Chemoinformatics: Directions Toward Combating Neglected Diseases

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FOREWORD

Chemoinformatics: Directions Towards Combating Neglected Diseases is the fruit of an original project from a group of Brazilian young scientists very concerned on the threat represented by neglected diseases today. These illnesses are so called because they mostly affect poor people from the third world being usually forgotten by the big pharmaceutical companies, that seems more interested in P&D against more profitable diseases like obesity, Alzheimer disease, Parkinson illness or sexual dysfunctions, than in tuberculosis or protozoan caused diseases, such as malaria, leishmaniosis, toxoplamosis and Chagas disease. As a consequence basically only the governmental agencies invest today in P&D of chemotherapy against neglected diseases. Such a behavior from the pharmaceutical companies could soon prove to be a terrible mistake because, as an outcome of globalization, these diseases are more frequently knocking the doors of the first world nations. MDR tuberculosis, for instance is quickly becoming a worldwide public healthy emergency while malaria is back, thanks to the resistance developed by *Plasmodium falciparum* against the chemotherapy available at present. Also the facilities of traveling today besides the huge amount of tourists attracted by the exotic rain forests worldwide (the main sources of all kind of neglected disease) have contributed to the spreading out of neglected diseases into EUA and Europe. If the developed nations take to long to wake up to this problem their populations will soon become victims again of diseases they have long left behind.

This project brings light back to this issue showing to the scientific community worldwide how the chemoinformatic techniques could be successfully employed to the design of new and more promising chemotherapy against such illnesses. Techniques like QSAR, SAR, homology modeling, molecular dynamics and docking are essential tools in the modern medicinal chemistry and have proved to be very efficient in the drug design against neglected diseases. This e-book, besides making a revision of the main aspects of these diseases, also describes several examples available in literature of successful applications of these techniques on studies of molecular targets on the parasites responsible for causing neglected diseases. The editors and authors are mostly theoretical chemists and hope, to highlight this emerging problem also to show the power of chemoinformatic techniques as cheap and efficient tools to the drug design, motivating young scientists, like them, to face the challenge represented by the fight against these terrible illnesses affecting more than a third of the world's population today.

Tanos Celmar Costa França

CHAPTER 2

Quantitative Structure Activity Analysis of Leishmanicidal Compounds

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Abstract: Several techniques have been used to study the mechanisms by which *receptors* recognize ligands, one of them being quantitative structure activity relationship analysis of compounds. This method facilitates the description of molecular details involved in drug recognition by molecular *receptors*, as well as the molecular mechanism involved. This technique constitutes an essential tool to investigate chemical, electronic, and structural features affecting the leishmanicidal activity of compounds. However, few studies address this topic in *Leishmania*. Efforts should be made to stimulate research in this area and thus describe the characteristics of leishmanicidal drugs and their interaction with molecular *receptors*. The present chapter summarizes progress made recently in quantitative structure activity relationship studies of leishmanicidal compounds in experimental and *in vivo* scenarios. The review highlights possible critical spots in drug design and discusses the potential activity of compounds against different strains of the parasite as a way to optimize the treatment of leishmaniasis.

Keywords: QSAR, leishmaniasis, Lipinski's rule.

1. INTRODUCTION

Arsenites and antimonials were amongst the first synthetic drugs used against infectious diseases at the beginning of the twentieth century, and still organic derivatives of the same heavy metals remain as the drugs of choice for the treatment of diseases caused by Trypanosomatids, including *Leishmania*. Simon Croft [1] wrote this statement in 1999 and unfortunately it still reveals, at least partially, the *state of the art* for chemotherapy against leishmaniasis. Indeed, Glucantime® and Pentostan® remain as the drugs of choice to fight against leishmaniasis in most endemic countries.

These drugs have several limitations like the need of parenteral administration, variable efficacy and high price and toxicity. However, these compounds are essential for control and treatment of the disease since alternative prevention measures, such as pesticide-impregnated bed-nets, fail or prove impractical and no new and efficient drugs exist [2].

Additionally, therapeutic failure attributed to altered drug pharmacokinetics, re-infection, or immunologic compromise of the host is a problem frequently observed in endemic areas [3]. Development of drug resistance further complicates the panorama of the disease, and strong indicators suggest that this may play an important role in therapeutic failure [4]. For this reason, there is an urgent need for markers of chemo-resistance that are easy to monitor in the laboratory and helpful to predict therapeutic prognosis [5-7], and for compounds less prone to induce drug resistance. Of note, despite intensive attempts, there are no effective vaccines for the prevention of leishmaniasis and this will not improve in the near future [8].

For a few years now, identification of the genome sequences of trypanosomatid parasites is either completed or underway (www.genedb.org). Extensive work is being done to characterize the biological function of the encoded proteins and to evaluate their value as antiparasitic drug targets [4]. However, in spite of the efforts made, very few of the identified pharmacophores have successfully entered the clinical pipeline [2, 9]. This means that finding lead drug-like compounds interesting enough to warrant an analysis of their biological activity must be a primary goal to uncover an appropriate cure for leishmaniasis [2].

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The cost of experimentally testing a compound relative to scoring it computationally is very high, and the methods are slow and tedious. In fact, corporate-sized databases, often exceeding 1 million entries, cannot be totally screened at a reasonable cost even by current technologies [10]. For this reason, computer-based methods are useful to suggest which subsets of compounds are most likely to be active. The computational study starts with an interesting biologically active molecule, *e.g.* an already screened inhibitor compound, and a search for the structural requirements responsible for the potency of the compound in a database of chemical structures. The ultimate goal is to find an active molecule different enough from the starting compound(s) that could be considered as a new class of therapeutic agent [10].

