Cheminformatics in Anti-Infective Agents Discovery

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Abstract: The existing chemical data such as those created by high throughput screening (HTS), structure-activity relationship (SAR) studies are converted into information as a result of storage and registration. Accessibility, manipulation, and data mining of such information make up the knowledge for drug development. Cheminformatics, exploiting the combination of chemical structural knowledge, biological screening, and data mining approaches is used to guide drug discovery and development and would assist by integrating complex series of rational selection of designed compounds with drug-like properties, building smarter focused libraries. This paper presents cheminformatics approaches and tools for designing and data mining of chemical databases and information. Many examples of success in lead identification and optimization in the area of anti-infective therapy have been discussed.

Key Words: Cheminformatics, database, descriptor modeling, anti-infective agent discovery.

1. INTRODUCTION

Drug research is a unique multi-disciplinary process heading towards the development of novel therapeutic agents in areas of medical need. The drug research can be divided functionally into two stages: discovery/design and development. Drug discovery/design consists of identification and characterization of new targets (enzymes or receptors), synthesis of new lead molecules, screening of new lead molecules for *in vitro* and/or *in vivo* biological activities, and physicochemical characterization of leads. Drug development focuses on evaluation of safety, toxicity and efficacy of new drug molecules and formulation combinations [1].

The random screening methods of pharmaceutical components have not been very successful in identifying antiparasitic compounds. One of the different approaches to search for novel pharmacological agents emerged from the field of combinatorial chemistry [2]. This technology includes a variety of techniques by which very large numbers of structurally distinct molecules are synthesized in a time and resource-effective way. The resulting pool of molecules reflects a library of diverse three-dimensional structures that is subsequently scrutinized for its pharmacological or diagnostic potential [3].

Cheminformatics is seen as an extension of chemical information, which is a well established concept covering many areas that employ chemical structures, data storage and computational methods, such as compound registration databases, on-line chemical literature, SAR analysis and molecule-property calculation [4]. This concept should not be mistaken with chemometrics which is the application of statistics to the analysis of chemical data from organic, analytical or medicinal chemistry and design of chemical experiments and simulations.

In addition to combinatorial chemistry, powerful computational methodologies for drug design and drug database screening and selection are now available. Equation systems linking quantitative structure-activity relationship, QSAR, studies are particularly relevant, and application of the mathematical models thereby obtained to large libraries of computer-generated compounds is known as virtual computational, or *in silico* screening. An important feature in these methods is the use of good structural descriptors that are representative of the molecular features responsible for the relevant biological activity; a very useful technique for describing molecular structure is molecular topology, a two-dimensional QSAR method which takes into account the internal atomic arrangement of compounds. The structure of each molecule can be represented by specific subsets of topological indices (TIs) [5]. Correlating all the advances in cheminformatics is better understood if it is linked to the progress in molecular biology.

The increasing availability of the entire genetic code of microorganisms forms a considerable potential to the drug discovery process. Most drugs now arise through discovery programs that begin with identification of a biomolecular target of potential therapeutic value through biological studies including, for example, analysis of mice with gene knockouts. Functional genomics, proteomics and computational biology offer promises for the future to prioritize genes that should be the focus of more intensive studies. In addition, they bring new directions that will be both distinct and complementary to traditional approaches. Functional genomics is devoted to the development and application of methods which allow investigators to efficiently study many genes simultaneously. For example, changes in gene expression can be monitored through the application of DNA microarray technology across thousands of genes [6]. Similarly, proteomic methods that allow investigators to view microbial protein expression are now beginning to be employed. Lastly, as more genes are characterized functionally in microorganisms, investigators are increasingly able to bear powerful computational approaches to identify candidate genes for more intensive analysis.

Applications of the above-mentioned methods should lead to the validation of candidate virulence genes. Once a

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candidate virulence gene has been identified, and perhaps its expression is modified, tests to confirm its role in virulence are necessary. First, the candidate gene must be reasonably involved in processes thought to be essential to virulence; second, inactivation of the gene should lead to a loss of virulence, and third, restoration of gene function should lead to restoration of virulence.

