Deana, A.; Zagotto, G.; Rosolen, A.; Pinna, L. A.; Meggio, F.; Moro, S. Identification of Ellagic Acid as Potent Inhibitor of Protein Kinase CK2: A Successful Example of a Virtual Screening Application. *J. Med. Chem.* **2006**, *49*, 2363-2366.

[59] Tintori, C.; Corradi, V.; Magnani, M.; Manetti, F.; Botta, M. Targets Looking for Drugs: A Multistep Computational Protocol for the Development of Structure-Based Pharmacophores and Their Applications for Hit Discovery. *J. Chem. Inf. Model.* **2008**, *48*, 2166-2179.

[60] Gohlke, H.; Klebe G. Approaches to the Description and Prediction of the Binding Affinity of Small-Molecule Ligands to Macromolecular Receptors. *Angew. Chem. Int. Ed.* **2002**, *41*, 2644-2676.

[61] Wolber, G.; Seidel, T.; Bendix, F.; Langer, T. Molecule-Pharmacophore Superpositioning and Pattern Matching in Computational Drug Design. *Drug Discov. Today* **2008**, *13*, 23-29.

[62] Langer, T.; Wolber, G. Pharmacophore Definition and 3D Searches. *Drug Discov. Today Technol.* **2004**, *1*, 203-207.

[63] Goodford, P. J. A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules. *J. Med. Chem.* **1985**, *28*, 849-857.

[64] Verdonk, M. L.; Cole, J. C.; Taylor, R. SuperStar: A Knowledge-Based Approach for Identifying Interaction Sites in Proteins. *J. Mol. Biol.* **1999**, *289*, 1093-1108.

[65] Gohlke, H.; Hendlich, M.; Klebe, G. Predicting Binding Modes, Binding Affinities and 'Hot Spots' for Protein-Ligand Complexes Using a Knowledge-Based Scoring Function. *Perspect. Drug Discov. Des.* **2000**, 20, 115-144.

[66] Wolber, G.; Langer, T. LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters. *J. Chem. Inf. Model.* **2005**, *45*, 160-169.

[67] Chen, J.; Lai, L. Pocket v.2: Further Developments on Receptor-Based Pharmacophore Modeling. *J. Chem. Inf. Model.* **2006**, *46*, 2684-2691.

[68] Ortuso, F.; Langer, T.; Alcaro, S. GBPM: GRID-Based Pharmacophore Model: Concept and Application Studies to Protein-Protein Recognition. *Bioinformatics* **2006**, *22*, 1449-1455.

[69] Böhm, H. J. LUDI: Rule-Based Automatic Design of New Substituents for Enzyme Inhibitor Leads. *J. Comput. Aided Mol. Des.* **1992**, *6*, 593-606.

[70] Eksterowicz, J. E.; Evensen, E.; Lemmen, C.; Brady, G. P.; Lanctot, J.K.; Bradley, E. K.; Saiah, E.; Robinson, L. A.; Grootenhuis, P. D.; Blaney, J. M. Coupling Structure-Based Design with Combinatorial Chemistry: Application of Active Site Derived Pharmacophores with Informative Library Design. *J. Mol. Graph. Modell.* **2002**, *20*, 469-477.

[71] Salum, L. B.; Polikarpov, I.; Andricopulo, A. D. Structure-Based Approach for the Study of Estrogen Receptor Binding Affinity and Subtype Selectivity. *J. Chem. Inf. Model.* **2008**, *48*, 2243-2253.

[72] Schindler, T.; Bornmann, W.; Pellicena, P. W.; Miller, W. T.; Clarkson, B.; Kuriyan, J. Structural Mechanism for STI-571 Inhibition of Abelson Tyrosine Kinase. *Science* **2000**, *289*, 1938-1942.

[73] Zimmermann, J.; Buchdunger, E.; Mett, H.; Meyer, T.; Lydon, N. B. Potent and Selective Inhibitors of the Abl-Kinase: Phenylamino-Pyrimidine (PAP) Derivatives. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 187-192.

