Drug design, biotechnology and medicinal chemistry: applications to infectious diseases

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Introduction

INFECTIOUS diseases are caused by pathogenic microorganisms (e.g., bacteria, viruses, fungi and parasites) that must invade host cells in order to reproduce. These diseases are serious public health problems affecting a significant portion of the world's population, and because of their socioeconomic aspect represent a major challenge for the twenty-first century, especially in the poorest and most vulnerable regions of the planet. According to the World Health Organization (WHO), infectious diseases account for about a third of the causes of death worldwide. The relationship between these diseases and the low income of the poorest populations is evidenced by the fact that infectious diseases rank first among the leading causes of death and permanent disability in developing countries (WHO, 2008).

Discovering and developing pharmaceutical drugs is a complex, lengthy and costly process, whose roots are deeply linked to scientific and technological innovations (Guido et al., 2008a). The significant advances in chemistry and biology and the better understanding of biochemical pathways, molecular targets and mechanisms that lead to the onset and progression of diseases have enabled the discovery of remarkable therapeutic innovations, providing significant improvements in the quality of life of many populations in the world.

Medicinal chemistry, which plays a recognized central role in drug R&D, is characterized by its relevant multidisciplinary nature, covering various specialties such as organic chemistry, biochemistry, pharmacology, information technology, and molecular and structural biology among others. According to the International Union of Pure and Applied Chemistry (IUPAC), medicinal chemistry is a discipline based on chemistry that involves the invention, discovery, planning, identification, preparation and interpretation of the molecular mechanism of action of biologically active compounds. Besides the discovery

of bioactive molecules, medicinal chemistry also incorporates studies of the metabolism and relationships between chemical structure and activity (Wermuth, 2003). This clearly shows the establishment of key interfaces between chemical, biological, pharmaceutical, medical, physical and computational sciences.

Discovery and development of new pharmaceutical drugs

In the implementation of drug design strategies, studies of evolutionary processes of molecular recognition in biological systems are of great importance, since they set up the fundamental basis for understanding properties such as potency, affinity and selectivity. Given this complex paradigm, biotechnological tools associated with medicinal chemistry methods play an outstanding role in the development of new molecules with biological activity.

The process of discovering and developing drugs, as shown in Figure 1, is divided into two major phases: (i) discovery (also known as pre-clinical or basic research phase); and (ii) development (or clinical phase) (Lombardino & Lowe, 2004). In the early stages of the discovery phase, research usually focuses on the identification and optimization of small molecules capable of representing New Chemical Entities (NCE) with potential for clinical development. Validation of the molecular target selected is essential for a number of reasons that range from establishing its relevance in the physiopathological process under study to characterizing the impact of its selective modulation in the treatment or cure of diseases or disorders in humans.

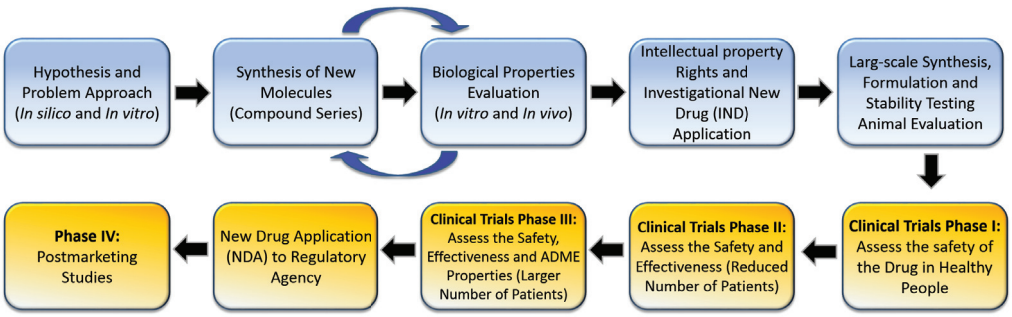

Figure 1 – Phases involved in the discovery and development of pharmaceutical drugs (ADME - absorption, distribution, metabolism and excretion; NDA - new drug application).