The use of computers and computational methods influences all aspects of drug discovery today. Computing tools provide the advantage of delivering new drug candidates faster and cheaper. Computer modeling algorithms and molecular simulations are used to predict QSAR and these data are used to optimize the design of new drugs by making a corelational model. Generally, successful modeling is rooted in the ability to identify and thereafter incorporate or predict the role of specific functional groups in the formation of the complex between drug and target. However, molecular modeling involving the drug interactions is limited by a lack of knowledge about the target structure, convenient 3D structure and available experimental data to verify these models.

One of the well-known processes using computational information techniques is structure-based drug design (SBDD). In SBDD, full structural knowledge of the protein target molecule provides information on how a potential drug interacts with the target. The success of SBDD, which has contributed to the introduction of 50 compounds into clinical trials and to numerous drug approvals, is well documented [7]. In SBDD, the role of computation consists of structure refinement using simulated annealing, development of the underlying molecular mechanics (MM), structure display, and building and MM evaluation of analogs.

The lack of development of new antimicrobial drugs together with increased resistance among the pathogenic and opportunistic microorganisms has been recognized as a potential threat to the public health [8-12]. In the area of anti-infective therapy, microorganism selection criteria include organism of sufficient prevalence in population with disease under study, organisms causes serious and severe disease, drug to which organism is resistant is commonly used in disease under study, limited available therapies as a result of multidrug-resistance, drug used to control spread of disease in population, and clinical correlation of *in vitro* resistance with poor clinical outcome [13]. In this mini-review, our focus would be the application of cheminformatics as has been largely influenced by computational techniques in the active research of anti-infective agents discovery, in which molecular libraries are screened, and the resulting leads are optimized in a cycle that features design, synthesis and assaying of numerous analogs.

2. TARGET SELECTION

The topic of target and target selection is one of the oldest subjects in drug discovery and normally design of a chemical scaffold would follow to produce compounds for screening. These processes originated from the knowledge gained through perceptive of some of the biological pathways and the screening that was done for an effect in a cell or even cells. Recently, many complete microbial genome sequences have been published and analyzed; *Saccharomyces jannaschii*, *Escherichia coli*, *Haemophilus influenzae*, *Mycoplasma genitalium*, *Methanococcus janaschii* and *Mycoplasma pneumoniae* are some of those organisms [14-18]. Data generated using genome projects are almost freely available. This information and the use of genomics and bioinformatics have had a great impact on drug discovery especially in the area of target selection. A number of conventional methods which can be used in target selection and target validation are shown in Table **1** with the emphasis on computer based target selection methods.

In the modern world of drug discovery, genetic information is now making the way for identification of single molecular targets. These are gained through knowledge of the genes of specific cell phenotypes that encode proteins that might be concerned with the pathogenesis of a particular disease state. The acceleration that has been made in genome sequencing and the identification of expressed genes will lead to the recognition of thousands of new targets, many of which will be applicable to the onset and resolution of a disease. Diverse set of genomic approaches for target selection is available nowadays. Target selection if not the major challenge, is surely one of the most important challenges in drug discovery for the treatment of infectious diseases. This can be due to the identification of targets that are essential for the microbial survival, but which are absent, or significantly divergent, in their mammalian host. For viral diseases, the small genome and relatively few viral proteins make this process fairly straightforward. However, for bacterial and fungal pathogens, there is a much larger potential pool from which to select targets. Various large-scale mutagenesis approaches are available for identification of essential fungal and bacterial genes [39]. A good example of the use of microarray for target selection would be the work carried out by Wilson [29] in which changes in gene expression after isoniazid compound treatment in *Mycobacterium tuberculosis* were used as being indicative of the mechanism-of-action of the compound. By examining the gene expression induced after treatment with an antimicrobial compound with unidentified mechanism, it will be feasible to deduce its mechanism-of-action. The studied alteration made in gene expression induced in *Mycobacterium tuberculosis* or other organisms can be used as a measuring tool for development of possible drug candidates.

Genomics has also been used for the identification of protein targets for vaccine development. Potential virulence factors can be identified by comparative genomics or *in vivo* methods. In addition, several computer programs are now available to search for secreted or membrane proteins that could be putative antigens. This *in silico* approach has been applied to the selection of antigens for Group B *Neisseria meningitidis* [38, 40] vaccine development and *Chlamydia pneumoniae* vaccine development [41].