[74] Hurst, T. Flexible 3D Searching - The Directed Tweak Technique. *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 190-196.

[75] Kurogi, Y.; Güner, O. F. Pharmacophore Modeling and Three-Dimensional Database Searching for Drug Design Using Catalyst. *Curr. Med. Chem.* **2001**, *8*, 1035-1055.

[76] Hindle, S. A.; Rarey, M.; Buning, C.; Lengaue, T. Flexible Docking Under Pharmacophore Type Constraints. *J. Comput. Aided Mol. Des*. **2002**, *16*, 129-149.

[77] Varady, J.; Wu, X.; Fang, X.; Min, J.; Hu, Z.; Levant, B.; Wang, S. Molecular Modeling of the Three-Dimensional Structure of Dopamine 3 (D3) Subtype Receptor: Discovery of Novel and

Potent D3 Ligands Through a Hybrid Pharmacophore- and Structure-Based Database Searching Approach. *J. Med. Chem*. **2003**, *46*, 4377-4392.

[78] Evers, A.; Klebe, G. Successful Virtual Screening for a Submicromolar Antagonist of the Neurokinin-1 Receptor Based on a Ligand-Supported Homology Model. *J. Med. Chem*. **2004**, *47*, 5381-5392.

[79] Kumar, A.; Chaturvedi, V.; Bhatnagar, S.; Sinha, S.; Siddiqi, M. I. Knowledge Based Identification of Potent Antitubercular Compounds Using Structure Based Virtual Screening and Structure Interaction Fingerprints. *J. Chem. Inf. Model*. **2009**, *49*, 35-42.

[80] Goetschi, E.; Angehrn, P.; Gmuender, H.; Hebeisen, P.; Link, H.; Masciadri, R.; Nielsen, J. Cyclothialidine and Its Congeners: A New Class of DNA Gyrase Inhibitors. *Pharmacol. Ther*. **1993**, *60*, 367-380.

[81] Andrade, C. H.; Salum, L. B.; Pasqualoto, K. F. M.; Ferreira, E. I.; Andricopulo, A. D. Three-Dimensional Quantitative Structure-Activity Relationships for a Large Series of Potent Antitubercular Agents. *Lett. Drug Des. Discov*. **2008**, *5*, 377-387.

[82] Steindl, T. M.; Schuster, D.; Laggner, C.; Langer, T. Parallel Screening: A Novel Concept in Pharmacophore Modeling and Virtual Screening. *J. Chem. Inf. Model*. **2006**, *46*, 2146-2157.

[83] Deng, J.; Sanchez, T.; Neamati, N.; Briggs, J. M. Dynamic Pharmacophore Model Optimization: Identification of Novel HIV-1 Integrase Inhibitors. *J. Med. Chem*. **2006**, *49*, 1684-1692.

[84] Alberts, I. L.; Todorov, N. P.; Dean, P. M. Receptor Flexibility in De Novo Ligand Design and Docking. *J. Med. Chem*. **2005**, *48*, 6585-6596.

[85] Barakat, K. H.; Torin Huzil, J.; Luchko, T.; Jordheim, L.; Dumontet, C.; Tuszynski, J. Characterization of an Inhibitory Dynamic Pharmacophore for the ERCC1-XPA Interaction Using a Combined Molecular Dynamics and Virtual Screening Approach. *J. Mol. Graph. Model*. **2009**. doi:10.1016/j.jmgm.2009.04.009.

[86] Muegge, I. Synergies of Virtual Screening Approaches. *Mini Rev. Med. Chem*. **2008**, *8*, 927-933.

[87] Brenk, R.; Irwin, J. J.; Shoichet, B. K. Here Be Dragons: Docking and Screening in an Uncharted Region of Chemical Space. *J. Biomol. Screen*. **2005**, *10*, 667-674.

[88] Andricopulo, A. D.; Montanari, C. A. Structure-Activity Relationships for the Design of Small-Molecule Inhibitors. *Mini Rev. Med. Chem*. **2005**, *5*, 585-593.