2. HOW TO DEFINE STRUCTURE ACTIVITY RELATIONSHIP AND QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP

In the last few years, the landscape of drug discovery and development for new anti-parasitic drugs has improved thanks to the financial support from not-for-profit organizations and the involvement of public-private partnerships [11].

For example, catalyzed by the Special Programme for Research and Training in Tropical Diseases (TDR) and with the collaboration of pharmaceutical companies, the search for new leads and anti-parasitic drugs has gained thousands of molecules required for the development of new medicaments against neglected diseases including leishmaniasis [11].

Computational methods and tools have been fundamental and are essential to screen the chemical structure databases and to identify leads to work with. Especially two of them focus on modeling the biological *receptors* and their binding drugs. The main goal of these methods is to optimize drug binding and help in the design of more potent or precise drugs. These methods are 'virtual screening', which is a computational technique that analyzes *in silico* libraries of chemical structures and identifies those most likely to bind to a drug target, and computer-aided design of molecules based on desired properties [12, 13]. This latter method determines which compounds match a specified set of (target) properties; it has a large potential since itpermits designing all kinds of chemical, bio-chemical and material products [13].

For a computational method to be successful, information must be available on theactivity of the compound, and how such activity relates to the molecule's structure, the so called Structure Activity Relationship (SAR). This method is also called structure-property relationship and can be defined as the process by which the chemical structure of molecules is quantitatively correlated with biological activity or chemical reactivity [14]. Although it may happen that different molecules use dissimilar binding modes or trigger diverse mechanisms, the basic assumption for SAR is that analogous molecules may interact with the same *receptor* and might have similar activities. Thus, the SAR analysis is a fundamental requirement for the manufacture of a molecule with specific desired characteristics.

On the other hand, the term Quantitative Structure Activity Relationship (QSAR) (see Fig. **1**) refers to predictive models derived from the application of statistical tools. QSAR correlates biological activity (including desirable therapeutic effect and undesirable side effects) of chemicals, (drugs/toxicants/environmental pollutants) with descriptors representative of molecular structure and/or properties [14]. Success of any QSAR model depends on accuracy of the input data, selection of appropriate descriptors and statistical tools and most importantly, validation of the developed model.

Drugs or ligands vary from the simple to the complex, but the *receptors* they bind to are extremely complex. Predicting the properties of a molecule based on structure is a non-linear and non-intuitive process that requires complex molecular modeling. As a consequence increasingly complicated molecules are designed every day [15]. Drug development is further complicated by the fact that it must function in a bio-molecular system composed of the biological *receptor* and the ligand, and inside a complex organism.

As already mentioned, the central tenet of SAR is that compounds with similar structure act at the same site and with the same mechanism. Unfortunately, many pharmaceutical computer-aided molecular design

systems predict the properties of either the ligands that operate on the *receptor* or the *receptor* itself, but usually not both. To overcome this situation, various organizations and web sites foster the validation of anti-infective drugs (http://www.dndi.org/) and of cellular targets (http://www.TDRtargets.org/), especially on diseases that are not a priority for the pharmaceutical industry as is the case for leishmaniasis.

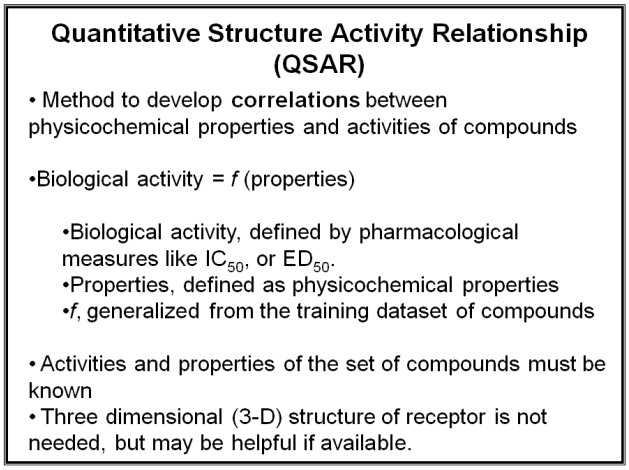

Figure 1: Definition of Quantitative Structure Activity Relationship. For description of the terms, see main text.

3. METHODS AND MODELS USED IN QSAR

The main goal of SAR and QSAR is to uncover correlations between physicochemical properties and molecular function of a group of compounds. To perform this task computational methods are needed and Molecular Descriptors (MD) should be described. According to the web page http://www.molecular-descriptors.eu, a MD is the result of a logic and mathematical procedure, which transforms chemical information encoded within the symbolic representation of a molecule, into a useful number or result of a standardized experiment. The characterization of MD constitutes a scientific field in it self; through it, scientists design strategies to define a feature. In fact, around 1600 MD have been listed and may be used to solve specific situations related to drug discovery and drug-*receptor* interactions [16]. This means that descriptor analysis is an extremely intricate procedure that adds complexity to the models that can be developed for SAR and QSAR.

Web pages (http://www.qsarworld.com/) have been designed to cover all the questions that may arise regarding the complexity of the methods and of the descriptors, and readers are invited to forward their search according to their individual interests. Here in we will remain as simple as possible and will only describe the minimal steps for SAR or QSAR analysis.