An earlier work showed promising results had been on a software called Computer Aided Target Selection (CATS). This software was used to study the *Saccharomyces cerevisiae* genome published in 1997 by Mewes [42]. The entire DNA sequence of the genome of *S. cerevisiae* was completed in 1996 and represents the first entirely decoded eukaryotic genome. The main human pathogenic fungi such as *Candida albicans* are closely related to *S. cerevisiae* on a molecular level, the sequence information can be used to

Table 1. Conventional Methods for the Identification of Targets in Drug Discovery

Technology	Application	Reference
Signature- tagged mutagenesis	Identification of genes required for pathogen survival in animal models	[19-23]
In vivo expression technology	Identification of pathogen genes induced *in vivo*	[24, 25]
Microarrays	- Understanding host response to pathogens - Correlating gene expression with pathogenicity - Identifying molecular targets of antimicrobial compounds - Inferring function of unknown genes	[26-32]
Comparative genomics	- Identification of pathogenicity related genes - Identification of antigens for vaccine development - Selecting targets conserved across multiple pathogens - Selecting targets with lowest homology to human proteins	[33-36]
Structural genomics	- Selecting targets conserved across multiple pathogens - Selecting targets with lowest homology to human proteins - Inferring function of unknown proteins	[37, 38]

identify and prioritize those genes that are most suitable as targets for antimycotic drug discovery. CATS allows an automated evaluation of all *S. cerevisiae* genes to be carried out with regard to their suitability as antifungal targets. The selected targets that are potent as antifungal targets are considered and a set of parameters generated and used in target selection. These parameters were named 'Quality', 'Occurrence', 'Specificity' and 'Assay development' then on the basis of mentioned parameters the CATS program calculates the total score. On the basis of these scores a number of 25 genes are selected, a few being previously described as good antifungal targets, the elongation factor 3 and the proton P-type ATPase, and the rest being novel targets like trehalose phosphate synthase, and SEC14 (essential protein used in production of secretary vesicles). To test the scoring, known antifungal targets are used and ranked using the above parameters, which showed promising results [34]. In this respect, it could be pointed out that CATS software can be used in order to reduce the targets, to be assessed manually to meaningful number and then choose the novel and most suitable target.

3. DATABASE

One could say the factor that revolutionized drug design was the emergence of databases. After the explosion in the amount of data that was generated through combinatorial chemistry (CC), a large number of compounds were made and the need arise to screen these molecules in a short time. The emerging challenges could slow down the process, for example, of chirality or scale of the synthetic compounds that were made or in terms of CC, a block of compounds that was not suitable as a drug nominee. The details for such databases can be found elsewhere [43] but the applications of such databases are shown wherever possible. A few publicly available databases are shown in Table **2**. The structures of the molecules found in these databases can be in 1D e.g., SMILES, 2D, or in some cases even in 3D format. Using data bases could lead to successful cases; for example the structure of a non-peptide inhibitor bound to HIV-1 protease was developed after the screening of the Cambridge Structural Database using the original compound, haloperidol, through a shape complementarily algorithm [44].

3.1. 2D Search in Databases

There are various classifications for molecular descriptors. One of the most commonly used is the identification of dimension used in the descriptor, such as 1D, 2D, and 3D. 1D algorithms are usually simpler and can be easily applied; however, the versatility of 1D descriptor usage is also limited since the specificity of chemical functions are not expressed. The 2D and 3D factors would contribute to major arrangements and spatial comparing factors. By defining a molecule as a graph it is possible to define a substructure as a sub-graph of such definition. An easy way to achieve a substructure study is to use a predefined record of fragments to see if each substructure is in a collection of molecules. 2D substructure database screening is comprised of the following steps: first, searching for a structure that grasp one or more fragment(s) in a virtual database, a procedure that would only takes few seconds in a database containing millions of molecules. After a query has finished, typically thousands of structure candidates that have been found are then reduced using another query by adding more substructures. In this way, the number of candidates will be further reduced. On the contrary of what is thought, 2D substructure search makes a powerful method for finding novel compounds which have no similarity in shape (mostly) with the original compounds [4, 51-52]. Forino designed, synthesized, and tested small-molecule inhibitors that were highly potent and selective against *Bacillus anthracis* lethal factor;