[89] Ji, H.; Li, H.; Martásek, P.; Roman, L. J.; Poulos, T. L.; Silverman, R. B. Discovery of Highly Potent and Selective Inhibitors of Neuronal Nitric Oxide Synthase by Fragment Hopping. *J. Med. Chem*. **2009**, *52*, 779-797.

[90] Cole, D. C.; Asselin, M.; Brennan, A.; Czerwinski, R.; Ellingboe, J. W.; Fitz, L.; Greco, R.; Huang, X.; Joseph-McCarthy, D.; Kelly, M. F.; Kirisits, M.; Lee, J.; Li, Y.; Morgan, P.; Stock, J. R.; Tsao, D. H.; Wissner, A.; Yang, X.; Chaudhary, D. Identification, Characterization and Initial Hit-to-Lead Optimization of a Series of 4-Arylamino-3-Pyridinecarbonitrile as Protein Kinase C Theta (PKCtheta) Inhibitors. *J. Med. Chem*. **2008**, *51*, 5958-5963.

[91] Wyatt, P. G.; Woodhead, A. J.; Berdini, V.; Boulstridge, J. A.; Carr, M. G.; Cross, D. M.; Davis, D. J.; Devine, L. A.; Early, T. R.; Feltell, R. E.; Lewis, E. J.; McMenamin, R. L.; Navarro, E. F.; O'Brien, M. A.; O'Reilly, M.; Reule, M.; Saxty, G.; Seavers, L. C.; Smith, D. M.; Squires, M. S.; Trewartha, G.; Walker, M. T.; Woolford, A. J. Identification of N-(4-piperidinyl)-4-(2,6-dichlorobenzoylamino)-1H-pyrazole-3-carboxamide (AT7519), a Novel Cyclin Dependent Kinase Inhibitor Using Fragment-Based X-Ray Crystallography and Structure Based Drug Design. *J. Med. Chem*. **2008**, *51*, 4986-4999.

[92] Lippa, B.; Pan, G.; Corbett, M.; Li, C.; Kauffman, G. S.; Pandit, J.; Robinson, S.; Wei, L.; Kozina, E.; Marr, E. S.; Borzillo, G.; Knauth, E.; Barbacci-Tobin, E. G.; Vincent, P.; Troutman, M.; Baker, D.; Rajamohan, F.; Kakar, S.; Clark, T.; Morris, J. Synthesis and Structure Based Optimization of Novel Akt Inhibitors. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 3359-3563.

[93] Morandi, S.; Morandi, F.; Caselli, E.; Shoichet, B. K.; Prati, F. Structure-Based Optimization of Cephalothin-Analogue Boronic Acids as Beta-Lactamase Inhibitors. *Bioorg. Med. Chem*. **2008**, *16*, 1195-1205.

[94] Walker, D. P.; Bi, F. C.; Kalgutkar, A. S.; Bauman, J. N.; Zhao, S. X.; Soglia, J. R.; Aspnes, G. E.; Kung, D. W.; Klug-McLeod, J.; Zawistoski, M. P.; McGlynn, M. A.; Oliver, R.; Dunn, M.; Li, J. C.; Richter, D. T;, Cooper, B. A., Kath, J. C.; Hulford, C. A.; Autry, C. L.; Luzzio, M. J.; Ung, E. J.; Roberts, W. G.; Bonnette, P. C.; Buckbinder, L.; Mistry, A.; Griffor, M. C.; Han, S.; Guzman-Perez, A. Trifluoromethylpyrimidine-Based Inhibitors of Proline-Rich Tyrosine Kinase 2 (PYK2): Structure-Activity Relationships and Strategies for the Elimination of Reactive Metabolite Formation. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 6071-6077.