In the initial steps of QSAR (see Fig. **2**) a group of compounds, all of which interact in a similar way with the same site in a molecule (*receptor*), must be selected. Afterwards the physicochemical characteristics or MD for each of these compounds must be calculated. Then, the compounds are separated in two sub groups, one to be used for **training** - that is a dataset with known biological values, helpful for designing a model to predict the biological effect of additional molecules- and a second dataset to be used for **testing** in the biological system [17]. This means that a biological attribute has to be measured. These attributes include the molar concentration of an inhibitor that modifies 50 percent the response (IC_{50}), or the amount of a drug that is therapeutic in 50 percent of the persons or animals in which it is tested (ED_{50}) [18].

The three dimensional (3-D) structure of the *receptor* is not an essential parameter; however, the information derived from it may be helpful to perform an integral analysis of the drug-receptor interaction. Once the data are collected, a model is constructed searching for a correlation between the properties and the biological activity. For doing so, regression analysis and statistical methods are chosen [19, 20].

Various automated prediction methods have been developed to annotate biological functions of molecules: molecular docking, molecular packing, Monte Carlo simulated annealing approach, pharmacophore modeling, protein cleavage site prediction, signal peptide prediction and structural bioinformatics, among others. We will not focus on their description but readers are invited to examine the following exhaustive good reviews [19, 21-24].

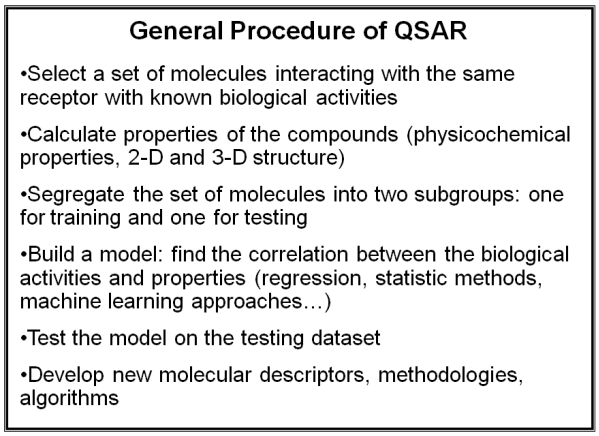

Figure 2: Minimal steps for QSAR.

These bioinformatics approaches are based on sequence alignment procedures. However, such methods perform poorly if there is low sequence homology between the examined sequence and the template sequences deposited in the databases. Alternative methods, such as the alignment-free Machine Learning methods have therefore been developed and are useful for predicting the function of the protein and explore its molecular diversity based on structural parameters independently of sequence-sequence similarity [18, 25]. Additional alternatives include graphic methods that provide useful insights into the biological function of proteins [18]. These graphical methods are useful for QSAR studies [17, 26].

On the other hand, in 1977 Chou published his key work about the molecular dynamics of bio-macromolecules [27]. The results obtained using this technique suggests that the low-frequency collective motions that exist in DNA and proteins hold a huge potential for revealing the dynamic mechanisms and the function of drugs in biological systems [18]. Indeed, methods like nuclear magnetic resonance support the certainty of this hypothesis by [28-30] and indicate that an understanding of the molecular interaction of drugs with their *receptors* must consider their static structures and their dynamical interactions. Thus, Molecular Dynamics has become the foremost computational technique to investigate structure and function of peptides (*receptors*) [18] and to predict activity against microbial species, growth inhibition of cancer lines and, in general mechanisms of action of any drug or pharmacophore.

4. LIPINSKI'S RULE OF 5

We have already mentioned that QSAR is a method that correlates the biological function (or activity) of a series of compounds with physicochemical and/or structural properties. For that reason it can be defined in terms of these simple equations:

$$\text{Activity} = f(\text{physicochemical properties}),$$

or,

$$\text{Activity} = f(\text{structural properties})$$

From these equations, it is easy to find out which physical and chemical characteristics should be fulfilled by the compounds to determine their activity. Lipinski's rule of five, described initially in 1997, lists which regulations the physicochemical properties should follow (see Fig. **3**) [31].

Most medically important drugs are relatively small and lipophilic molecules. Based on this observation, Lipinski's rule of 5 evaluates if a biologically active compound holds properties that would make it an efficient, orally active drug in humans. This has been called "drug likeness" of the compound; of note, this concept does not predict its pharmacological activity [31].

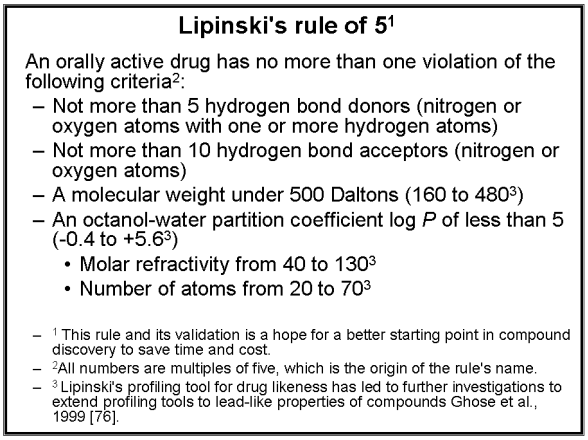

Figure 3: Minimal requirements for designing an orally active drug. Lipinski's rule of 5.

The key to drug discovery is to identify chemical structures with good inhibitory effects on specific targets, without or with minimal toxicity (non-specific activity). Modification of the molecular structure of compounds results in drugs with higher molecular weight, more rings and bonds with free rotation, and a higher lipophilicity. These features do not guaranty a stepwise increase in activity and selectivity, and in drug-like properties [31]. For this reason Lipinski's rule of 5 must be strictly followed during the active lead structure optimization and is fundamental for drug development and consequently for QSAR.