Table 2. The Publicly Available Compound Databases

Database	Compounds/Entry Number	Reference or Website
The Cambridge Structural Database	355,000 crystal structures of organic and metal-organic compounds	[45]
ChemDB	4.1 million	[46]
Medchem Database	55,000	[47]
ChemIDplus	370,000	[48]
NCI	400,000	[49]
ZINC	3.3 million	[50]

using this method, he identified 22 compounds (among ~ 680 analogues) applying a 2D substructure search [53].

3.2. 3D Searching in Databases

2D Substructure despite being powerful method has its limitations. Does a target recognize a substructure or an entire molecule? The three dimensional stereoelectronic features of the structure play a key role in the binding affinity of the molecule to its target. There are different types of 3D database searching: considering whether the structure of the target molecule is available or not, there are two kinds of approaches. In case that the target has a known structure obtained by NMR study, crystallography or comparative modeling [54], molecules fitting that structure or those of the known ligand could be searched. However, lack of availability of such data means we have to draw a pharmacophore which is an indicative of some of the key features of a set of active molecules [55-56].

3.2.1. Pharmacophores

IUPAC describes receptor mapping or better known as pharmacophore mapping, as a procedure that is used to express the geometric and/or electronic characteristics of a binding site when inadequate structural data of the binding site are present. Narrative of a pharmacophore claims a well-organized method for the study of the data available for the features that affect the binding of the molecule. The difficulties faced when trying to design a pharmacophore can be clustered into two categories. The first one is the evaluation of the ligands, superimposition, molecular likeness and conformational aspects. The second difficulty is the fact that a logical pharmacophore has to include some unique effects that take place in actual binding which generally are not understood by means of a straightforward comparative analysis of the ligands [57]. Recently, database-pharmacophore sieves have been launched that offer simple sensitive conventions to categorize impending drugs [58]. A simple scheme representing a pharmacophore can be categorized in a few steps. The first step is deriving a 3D pharmacophore using 3D structures of the ligands or the target (binding site) then to search a 3D database. If a compound is found, it can be optimized so that a new lead is found or a *de novo* design to build a molecule for the pharmacophore is applied. This part will be discussed more comprehensively in the *de novo* section. Conformational flexibility is one of the foremost difficulties in pharmacophore generation, because the molecular conformations under biological conditions are not known. Several softwares are used for building pharmacophore based on ligand conformations. Catalyst (Accelrys, Inc.) [59] is one of the most frequently used softwares; swift 3D database exploring algorithms along with flexibility throughout pharmacophore creation is the main reason for its numerous use. DiscoTech, and Gasp (Tripos, Inc.) are also pharmacophore generating softwares which have also shown winning tales [60-62]. What makes these softwares differ from each other is mostly the algorithm that lies behind the pharmacophore generation. When no information on the ligand is available using the structure based algorithm within softwares such as Cerius package (Accelrys, Inc.), one thing to try is building binding-site pharmacophore. First of all, using LUDI algorithm, an interaction site calculation is carried out, then the next step is to cluster the hydrogen bond donors (HBD), hydrogen bond acceptors (HBA) and hydrophobic regions and the last step is to convert the obtained clusters into a characteristic pharmacophore which will represents the HBA, HBD, and hydrophobic regions [63].

Zhang introduced a recent pharmacophore based virtual screening approach to identify SARS-coronavirus proteinase inhibitors. That was a victorious approach given that among the found by the screening are six compounds that already exhibited anti-SARS-CoV activity experimentally [64]. In another study, Dayam used beta-diketo acid pharmacophore hypothesis for the discovery of a novel class of HIV-1 integrase inhibitors (IN). They found that compounds containing both salicylic acid and a 2-thioxo-4-thiazolidinone (rhodanine) group showed significant inhibitory potency against integrase, while the presence of either salicylic acid or a rhodanine group alone did not present such activity [65]. Steindl [66] used neuraminidase inhibitors of influenza virus to deliver structure-based pharmacophore hypotheses using in virtual screening of chemical databases. The unique aspect of this work is the strategies they used to prevail over the limitations of Catalyst data format, since multiple interactions of one chemical function cannot be included at the same time. Brenk [67] developed a pharmacophore hypothesis for tRNA-guanine transglycosylase inhibitors for *Shigellae*, and several new inhibitors of micro molar binding affinity were discovered.