[95] Sweeney, Z. K.; Harris, S. F.; Arora, N.; Javanbakht, H.; Li, Y.; Fretland, J.; Davidson, J. P.; Billedeau, J. R.; Gleason, S. K.; Hirschfeld, D.; Kennedy-Smith, J. J.; Mirzadegan, T.; Roetz, R.; Smith, M.; Sperry, S.; Suh, J. M.; Wu, J.; Tsing, S.; Villaseñor, A. G.; Paul, A.; Su, G.; Heilek, G.; Hang, J. Q.; Zhou, A. S.; Jernelius, J. A.; Zhang, F. J.; Klumpp, K. Design of Annulated Pyrazoles as Inhibitors of HIV-1 Reverse Transcriptase. *J. Med. Chem*. **2008**, *51*, 7449-7458

[96] Lucas, S.; Heim, R.; Negri, M.; Antes, I.; Ries, C.; Schewe, K. E.; Bisi, A.; Gobbi, S.; Hartmann, R. W. Novel Aldosterone Synthase Inhibitors With Extended Carbocyclic Skeleton by a Combined Ligand-Based and Structure-Based Drug Design Approach. *J. Med. Chem*. **2008**, *51*, 6138-6149.

[97] Young, W. B.; Kolesnikov, A.; Rai, R.; Sprengeler, P. A.; Leahy, E. M.; Shrader, W. D.; Sangalang, J.; Burgess-Henry, J.; Spencer, J.; Elrod, K.; Cregar, L. Optimization of a Screening Lead for Factor VIIa/TF. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 2253-2256.

[98] Liao, J. Molecular Recognition of Protein Kinase Binding Pockets for Design of Potent and Selective Kinase Inhibitors. *J. Med. Chem*. **2007**, *50*, 409–424.

[99] Cramer, R. D.; Patterson, D.; Bunce, J. Effect of Shape on Binding of Steroids to Carrier Proteins. *J. Am. Chem. Soc*. **1998**, *110*, 5959-5967.

[100] Klebe, G.; Abraham, U. On the Prediction of Binding Properties of Drug Molecules by Comparative Molecular Field Analysis. *J. Med. Chem*. **1993**, *36*, 70-80.

[101] Hansch, C.; Kurup, A.; Garg, R.; Gao, H. Chem-Bioinformatics and QSAR: A Review of QSAR Lacking Positive Hydrophobic Terms. *Chem. Rev*. **2001**, *101*, 619-672.

[102] Winkler, D. A. The Role of Quantitative Structure-Activity Relationships (QSAR) in Biomolecular Discovery. *Brief Bioinform*. **2002**, *3*, 73-86.

[103] Gedeck, P.; Lewis, R. A. Exploiting QSAR Models in Lead Optimization. *Curr. Opin. Drug Discov. Dev*. **2008**, *11*, 569-575.

[104] Klebe, G.; Abraham, U.; Mietzner, T. Molecular Similarity Indices in a Comparative Analysis (CoMSIA) of Drug Molecules to Correlate and Predict their Biological Activity. *J. Med. Chem*. **1994**, *37*, 4130-4146.

[105] Cramer, R. D. Topomer CoMFA: A Design Methodology for Rapid Lead Optimization. *J. Med. Chem*. **2003**, *46*, 374-388.

[106] Salum, L. B.; Polikarpov, I.; Andricopulo, A. D. Structural and Chemical Basis for Enhanced Affinity and Potency for a Series of Estrogen Receptor Ligands: 2D and 3D QSAR Studies. *J. Mol. Graph. Model*. **2007**, *25*, 434-442.

[107] Guido, R. V. C.; Oliva, G.; Montanari, C. A.; Andricopulo, A. D. Structural Basis for Selective Inhibition of Trypanosomatid Glyceraldehydes-3-Phosphate Dehydrogenase: Molecular Docking and 3D QSAR Studies. *J. Chem. Inf. Model*. **2008**, *48*, 918-929.

[108] Castilho, M. S.; Postigo, M. P.; de Paula, C. B.; Montanari, C. A.; Oliva, G.; Andricopulo, A. D. Two- and Three-Dimensional Quantitative Structure-Activity Relationships for a Series of Purine Nucleoside Phosphorylase Inhibitors. *Bioorg. Med. Chem*. **2006**, *14*, 516-527.