To help scientists to design the best compounds, a web page has been developed (http://www.chemaxon.com/marvin/sketch/index.jsp) to calculate the relevant physicochemical parameters that should be followed to design a drug according to Lipinski's rule of 5.

5. ADVANTAGES AND DISADVANTAGES OF QSAR

Drug discovery both in the pharmaceutical industry and in the academia is a very challenging process; therefore, the preparation of virtual libraries of compounds for high through-screening is often desired [10, 18]. The design and preparation of such libraries is time consuming and challenging and the characterization of drug-like properties of compounds within the libraries must correlate two-dimensional (2-D) and three-dimensional (3-D) descriptors with drug action; otherwise these properties will be of limited use [18].

As already mentioned, QSARs, the basis of rational drug design, assume a unique mechanism for all compounds that belong to a data set, and are therefore fundamental for developing predictive relationships to improve the profile of virtual libraries. As the main goal of SAR and QSAR is to uncover correlations between physicochemical properties and molecular function of a group of compounds, at least two advantages derive from this quantification: 1) An understanding of the relationship that exists between structure and activity. 2) An understanding of the effect that a given molecular structure may have on activity. This information is extremely helpful for predictions leading to the synthesis of novel and effective analogues. Last but not least, QSAR reinforce the identification of active molecules for a selected target when compared with random selection or other traditional methods (http://www.qsarworld.com).

However, systems that depend on the structure of a biological *receptor* face extreme difficulties: 1) Membrane-bound *receptors* are elusive for the determination of their 3-D structure, either by nuclear magnetic resonance (NMR) or X-ray crystallography. 2) The data collections describe only a few thousand

receptors; these structures are artificially modeled in water or organic solvents, far away from the weak salt solutions where the *receptors* function. 3) *Receptors* suffer conformational changes when they accept the ligand; this implies an even more complicated binding kinetics, and makes the unbound *receptor* not necessarily relevant for QSAR. 4) The determination of 3-D structures of ligand bound *receptors* adds extra complexity to the procedure. 5) QSAR techniques are accurate on a small scale when determining the properties of specific regions, such as the active site in an enzyme; however, they do not produce an accurate global description of the whole molecule. 6) False correlations may arise because biological data are subject to considerable experimental error (noisy data). 7) If the training dataset is not large enough, the data collected may not reflect the complete molecular space (http://www.qsarworld.com).

We can thus conclude that although the results can seldom be used to predict the most likely compounds with best activity, the information obtained through 3-D QSAR has led to better characterization of molecules and compounds and improved calculation of their properties. The main drawback is that QSAR methods are unable to model steric interactions accurately, particularly when these interactions involve large regions of the molecular surface [32]. This is especially relevant when functional areas of the drug that participate in binding easily identified with QSAR, heavily contribute in determining the molecule's activity and defining the "pharmacophore" areas of the molecule [33].

6. QSAR IN *LEISHMANIA*

The first initiatives to perform SAR in *Leishmania* used antifolates as leishmanicidal drugs, and measured the capacity of triazine derivatives to inhibit cell growth by targeting the enzyme dihydrofolate reductase [34]. Linear free energy descriptors through Molecular Shape Analysis (MSA) were used to develop the QSARs. The MSA-QSAR results suggested that the activity of the antifolates was independent of the conformation assigned to the large flexible substituents [34, 35]. Since then, different types of compounds have been screened against *Leishmania*. The structure of the isolated (natural) and designed (synthetic) molecules include acridines, phenothiazines, purines, pyrimidines and quinolines that contain nitrogen heterocycles [36] (see Fig. **4**), although certain amino acids, amides and esters, aniline derivatives, flavonoids and quinones have also been reported to be leishmanicidal [37]. In spite of many efforts done, few substances have reached the pre-clinic and the clinic stage. Here in we will present a summary of the leishmanicidal compounds SAR analyses published until now. In many cases the mechanism of action of the compounds is unknown; we will address this issue when the information is available.

Lead compounds used for QSAR in *Leishmania*

Arylisoquinolines	[54, 55]
Aurones	[77]
Azatrypanthrin derivatives	[56]
Bisphosphonate analogs	[69, 70]
Chalcones	[38, 40, 41, 42]
Dinitroaniline sulfonamide derivatives	[62, 63]
Folate Antagonists	[34, 35]
Lactones	[48]
Lignans benzoflurans	[53, 78]
Miltefosine analogs	[68]
Nifuroxazide	[58]
Phloroglucinol	[47]
Pyrazol pyridines	[59]
Sesquiterpenes	[45,46]
Trypanothione antagonists	[60]

Figure 4: Examples of compounds used for QSAR in *Leishmania*. Brackets, main references.

7. NATURAL PRODUCTS AND DERIVATIVES

Lidochalcone A, isolated from Chinese licorice, efficiently inhibits the proliferation of *Leishmania* (*L.*) (*L. major* and *L. donovani)*. It does so by interfering with the function of parasite's mitochondria [38]. This

compound has a dual -maybe unwanted- effect since it also inhibits the phytohemmaglutinin A-induced proliferation of human lymphocytes [39]. However, changes in the substitution pattern of lidochalcone A modulate the activity against parasites and macrophages in an opposite way, making it possible to prepare chalcones with high selectivity against *Leishmania* [38].