3.2.2. Docking

Molecular recognition is the fundamental basis for drug action. Emil Fischer, in 1894 [68], first proposed the 'lock-and-key' principle whereby steric and electronic complementarily between proteins and their ligands drive the complexation process. Molecular docking algorithms attempt to create and recognize the most harmonizing match between a ligand and its macromolecular target. There are three steps to the docking process: study of the target and the ligand, creation of recognized complexes (sampling), and calculation of the 'fitness' of the complex (scoring) [69]. Various programs are used in a docking algorithm such as DOCK, FLEXX, FRED, GLIDE, GOLD, SLIDE, SURFLEX, and QXP. The main features of such docking tools have been explained below and drawn separately in Table **3**. The usage of these docking softwares would be different and our survey shows that the percentage of applications in different aspects of drug discovery according to collective citation in Pubmed and Scirus is highest for DOCK, AUTODOCK, GOLD and FLEXX respectively. It is noteworthy to mention that the capability to precisely place a ligand in the binding site of the target under study is the key to a winning breakdown. In a recent study [70], Kellenberger performed a comparative study on eight docking softwares for their ability to fit the ligand to the target. The study showed that the best softwares for docking accurately based on their tendency to recover the X-ray pose of 100 small-molecular-weight ligands, and for their capacity to discriminate known inhibitors of an enzyme (thymidine kinase) from randomly chosen "drug-like" molecules were GLIDE, GOLD, and SURFLEX. A key factor that plays a major role in docking accuracy is the consideration of ligand and protein flexibility. In another study conducted by Erickson [71], the ligand flexibility using four docking algorithms, DOCK, FlexX, GOLD, and CDOCKER was applied. Each algorithm performed well with an accuracy of over 50%, but the finding showed that docking accuracy decreases significantly for ligands with eight or more rotatable bonds. Only CDOCKER algorithm was able to accurately dock most of those ligands with eight or more rotatable bonds to include an accuracy of 71% [71]. For consideration of receptor flexibility multiple protein structures (MPS) would be the best option [72]. These structures can come from NMR studies, multiple crystal structures, or mul-

Table 3. Comparison of some of the Docking Programs

Software	Docking Algorithm	Scoring Utility	Ligand Flexibility	Target Flexibility	Web Address	Representative Applications in Anti-Infective Drug Design
Dock	Incremental	Contact score, Force field interaction, Electrostatic energy score (DelPhi)	✓	×	http://www.cmpharm.ucsf.edu/kuntz/	AmpC β-Lactamase [77]; Thymidylate synthase [81]
FlexX	Incremental	Empiric score	✓	×	http://cartan.gmd.de/flexx/	Dihydrofolate reductase [82]; Deoxycytidine kinase [83]; Metallo-beta-lactamase [84]
SLIDE	Conformational groups	Empiric score	✓	✓	http://www.bch.msu.edu/labs/kuhn	Dihydrofolate reductase [85]
AutoDock	Monte Carlo simulated annealing; The Lamarckian Genetic Algorithm (LGA)	Force field interaction	✓	✓	http://www.scripps.edu/pub/olson-web/doc/autodock/	BMS-378806 [86]; Reverse transcriptase HIV-1 [80]
LigandFit	Monte Carlo	Empiric score	✓	×	http://www.accelrys.com/insight/affinity.html	CDC25 phosphatase inhibitory activity [87]; Oxazolidine-2-thiones [88]
Fred	Conformational groups	Gaussian score or/and Pragmatic score	×	×	http://www.eyesopen.com	No reported application
Gold	Genetic algorithm	Empiric score	✓	Partial	http://www.ccdc.cam.ac.uk/prods/gold/index.html	Tripeptidyl peptidase II [89]; Lactate dehydrogenase [90]
Glide	Exhaustive Monte Carlo	Empiric score	✓	✓	http://www.schrodinger.com/Products/glide.html	No reported application
ICM	Brownian, local minimization	Force field and Empiric score	✓	✓	http://abagyan.scripps.edu/	No reported application