[109] Honorio, K. M.; Garratt, R. C.; Polikarpov, I.; Andricopulo, A. D. 3D QSAR Comparative Molecular Field Analysis on Nonsteroidal Farnesoid X Receptor Activators. *J. Mol. Graph. Model*. **2007**, *25*, 921-927.

[110] Salum, L. B.; Dias, L. C.; Andricopulo, A. D. Fragment-Based QSAR and Molecular Modeling Studies on a Series of Discodermolide Analogs as Microtubule-Stabilizing Anticancer Agents. *QSAR Comb. Sci*. **2009**, *28*, 325-337.

[111] Muegge, I.; Podolgar, B. L. 3D-Quantitative Structure Activity Relationships of Biphenyl Carboxylic Acid MMP-3 Inhibitors: Exploring Automated Docking as Alignment Methodol. *QSAR Comb. Sci*. **2001**, *20*, 215-222.

[112] Trossini, G. H.; Guido, R. V. C.; Oliva, G.; Ferreira, E. I.; Andricopulo, A. D. Quantitative Structure-Activity Relationships for a Series of Inhibitors of Cruzain from Trypanosoma cruzi: Molecular Modeling, CoMFA and CoMSIA Studies. *J. Mol. Graph. Modell*. **2009**. doi:10.1016/j.jmgm.2009.03.001.

[113] Sutherland, J. J.; O'Brien, L. A.; Weaver, D. F. A Comparison of Methods for Modeling Quantitative Structure-Activity Relationships. *J. Med. Chem*. **2004**, *47*, 5541-5554.

[114] Baroni, M.; Costantino, G.; Cruciani, G.; Riganelli, D.; Valigi, R.; Clementi, S. Generating Optimal Linear PLS Estimations (GOLPE): An Advanced Chemometric Tool for Handling 3D-QSAR Problems. *QSAR Comb. Sci*. **1993**, *12*, 9-20.

[115] Ferguson, A. M.; Heritage, T.; Jonathon, P.; Pack, S. E.; Phillips, L.; Rogan, J.; Snaith, P. J. EVA: A New Theoretically Based Molecular Descriptor for Use in QSAR/QSPR Analysis. *J. Comput.-Aided Mol. Des*. **1997**, *11*, 143-152.

[116] Silverman, B. D.; Platt, D. E. Comparative Molecular Moment Analysis (CoMMA): 3D-QSAR Without Molecular Superposition. *J. Med. Chem*. **1996**, 39, 2129-2140.

[117] Gohlke, H.; Klebe, G. DrugScore Meets CoMFA: Adaptation of Fields for Molecular Comparison (AFMoC) or How to Tailor Knowledge-Based Pair-Potentials to a Particular Protein. *J. Med. Chem*. **2002**, *45*, 4153-4170.

[118] Doweyko, A. M. The Hypothetical Active Site Lattice. An Approach to Modelling Active Sites from Data on Inhibitor Molecules. *J. Med. Chem*. **1988**, *31*, 1396-1406.

[119] Harada, T.; Nakagawa, Y.; Wadkins, R. M.; Potter, P. M.; Wheelock, C. E. Comparison of Benzil and Trifluoromethyl Ketone (TFK)-Mediated Carboxylesterase Inhibition Using Classical and 3D-Quantitative Structure-Activity Relationship Analysis. *Bioorg. Med. Chem*. **2009**, *17*, 149-164.

[120] Ye, M.; Dawson, M. I. 3D-QSAR and QSSR Studies of 3,8-Diazabicyclo[4.2.0]Octane Derivatives as Neuronal Nicotinic Acetylcholine Receptors by Comparative Molecular Field Analysis (CoMFA). *Bioorg. Med. Chem. Lett*. **2009**, *19*, 127-131.