Therefore, various groups have synthesized numerous chalcones and their derivatives, based on statistical and rational design methods, and have performed QSAR analysis [38-42]. For example, the group of Nielsen [38] developed a method to correlate the biological activity of the synthesized chalcones to physicochemical parameters like molar refractivity, lipophilicity and changes in the type of aromatic reactant substitutes (Hammet electronic effect) [43]. They concluded that positions 4 and 4' in the molecule are crucial for the biological activity of the derivatives and that steric interactions between the chalcones and the target molecules are of major importance for the potencies of the compounds; additionally, these results suggest that substitutions in positions 4', 2, 3 and 4 of the parent compound decrease the toxicity towards lymphocytes [38]. Unfortunately, these substitutions interfere at the same time with the solubility of the molecules and decrease the activities against *Leishmania* [38]. A fundamental conclusion essential for the development of leishmanicidal compounds aroused from this work: it is possible to separate the leishmanicidal activity from the antilymphocite properties of the molecules. Similar conclusions were obtained by the group of Lunardi [40] that designed chalcones by substitutions in the ring structures; indeed, their best compound was active *in vitro* against *L. braziliensis* at 13.7 μM, without affecting macrophages even at concentrations of 300 μM.

Chalcones have also been synthesized as a set of 4-methoxychalcone derivatives to identify if sulfonamide and methoxy substitutions could be promising adding-groups for the design of lead antiparasitic compounds. SAR analysis of these derivatives was devoted to determining structural and stereo-electronic features that could direct the leishmanicidal activity to the sulfonamide moiety [41]. This study evaluated *in vitro* the activity of the best molecules against promastigotes and their capacity to decrease the infection rate of *Leishmania*-infected macrophages. Additionally, the study evaluated the cytotoxicity of the compounds against mouse peritoneal macrophages to determine the selectivity index [41]. The MD used to compare with the activity of the compounds were the dipole, the Highest Occupied Molecular Orbital (HOMO), the Lowest Occupied Molecular Orbital (LUMO), the calculated log *P* (octanol/water partition), the molecular weight and the molecular volume [44]. The SAR analysis of these sulfonamide 4-methoxychalcone derivatives suggests that the molecular volume, the HOMO density concentrated in the chalcone moiety, and the conformational configuration of the compounds are fundamental structural and stereo-electronic features for the leishmanicidal activity. Of note, these compounds were designed according to Lipinski's rule of 5 and their "drug likeness" was similar to that of classical leishmanicidal drugs. Interestingly, there were very minor structural differences among the best and worst compounds and these differences were related to only one additional spacer (carbon residue) present in the structure of the best compound [41]. This key work for SAR in *Leishmania* demonstrated an enhanced leishmanicidal activity of derivatives due to the combination of two different pharmacophoric groups (*i.e.* chalcone and sulfonamide).

Natural products isolated from plants of the order Celastraceae have also been useful in the discovery of leishmanicidal compounds. These plants produce active secondary metabolites, the so called sesquiterpenes, of variously polyoxygenated tricyclic scaffolds, all based on a core C15 skeleton known as dihydro-â-agarofuran[5,11-epoxy-5â, 10R-eusdesm-4(14)-ene]. Many sesquiterpenes isolated from Celastraceae are lipophilic and cross cell membranes have a wide range of biological actions, and probably interact with a variety of cellular targets. Interestingly they have been described as useful in reversing multidrug resistance (MDR) and as efficient antitumor-promoting compounds [45, 46], two properties which make them clinically interesting.

The biological evaluation of sesquiterpenes as modulators of the MDR phenotype in *L. tropica* included a 3-D-QSAR both for natural sesquiterpenes from *Maytenus cuzcoina* and semi synthetic derivatives. The 3-D-QSAR was performed using the Comparative Molecular Similarity Indices Analysis (COMSIA), an extension of the Comparative Molecular Field Analysis Methodology (COMFA). This technique permitted

the comparison of various sesquiterpenes bearing substituents at different positions of the dihydro-â-agarofuran skeleton, and allowed the characterization of the steric, electrostatic, hydrophobic, and hydrogen-bond-donor and acceptor requirements needed at the active sites of the *receptors* for ligand recognition. The 3-D-QSAR showed that the electrostatic features represented 58.9 percent of the total requirements needed to act as modulators at the P-glycoprotein-like transporter, being the most salient features those related to the H-bond interaction between the substituents at the C-2 and C-6 positions of the molecule with the receptor [45, 46]. Of note, these compounds selectively decreased the phenotype of drug-resistant parasites without significant toxicity.

Agelasine D, benzofuranes, flavonoids, lactones and phloroglucinol compounds are active antiparasitic compounds [47-51]. Many of these molecules are isolated from bacteria, plants or sponges and constitute lead compounds that when modified at their side chains display good selectivity against parasites. In fact, analogs of the O-prenylated phloroglucinol derivative based O-alkylated and formylated acylphloroglucinols, have a demonstrated leishmanicidal activity against *L. donovani* promastigotes (IC_{50} ~ 5 μg ml^{-1}). In these compounds the prenyl, methyl and isovaleryl side chains were replaced with allyl, isopentyl, formyl, acetyl and isopentyl groups. The O-alkylated phloroglucinols bearing formyl/isovaleryl moieties are the most active molecules against *L. donovani* compared to compounds bearing formyl/acetyl, acetyl/acetyl or isovaleryl/isovaleryl moieties. These results suggest that the activity of the acylphloroglucinol molecules depends on the length of the acyl side chain and that substitution of the acyl group with the alkyl group results in loss of activity [47].