The three-dimensional (3d) structure of the selected biological target (e.g., protein, DNA or RNA) can be known or unknown, and this determines the prioritization of design strategies (Andricopulo et al., 2009). Great advances in genomics and proteomics, coupled with evolving X-ray crystallography and nuclear magnetic resonance (NMR) techniques provide a significant increase in the number of molecular targets with 3D structures available in the Protein Data Bank (PDB).

Bioactive molecules (or ligands) can be identified from actual (e.g., biological, biochemical) or virtual (e.g., computational) screenings of natural products, synthetic compounds or combinatorial collections, or by means of rational planning. It should be emphasized, however, that in all cases the biological properties must be determined experimentally, and the performance of standardized and validated high quality tests is essential. In general, low potency and affinity molecules are identified in the early design phases and should be optimized with respect to a series of pharmacodynamic (e.g., potency, affinity, selectivity) and pharmacokinetics (e.g., absorption, metabolism, bioavailability) properties. The optimized compounds are selected as lead compounds for further development of a drug candidate (NCE) (Guido & Andricopulo, 2008).

With the aid of medicinal chemistry methods it is possible to exploit the vast chemical space outlining the work involving the identification, selection and optimization of molecules capable of interacting, with high affinity and selectivity, with the selected molecular target (e.g., enzyme, receptor), which represents the biological space. Several strategies can be used to investigate the chemical- biological space, such as: organization of databases, the use of molecular filters, high-throughput screening and virtual screening.

Knowledge of the structures of macromolecular targets or of ligand-receptor complexes enables structure-based drug design. In contrast, when the structure of the selected target is unknown, ligand-based drug design methods can be used, exploiting the properties and characteristics of series of bioactive ligands. In many cases, the use of integrated SBDD and LBDD strategies can generate useful information for designing NCE, through the synergy and complementarity of knowledge between the strategies (Guido et al., 2008a; Andricopulo et al., 2009). The skilled management of information is a very important factor in the present day, as it enables organizing and analyzing the vast amount of data available.

Biotechnology and drug discovery

The biotechnology revolution (e.g., genomics, functional genomics, proteomics, metabolomics and cytomics) has provided extremely useful information for drug discovery. The suffix "omics" indicates a number of new disciplines and procedures aimed at the functional and/or structural identification of tissues, cells, gene expression patterns and metabolic characteristics (Bhogal & Balls, 2008). These strategies have a variety of applications through the monitoring of biochemical or cellular indicators (e.g., transcription of a specific gene or variation in the expression/function of a particular protein), from the phase of identification of physiological and/or metabolic changes induced by the stage of the disease, to the evaluation of the effects of drugs in the human body (Table 1). Although promising, these technologies have some limitations, including (i) the need for methods capable of interpreting and correlating in an optimized way the huge amount of information generated; and (ii) the rational and effective application of biological data in drug design.

Functional genomics (or transcriptome) focuses on the complete knowledge of the set of transcripts (messenger RNA, ribosomal RNA, carrier RNA and microRNA) of a given organism, tissue or cell strain. Functional genomics studies are based on microarray techniques capable of identifying and quantifying mRNA transcripts in cells. The analysis of microarrays of RNA interference (RNAi) has contributed significantly to the evaluation of gene function through gene silencing or decreased expression of proteins (Mousses et al., 2003). However, post-translational events that are important for cellular regulation and signaling, such as phosphorylation, glycosylation, membrane-anchoring and folding are rarely detected by this technique. Proteomic methods (Blackstock & Weir, 1999) are useful alternatives in these cases and, although less suitable for large-scale trials, provide more accurate and detailed data, since the strategies involved (e.g., mass spectroscopy) are based on the resolution and detection ability of specific proteins isolated from several sources.