[121] Kang, N. S.; Ahn, J. H.; Kim, S. S.; Chae, C. H.; Yoo, S. E. Docking-Based 3D-QSAR Study for Selectivity of DPP4, DPP8, and DPP9 Inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3716-3721.

[122] Vrtacnik, M.; Voda, K. HQSAR and CoMFA Approaches in Predicting Reactivity of Halogenated Compounds with Hydroxyl Radicals. *Chemosphere* **2003**, *52*, 1689-1699.

[123] Fratev, F.; Benfenati, E. A Combination of 3D-QSAR, Docking, Local-Binding Energy (LBE) and GRID Study of the Species Differences in the Carcinogenicity of Benzene Derivatives Chemicals. *J. Mol. Graph. Model*. **2008**, *27*, 147-160.

[124] Peng, C. C.; Rushmore, T.; Crouch, G. J.; Jones, J. P. Modeling and Synthesis of Novel Tight-Binding Inhibitors of Cytochrome P450 2C9. *Bioorg. Med. Chem*. **2008**, *16*, 4064-4074.

[125] Korhonen, L. E.; Turpeinen, M.; Rahnasto, M.; Wittekindt, C.; Poso, A.; Pelkonen, O.; Raunio, H.; Juvonen, R. O. New Potent and Selective Cytochrome P450 2B6 (CYP2B6) Inhibitors Based on Three-Dimensional Quantitative Structure-Activity Relationship (3D-QSAR) Analysis. *Br. J. Pharmacol*. **2007**, *150*, 932-942.

[126] Arimoto, R. Computational Models for Predicting Interactions with Cytochrome P450 Enzyme. *Curr. Top. Med. Chem*. **2006**, *6*, 1609-1618.

[127] Chohan, K. K.; Paine, S. W.; Waters, N. J. Quantitative Structure Activity Relationships in Drug Metabolism. *Curr. Top. Med. Chem*. **2006**, *6*, 1569-1578.

[128] Hillebrecht, A.; Klebe, G. Use of 3D QSAR Models for Database Screening: a Feasibility Study. *J. Chem. Inf. Model*. **2008**, *48*, 384-396.

[129] Tropsha, A.; Golbraikh, A. Predictive QSAR Modeling Workflow, Model Applicability Domains, and Virtual Screening. *Curr. Pharm. Des.* **2007**, *13*, 3494-3504.

[130] Shen, M.; Beguin, C.; Golbraikh, A.; Stables, J. P.; Kohn, H.; Tropsha, A. Application of Predictive QSAR Models to Database Mining: Identification and Experimental Validation of Novel Anticonvulsant Compounds. *J. Med. Chem.* **2004**, *47*, 2356-2364.

[131] Oloff, S.; Mailman, R. B.; Tropsha, A. Application of Validated QSAR models of D1 Dopaminergic Antagonists for Database Mining. *J. Med. Chem.* **2005**, *48*, 7322-7332.

[132] Moro, S.; Bacilieri, M.; Cacciari, B.; Bolcato, C.; Cusan, C.; Pastorin, G.; Klotz, K. N.; Spalluto, G. The Application of a 3D-QSAR (autoMEP/PLS) Approach as an Efficient Pharmaco-dynamic-Driven Filtering Method for Small-Sized Virtual Library: Application to a Lead Optimization of a Human A3 Adenosine Receptor Antagonist. *Bioorg. Med. Chem.* **2006**, *14*, 4923-4932.

[133] Murcia, M.; Ortiz, A. R. Virtual Screening with Flexible Docking and COMBINE-Based Models. Application to a Series of Factor Xa Inhibitors. *J. Med. Chem.* **2004**, *47*, 805-820.

[134] Setny, P.; Trylska, J. Search for Novel Aminoglycosides by Combining Fragment-Based Virtual Screening and 3D-QSAR Scoring. *J. Chem. Inf. Model*. **2009**, *49*, 390-400.

[135] Hermann, T. Drugs targeting the ribosome. *Curr. Opin. Struct. Biol*. **2005**, *15*, 355-366.

Received March 19, 2009 Accepted June 29, 2009