Synthetic aliphatic and aromatic lactones and dimers are active against *L. panamensis* at 0.8 μg ml^{-1} but are still very cytotoxic. The SAR demonstrated that the aliphatic side chain enhances the biological activity and decreases the cytotoxicity. This means that a reduced size of the lactone ring increases the selectivity index, although it also decreases the activity of the compound [48].

Inhibitors of tubulin polymerization at the colchicine binding site constitute an example worth to mention. A series of synthetic dihydrobenzofuran-lignans and related benzofurans have thus been tested against *Leishmania* [50]. The results suggest that compound 2g may function as a promising leishmanicidal dihydrobenzofuran derivative lead compound. Interestingly the concept of quasi-atomistic receptor surface modeling was used to develop a 4D-QSAR study of this interaction (http://www.biograf.ch) [52, 53].

A final example we would like to address is the SAR analysis of the naturally occurring plant-derived naphthylisoquinoline alkaloids. This is an interesting model since, besides studying the *in vitro* activity of the compounds, the decrease in the infection rate of infected macrophages and the cytotoxicity of the compounds against different mammalian cell types were investigated. The initial results and the SAR studies, where the aryl portion of the lead substrate was modified, demonstrated that the alkaloids ancistrocladinium A and B and the synthetic isoquinolinium salt decrease the macrophage infection rate by acting directly on the intracellular amastigotes [54, 55]. Additionally, the new isoquinolines act synergistically with amphotericin B and do not interact with cytochrome P450 enzymes involved in the metabolism of leishmanicidal drugs, thus making naphthylisoquinoline alkaloid derivatives promising candidates to be considered as leishmanicidal pharmacophores [55].

8. RANDOM AND RATIONAL DESIGN OF COMPOUNDS

The mode of action of many active leishmanicidal compounds is yet unknown and information on the molecular mechanisms is very scarce to support a rational design or selection of active molecules. However, since normally steric and electronic properties are responsible for the recognition of drugs by *receptors*, analysis of these properties in series of compounds should help obtain better insights on the mechanism of action and design more efficient analogues [56]. Within this aim, many groups have devoted their studies to finding out which electronic structure of specific molecules and derivatives should guide the most efficient activity and receptor-drug interaction to obtain lead compounds against *Leishmania*.

For example, the indolo[2,1-b]quinazoline-6,12-dione derivatives or azatrypanthrin derivatives [57] have been analyzed and tested for *in vitro* leishmanicidal activity. Their molecular characteristics in a 3-D space generated a satisfactory model that correlates experimental and estimated activity of the trypanthrins and confirms the stereo-electronic and redox potential analyses of SAR. The results are interesting and extend to structurally diverse classes of compounds, especially since the drugs are less toxic to mammalian cell lines than to *Leishmania*, making tryptanthrins suitable for further studies as potential leishmanicidal candidates [56].

In another study fifty-three nitro molecules were designed based on the antibacterial agent nifuroxazide backbone. The core structure permitted different substitution patterns in the aromatic ring, thus generating several analogues with diverse physicochemical properties but with similar steric characteristics. The side chain groups were chosen based on the relative electronic and lipophilic contributions of an appropriate substituent to the global structure. The results of using mono- and di-substituted analogues and 2-D QSAR studies suggest that there are compounds that exhibit IC_{50} values lower than the standard drugs pentamidine and amphotericin and are good candidates for further *in vivo* leishmanicidal assays. Additionally the results indicate that nitrothiophene derivatives were more active than the nitrofuran analogues [58].

Our final example relates to fused heterocyclic systems containing a pyrazole ring, for example 1H-pyrazolo[3,4-b]pyridine. These compounds are considered among the most versatile bioactive molecules. Pyrazolo[3,4-b]pyridines are potential specific antagonists of nucleic acid metabolism, and derivatives of this heterocyclic ring system are substrate inhibitors of purine-requiring enzymes [59]. The pyrazolo-pyridine is considered an aminoquinoline analogue, whose derivatives such as chloroquine and amodiaquine are antimalarial. Amodiaquine had never been used against leishmaniasis and de Mello and collaborators synthesized three series of 4-anilino-1H-pyrazolo[3,4-b]pyridine-5-carboxylic esters to study their potential leishmanicidal activity. The compounds were tested against promastigote forms of *L. amazonensis* and the activities correlated with the octanol/water partition parameter, log *P*. The results demonstrated that 3′-diethylaminomethyl-substituted compounds are the most active, with IC_{50} between 0.39 and 0.12 μM. Molecular modeling predicted the log *P* and steric parameters as the most significant contributions to biological activity [59].

9. TARGET ORIENTED DESIGN OF COMPOUNDS

The design of selective toxic agents should exploit key differences in metabolism between the host and pathogen, to exploit these differences in the design of selective toxic agents. We will now comment on a few examples that have used rational design to devise compounds directed towards known validated targets and new molecular targets in *Leishmania* pathogens [18].