Metabolomic studies allow the identification of low molecular weight metabolites through very sensitive techniques such as NMR (Lindon & Nicholson, 2008), which requires only 20 mg of tissue or biofluids (Bollard et al. 2005). In addition, the procedures involved can be widely applied in studies to evaluate pharmacokinetic properties of drug candidates, both in the preclinical phase in animal models and in human clinical trials (Figure 2). The use of the Human Metabolome Database) (Wishart et al., 2007) assists in the evaluation of the impact of diseases on the metabolism of different organisms.

Cytomics, which is characterized by the study of cellular systems, is based on a less reductionist assumption that integrates data from functional genomics and metabolomics to elucidate genetic and biochemical events in a single cell (Valet, 2006). Techniques previously exclusive to cellular studies, such as flow cytometry, confocal microscopy, bioimaging and fluorescence are being applied in drug R&D because they allow data collection in real time, showing a realistic scenario of the biological phenomena.

Table 1 – Biotechnological strategies and methods

Strategy	Sample	Technology	Information	Advantages	Limitations
Genomics	DNA	DNA Sequencing	Nucleotide sequence	Efficiency in data generation	Excessive data generation
Functional genomics	RNA	Determination of transcripts - mRNA	mRNA hybridization patterns	Efficiency in data generation	Extrapolation of mRNA transcript to protein expression
Proteomics	Proteins	Mass spectroscopy, gel electrophoresis, protein chips, antibody arrays	Amino acid sequence, post-translational changes, interactions between proteins	Data generated on a medium-scale; relevant information for discovering new targets	Low efficiency in data generation, associated noise
Metabolomics	Metabolites	Mass spectroscopy, NMR, HPLC	Metabolite molecules	High-scale screening with significant transfer capability for studies	Complex mixtures, associated noise, reproducibility
Cytomics	Cells	Digital imaging, fluorescence, immunohistochemistry	Photons	Optimization of temporal and spatial visualization of cellular effects of drugs	Difficulty in obtaining relevant biomarkers

In general, the different biotechnological strategies discussed produce large amounts of data that need be analyzed quickly and effectively. In this context, bioinformatics methods play a key role by enabling the organization, management, visualization and interpretation of the information generated. The goal is the establishment of correlation patterns between different biochemical and cellular events involved in the disease state (or during treatment with a drug candidate). The integrated analysis of these data leads to interactomics (Cusick et al., 2005), which is characterized by the mapping of interaction networks among the various biological phenomena (e.g., molecular, biochemical, cellular). A significant number of data containing maps of the interaction of various biological systems is available in databases accessible to the public, such as: (i) PSI-MAP - Protein Structural Interactome Map (Gong et al., 2005); (ii) DIP - Database of Interacting Proteins (Xenarios et al., 2000); (iii) BIND - Biomolecular Interaction Network Database (Bader et al., 2003); and (iv) MINT - Molecular Interaction Database) (Zanzoni et al., 2002).

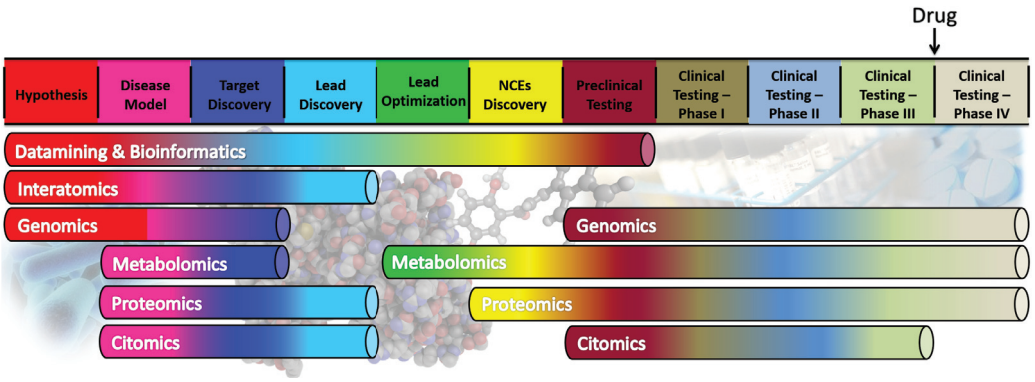

Figure 2 – Integration of biotechnology in drug R&D.