tiple conformations generated by computational routines. In general, the techniques can be divided into methods that employ experimentally determined structures and those that use computer-generated conformations. The computational methods that have been used include molecular-dynamics routines, low-frequency normal modes, simulated annealing, and other techniques [73]. One of the first works on MPS was carried out by Kuntz using DOCK software [74]. In this respect, the other package is FlexE, which can take account of protein flexibility by means of MPS from crystallographic data [75].

Scoring Functions

A scoring function assists in calculating the exact fitness score, although the present algorithms might not allow a very close to exact level. Scoring functions are based on the hypothesis that free energy can be expressed as a sum of independent terms. From this point of view, all scoring functions experience a considerable size reliance of the score: the larger a molecule, the higher is the probability that it is scored favorably. Although this simple model of molecular identification has been extremely valuable, one should keep in mind that good complementarities between receptor and ligand is certainly a qualification of a binding and not an entirely realistic fact [76]. Shoichet [77] used a database of over 200,000 compounds for docking in the active site of AmpC β-lactamase to identify potential inhibitors. After testing 56 of the best scoring compounds, three had K_i values of 650 μM or better. James [78] developed a computational approach to screen a large chemical library for binding to a three-dimensional RNA structure of HIV-1. From the ranked list of compounds predicted to bind TAR, 43 were assayed for inhibition of the Tat-TAR interaction *via* electrophoretic mobility shift assays. Eleven compounds inhibited the Tat-TAR interaction with a value of between 0.1 and 1 μM, and some inhibited Tat transactivation in cells [78]. Davies [79] carried out a novel study on NAT (*N*-acetyltransferases) activity. This assay has been utilized to identify novel substrates for pure NAT from *Salmonella typhimurium* and *Mycobacterium smegmatis,* which show a relationship between the lipophilicity of the arylamine and its activity as a substrate, that lead to finding an endogenous role of NAT in the protection of bacteria from aromatic and lipophilic toxins. The results showed that NAT could be a potential drug target [79]. Another approach would be to evaluate a given inhibitor and study the coordinates of the molecule when binding to the target site. Potent non-nucleoside reverse transcriptase inhibitors (NNRTIs) of the pyridinone derivative type were docked by Castillo & his colleagues [80] into nine NNRTIs binding pockets of HIV-1 reverse transcriptase (RT) structures. The docking results indicated that pyridinone analogues adopt a butterfly conformation and share the same binding mode as the *inhibitors co-crystallized with reverse transcriptase* in the pocket geometries of nevirapine.

3.2.3. De Novo Drug Design

When searching a database, the bottleneck is that there is no access to novel virtual compounds but with *de novo* design it is possible to generate novel compounds based on 3D pharmacophore or 3D structure of receptor binding site. There are two basic approaches toward *de novo* design, the first one called inside out, in which the molecules are grown in the binding site and then scored using various energetic functions. The second approach is based on screening of the binding site to determine potential binding places such as HBD, HBA and hydrophobic regions. The next step would be to evaluate which binding groups would bind tightly and the last step is comparing the connection of these functional groups together.

In 1996 and 1997, the first studies based on *de novo* methods for generation of enzyme inhibitors were published. *De novo* methods have been used to modify and significantly improve the binding affinity of known inhibitors. A number of cases of successful *de novo* design of a protein ligand have already been disclosed [91]. These studies show that indeed this approach is feasible and can lead to useful new structures. In addition, methods are being developed for the automatic computer generation of virtual molecular libraries which can be searched to identify molecules to match a pharmacophore or fit into a binding site, which have been discussed in the pharmacophore section. One of the most widely used programs for *de novo* design is GRID. This software allows a gird to be implanted on the binding site. A probe is then placed on the vertices of the grid and the interaction energy of the probe is then calculated and using the interaction energies a novel structure is built [92] which then can be evaluated using docking algorithms. The final step is to make a molecule that actually exists and can be synthesized easily. Even peptides can be built using these *de novo* methods, but using the inside out scheme would be easier for these kinds of studies due to its simplicity when coming down to the synthesis of the compounds [93].