For example, the thiol metabolism of trypanosomatids depends on the hexapeptide trypanothione (N1,N8-bis(glutathio-nyl)spermidine) (T(SH)2), an antioxidant replacing glutathione, the major antioxidant in other eukaryotic cells. Trypanothione may thus be an important antiprotozoal drug target and in fact some trypanocidal drugs, notably the arsenicals (melarsoprol) and difluoromethylornithine (eflornithine), may work by interfering with the metabolism or synthesis of this hexapeptide. The central role of trypanothione in *Trypanosoma* and *Leishmania* parasites make thiol-dependent enzymes potential targets for the development of chemotherapeutic drugs. Daunes and collaborators synthesized a series of N-S-blocked glutathione monoester and diester derivatives based on N-benzyloxy-carbonyl-S-(2,4-dinitrophenyl)glutathione and evaluated them for activity against *Trypanosoma* (*T.*) *brucei* (*b.*) brucei, *T. cruzi*, and *L. donovani in vitro*. Only monoesters with a log *P* value > 2.7 inhibited the growth of *T. b. brucei* bloodstream form trypomastigotes. Diester compounds were better inhibitors of *T. b. brucei* growth than monoester compounds, and some displayed high activity against *T. cruzi* and *L. donovani* [60]. Again, analysis of the inhibition data *vs*. calculated log *P* values provided evidence to support membrane penetration and steric factors as the key components in the activity of these compounds.

Dyneins are important proteins of *Leishmania* governing fundamental processes such as cilia and flagella motion, nuclear migration, organization of the mitotic spindle, and chromosome separation during mitosis. The first QSAR for dynein proteins in *Leishmania* contained 411 protein sequences of different species. This analysis discriminated more than 92 % of dyneins and other proteins in four different training and cross-validation datasets [61]. The analysis allowed the description of a new dynein sequence through a combined experimental and theoretic analysis and illustrated the usefulness of the model to search for potential drug targets. This was possible by the use of a combined strategy of 2D-electrophoresis analysis of *L. infantum* biological samples, followed by the excision from 2D-E gels of the spot of interest - unknown protein or protein fragment in the region M < 20,200 and isoelectric point < 4-. Afterwards, and through the Modular Approach to Software Construction Operation and Test (MASCOT) search engine (http://www.matrixscience.com), which uses mass spectrometry data to identify proteins from primary sequence databases, a *L. infantum* protein containing a dynein heavy chain was successfully identified [61].

Dinitroaniline sulfonamide compounds are known for their leishmanicidal activity. A virtual screen analysis of drug-like compounds belonging to the Maybridge® database led to the identification of the compound **BTB 06237** (2-[(2,4-dichloro-5-methylphenyl)sulfanyl]-1,3-dini-tro-5-(trifluoromethyl)benzene, 1), with potent activity against *L. donovani* axenic amastigotes (IC_{50} = 0.52 ± 0.20 μM) [62]. The leishmanicidal activity of **BTB 06237** correlated with the production of reactive oxygen species and the dissipation of the mitochondrial membrane potential; at the end, the results of SAR studies suggested that **BTB 06237** is a promising leishmanicidal pharmacophore [63].

The phosphocoline analogue miltefosine was approved in 2000 as an orally active drug for improving the classical therapies against leishmaniasis, *i.e.*, meglumine antimoniate and sodium stibogluconate. Still, its side effects remain challenging, especially teratogenicity. However, the fact that its cellular target is known represents an advantage in the effort to design that are active against leishmaniasis but lack the above mentioned drawback. With this aim, series of compounds have been designed as ring-substituted ether phospholipids carrying *N,N,N*-trimethylammonium, N-methylpiperidino, or N-methylmorpholino head groups [64]. Interestingly, the leishmanicidal activity of the different series demonstrated that some analogues selectively act against the promastigote forms of *L. donovani* or *L. infantum*. Additionally, some compounds were even more potent than miltefosine (hexadecylphosphocholine) against either *L. donovani* and *L. infantum* or both. Interestingly, Differential Scanning Calorimetry (DSC) demonstrated that the active compounds affect the thermotropic properties of a model membrane bilayer to a lesser extent than the less active ones [64]. Miltefosine is a ring-substituted ether phospholipid that challenges the use of SAR analysis since it is a rigid molecule and normally the analogues have increased structural flexibility [65]. This means that to perform a 3-D-QSAR the optimized 3-D-conformations of molecules are needed [66-68]. This challenging situation has been successfully addressed and a systematic procedure correlating the leishmanicidal activity of ring-substituted ether phospholipids against *L. infantum* and *L. donovani* has been performed and correlated with electrostatic, hydrophobic and steric parameters [65]. As a result, good COMFA and COMSIA activity models with similar results were obtained and suggest that the steric effect determines the activity of the miltefosine type ether phospholipid analogues. Interestingly this work demonstrated that the high leishmanicidal activity of these compounds can be achieved by a combination of an adamantylidene group in the lipohilic part of the choline at the head group of specific compounds [65].

Using the mevalonate/isoprene biosynthesis pathway enzyme, farnesyl pyrophosphate synthase, the activity of 62 bisphosphonates has been evaluated for inhibition of the enzyme from *L. major*. The investigated compounds exhibit activities (IC_{50} values) ranging from 100 nM to 80 μM (corresponding to Ki values as low as 10 nM). Bisphosphonates containing longer or multiple (N,N-) alkyl substitutions were inactive, as were aromatic species lacking an ortho- or meta-nitrogen atom in the ring, or possessing multiple halogen substitutions or a para-amino group. The active compounds were the bisphosphonates containing short (n) 4, 5) alkyl chains [69, 70]. The COMSIA fields were useful for investigating which structural features correlated with high activity, and indicated that a positive charge in the bisphosphonate side chain and a hydrophobic prospect significantly contributed to the activity of the compounds. These results represent the first detailed

QSAR of the inhibition of an expressed farnesyl pyrophosphate synthase enzyme by bisphosphonate inhibitors, and demonstrate that the activity of these inhibitors can be predicted using 3-D-QSAR techniques [70].

10. BEYOND STRUCTURE-BASED DRUG DISCOVERY: FRAGMENT APPROACHES, MULTITASKING QSAR AND POLYPHARMACOLOGY

QSAR models predict the biological activity of drugs against only one target. However, the interaction of a compound with multiple targets is more common than previously recognized. In fact the terms promiscuous targets, dirty drugs, and complex pharmacology are in common use now days. Consequently, the prediction of drug activity against different targets is an interesting challenge, usually difficult to achieve. Fragment approach drug design, multitasking QSAR and pharmacological networks are approaches that go beyond QSAR. When successful these approaches may yield opportunities to construct and map the contribution of sub-structures that function for multiple targets and species [52, 71, 72]. This would be especially interesting when dealing with a parasite like *Leishmania* where at least 20 species (with different isoforms of proteins or targets) could be pathogenic for humans.

Although efforts to deal with this situation have already begun, the obtained information is still scarce and difficult to understand. However, if successful, these methods would allow identifying compounds according to their profile of biological activity [17, 73, 74] and will constitute a fundamental methodology for designing drugs, including those for leishmaniasis.

11. FINAL COMMENTS

Parasitic diseases are still a threat to mankind in the 21st Century. The lack of effective chemotherapy for tropical diseases, and the alarming decrease in our arsenal of effective antiparasitic drugs due to the development of drug resistance emphasize the need for new therapeutic agents. In determining the best drug candidates, it is critical to keep in mind that any new drug must be affordable to those affected by these diseases, of course, including leishmaniasis [75].

New leishmanicidal products should be structurally simple enough to serve as leads for easy analogue synthesis or be available in sufficient quantities to permit economical access by affected populations.

Several techniques have been used to study the mechanisms by which *receptors* recognize their ligands, and QSAR facilitates the description of the molecular details involved in the mechanism of action and/or behavior of a specified interaction and recognition model. Given the tremendous chemical diversity, the successful development of antiparasitic products based in this method could have a dramatically positive impact on the treatment of leishmaniasis [76]. In the case of *Leishmania* QSAR constitute an essential tool to investigate chemical, electronic, and structural features affecting the leishmanicidal activity of compound libraries either with known mechanism of action or chosen at random. However, the information regarding *Leishmania* QSAR is rather scarce and major efforts are needed to stimulate this type of research in order to collect information that is fundamental for describing the determinants of leishmanicidal drugs.

Table **1** summarizes efforts made in this direction. Although many of the studies include an evaluation of the cytotoxicity of compounds against mammalian cells, only two describe *in vitro* the decrease in percentage of macrophage infection, and unfortunately only one has gone as far as *in vivo* testing of compounds. This means that a critical spot in leishmanicidal drug design is to encourage researchers to perform *in vivo* experiments and go into pre-clinical and clinical phases of research to reach the patient as soon as possible. We can never forget that our ultimate goal is to cure those suffering from the disease.

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Table 1: *In vitro* and *In vivo* activity of leishmanicidal compounds in selected SAR analysis.

Strain	Compound Type	*In Vitro* Activity µg / ml		*In Vitro* Activity µM		Cytotoxicity		*In Vivo*	References*
		Promastigotes	Percentage ↓ of Infection	Promastigotes	Percentage ↓ of Infection	µg / ml	µM	Percentage ↓ of Infection	
L. amazonensis	pyrazol pyridines	0.44	ND	1.23	ND	ND	ND	ND	de Mello *et al.*,2004
L. braziliensis	chalcones	ND	ND	13.7 (9.9-18.9)	ND	ND	> 300	ND	Lunardi *et al.*, 2003
L. braziliensis	chalcones	ND	ND	3.5 ± 0.6	ND	ND	69.0 ± 3.7	ND	Andriguetti-Fröhner *et al.*, 2009
L. donovani	aurones	0.45	1.40			<2.32>25	ND	ND	Kayser *et al.*, 1999
L. donovani	chalcones	ND	ND	3.4 ± 0.5	ND	ND	3.7 ± 0.2	ND	Nielsen *et al.*, 1998
L. donovani	trypanothione antagonists	ND	ND	7.8	ND	ND	269	ND	Daunes et al, 2001
L. donovani	dinitroaniline sulfonamide	ND	ND	0.67 ± 0.24	2.6 ± 1.2	ND	11.0 ± 2.9	28	Delfín *et al.*, 2009
L. donovani	Pentamidine analogs	ND	ND	1.17 [1, 2]	ND	ND	6.40 [1]	ND	Bakunova *et al.*, 2009
L. major	bisphosphonates	ND	ND	0.11	ND	ND	ND	ND	Sanders *et al.*, 2003
L. major	arylisoquinolines	ND	ND	2.65 ± 1.51	0.092	ND	12.67 ± 3.07	ND	Ponte-Sucre *et al.*, 2009
L. panamensis	lactones	2.8 ± 0.8	33.9 ± 1.4	ND	ND	ND	ND	ND	Castaño *et al.*, 2009

* de Mello *et al.*,2004, [59]; Lunardi *et al.*, 2003, [40]; Andriguetti-Fröhner *et al.*, 2009, [41]; Kayser *et al.*, 1999, [77] ; Nielsen *et al.*, 1998, [38] ; Daunes et al, 2001, [60]; Delfín *et al.*, 2009, [63]; Sanders *et al.*, 2003, [69]; Ponte-Sucre *et al.*, 2009, [55]; Castaño *et al.*, 2009, [48] Bakunova *et al.*, 2009 [79]. ND= not determined. [1] average of two determinations. [2] axenic amastigotes.

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