LeapFrog, a *de novo* drug design program was used to design novel, potent, and selective inhibitors of HIV-1 integrase [94]. The designed compounds were synthesized and tested for *in vitro* inhibition of HIV-1 integrase. Out of the 25 compounds that were designed and synthesized, four molecules showed moderate to low inhibition of HIV-1 integrase for 3'-processing and 3'-strand transfer activities. Nonetheless, these compounds possess structural features not seen in known HIV-1 integrase inhibitors and thus can serve as excellent leads for further optimization of anti-HIV-1 integrase activity [94].

De novo ligand design methods have been applied to the X-ray crystal structure of bacterial neuraminidase in the presence of some selected water molecules. The results showed that the complete removal of all bound water molecules can lead to difficulties in generating any potential ligands. Although with limitation, this example shows that, only in some cases, bound water molecules can be more accessible for hydrogen bonding to an incoming ligand than the actual protein binding sitepoints associated with them. From the point of view of *de novo* ligand design, water molecules can thus act as versatile amphiprotic hydrogen-bonding site points and reduce the conformational constraints of a particular binding site [95].

MCSS and GRID are two methods, based on significantly different algorithms, which are used for this purpose. A comparison of the two methods for the same functional groups has been reported [96]. Calculations were performed

for nonpolar and polar functional groups in the internal hydrophobic pocket of the poliovirus capsid protein, and on the binding surface of the src SH3 domain. The two approaches are shown to agree qualitatively; for example the global characteristics of the functional group maps generated by MCSS and GRID are similar. However, there are significant differences in the relative interaction energies of the two sets of minima, a consequence of the different functional form used to evaluate polar interactions (electrostatics and hydrogen bonding) in the two methods. The single sphere representation used by GRID affords only positional information, supplemented by the identification of hydrogen bonding interactions. By contrast, the multi-atom representation of most MCSS groups yields in both positional and orientational information. The two methods are most similar for small functional groups, while for larger functional groups MCSS yields results consistent with GRID but superior in detail. These results are in accordance with the somewhat different purposes for which the two methods were developed. GRID has been used mainly to introduce functionalities at specific positions in lead compounds, in which case the orientation is predetermined by the structure of the latter. The orientational information provided by MCSS is important for its use in the *de novo* design of large, multifunctional ligands, as well as for improving lead compounds [96].

4. ARTIFICIAL NEURAL NETWORKS (ANN)

Artificial neural network (ANN) methods are conventional methods for dimension reduction. As in Multi Dimensional Scaling (MDS), ANN is part of a non-linear mapping procedure to "rearrange" data objects in an efficient manner, and therefore to make a configuration that best approximates the experimental spaces. It moves objects around in the space defined by the specified number of possibilities or training sets, and then checks how well the distances between objects can be reproduced by the new configuration. In other words, ANN uses a minimization algorithm that evaluates different configurations with the goal of maximizing the goodness-of-fit, and to include this to the least set error as compared with the target value [4, 98].

QSAR techniques endeavor to find relationships among the properties of bioactive molecules and the biological response they elicit when applied to a biological systems. The primary hypothesis is that changes in molecular properties give rise to different biological responses. There is a growing interest in the application of neural networks (NNs) in QSAR modeling. The special interest in NNs arises from their ability to carry out nonlinear mapping of the physicochemical descriptors to the corresponding biological activity implicitly [97-98]. A topological method that makes it possible to predict the properties of molecules on the basis of their chemical structures was applied for quinolone anti-microbial agents [99]. In this method it was made possible to verify the minimal inhibitory concentration (MIC) of quinolones. Scrutiny on the results showed that the experimental and calculated values have a high correlation that makes it possible to gain a QSAR explanation of the information contained in the network after training has been carried out. A nonlinear quantitative structure-anti-HIV-1-activity relationship (QSAR) study was realized in a series of 1-[2-hydroxyethoxy-methyl]-6-(phenylthio) thymine] (HEPT) derivatives acting as non-nucleoside reverse transcriptase inhibitors (NNRTIs). The usefulness of the model and the nonlinearity of the relationship between molecular descriptors and anti-HIV-1 activity have been clearly demonstrated [100]. Anti-HIV-1 activities of 20 tetrapyrroles (hematoporphyrin derivatives, meso-tetraphenylporphyrins, a chlorine, and a phthalocyanine) were predicted based on their molecular structures using four nonlinear models with good predictive ability [101]. Since toxicity is a general phenomenon observed in many groups of antibiotic chemical classes and yet a typical drug discovery program should include a screening of toxicity, therefore, ANN has recently been applied in toxicity predictions, including several types of algorithms: back-propagation neural network, Bayesian-Regularized Neural Networks, and self-organization map (SOM) [102]. All these cases have proved ANN to be a powerful tool in modeling the nonlinear data to be used in quantitative or qualitative modeling of various bioactive molecular designs.

5. CONCLUSION

Synergy between theoretical and experimental data has proven to be a very powerful tool to elucidate and open new gates not only in chemistry related topics but also n drug discovery [103]. When the experimental results are in agreement with computational ones, it usually indicates a good consideration of influencing factors to result in a superior model to be used as a discovery tool. The *in silico* methodologies discussed in this paper can be used in simulating or predicting the aspect of interaction between a main cellular target and the drug, as well as in the kinetics and entrance pathway through membranes, which may play a significant role in the antibiotic action. An *in silico* model is to produce close to experimental values; therefore, the key aspects affecting such processes should be studied and fully elucidated for the infections, in particular those that are caused by intracellular agent that seem more problematic to larger number of people around the globe.

A drug discovery hypothesis enjoys favorable consideration if there are various means of testing it. Such approach can nicely satisfy the existing criteria predicting the drug target interactions and finally the biological activity. Computational studies by the help of quantum chemical methods have recently reached the levels of efficacy and accuracy that permit their application to the elucidation of active molecule mechanisms. While some parameters involved in the drug interactions and biological processes have been fairly well established by experimentation, such interactions may have not yet been probed by rigorous computational methods. The size of these molecules and the nature of the interactions involved would place the anticipated computational investigations near the limits of state-of-the-art quantum chemical methods. Nevertheless, it is now feasible to treat this problem by the application of high-level *ab initio* methods, which predict chemical properties. However, exact mechanistic solutions are impossible for all but the simplest physical systems, and practical calculations always demand some level of approximation. In the case of small molecules, these approximations can be sufficiently valid that experimental properties are reliably predicted by computations. In this

sense, the predictions for larger molecules have to wait for sufficient calculated data, as well as computing capability.

Structure-based virtual screening utilizing docking algorithms has become an essential tool in the drug discovery process, and significant progress has been made in successfully applying the technique to a wide range of receptor targets. *In silico* validation of virtual screening protocols before application to a receptor target using a corporate or commercially available compound collection is the key to establishing a successful process. The impact of ligand database preprocessing has yet to be examined in the context of virtual screening and prioritization of compounds for biological evaluation. In our paper, we provided an insight into the implications of cheminformatics and particular examples of anti-infective applications were mentioned.

The CPU consumption is well in relation to the docking protocol and finding the active molecules success rates in the screenings. Assessment of these parameters and the following enrichments are highly dependent on the initial cheminformation treatment used in database construction. The interplay of SMILES representations, stereochemical information, protonation state enumeration, and ligand conformation ensembles are critical in achieving optimum enrichment rates in such screening. Considering the lipophilic parameters, they are useful for QSAR studies and the experimental values would bring a large participation in obtaining the stable outcome, of such experimental values, those obtained from reverse phase HPLC studies that can produce the lipophilicity data and log P are the more reliable ones. The QSAR study of bioactive compounds with anti-infective property had indicated that although the electronic and the steric parameters can best describe the antibacterial activity, the topologic parameters are of the most important group. The lipophilic properties by themselves do not improve the correlation, when they are included in the regression and neural network analysis; while topological indices play a superior role in the antibacterial activity prediction [104]. Cheminformatics with the capabilities providing in the field of drug discovery make numerous possibilities for further research, including some which may have practical utility in relation to general practice of treating infectious diseases and providing more efficient drugs.

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