Neglected tropical diseases

Neglected tropical diseases (NTD) are striking consequences of social underdevelopment, depicting in detail the situation of the poorest and most disadvantaged regions on the planet (Beyrer et al., 2007). NTD are endemic in several important geographic regions (Dias et al., 2009), affecting millions of people and determining high morbidity and mortality rates. For the majority of priority NTD in WHO special programs (e.g., malaria, African trypanosomiasis (sleeping sickness), American trypanosomiasis (Chagas' disease), dengue fever, leprosy, leishmaniasis, schistosomiasis, and onchocerciasis, among others), the therapeutic options are inadequate and pose a number of problems such as low effectiveness, high toxicity and the emergence of resistant strains (Guido & Oliva, 2009). This scenario is compounded by the limited number and lack of innovation in drug R&D programs in the area of NTD (Nwaka & Hudson, 2006; Nwaka et al., 2008). Thus, the situation calls for concentrated global efforts (government - academia - industry) for the creation and maintenance of R&D programs aimed at the discovery of new alternative therapies for the treatment and control of these diseases (Guido & Oliva, 2009).

The WHO Special Program for Research and Training in Tropical Diseases (TDR), the Medicines for Malaria Venture (MMV) Program and the Drugs for Neglected Diseases Initiative (DNDi) are examples of public-private partnerships that aim to create, stimulate and invest in R&D of drugs against a variety of NTD (Dias et al. 2009). Some examples of drugs that have reached the market through public-private partnerships are provided in Table 2.

Table 2 – Anti-DTN drugs developed through public-private partnerships

Drug	Registry	Therapeutic Indication	Pubic-private partnerships
Praziquantel	1976	Schistosomiasis	Bayer / TDR
Mefloquine	1984	Malaria	Hoffman La Roche / Wrair* / TDR
Ivermectin	1987	Onchocerciasis	Merck / TDR
Halofantrine	1988	Malaria	Smith Kline Beecham, Wrair* / TDR
Eflornithine	1991	Sleeping sickness	Marion Merrel Dow / TDR
Liposomal Amphotericin B	1994	Leishmaniasis	NeXstar / TDR
Artemisinin	1997	Malaria	Rhone Poulenc Rorer / Kunming / TDR
Artemisinin-lumefantrine	1999	Malaria	Novartis / Chinese government
Artemotil	2000	Malaria	Artecef / WRAIR / TDR
Miltefosine	2002	Leishmaniasis	Zentaris, Indian CMR / TDR
Artesunate-amodiaquine	2007	Malaria	Sanofi-Aventis / DNDi
Artesunate-mefloquine	2008	Malaria	Farmaguinhos / DNDi

* WRAIR - Walter Reed Army Institute of Research.

National R&D initiatives in the area of NTD

Extremely important initiatives are being successfully implemented to include Brazil in an increasingly significant science and technology scenario. Three examples are presented to illustrate the breadth and diversity of networks and partnerships that have provided great opportunities and challenges in the area of NTD.

The Center for Structural Molecular Biotechnology (CBME) is the result of solid collaborations established in drugs R&D projects involving: (i) Protein Crystallography and Structural Biology and Medicinal and Computational Chemistry Laboratories (LQMC) of the Institute of Physics of São Carlos University in São Paulo (IFSC-USP); (ii) the National Synchrotron Light Laboratory (LNLS) in Campinas; and (iii) the Chemistry (DQ), Genetics and Evolution (DGE) and Physiological Sciences (DCF) departments of the Federal University of São Carlos (UFSCar). The CBME is one of the ten Research, Innovation and Dissemination Centers (CEPID) supported by the São Paulo Research Foundation (FAPESP) (CBME, 2010). The main objectives of the CBME include conducting basic and applied research, generating scientific and technological

innovation, and disseminating knowledge and education in all areas of biotechnology. To meet its objectives, the CBME has adopted an integrated multidisciplinary approach, which includes the use of molecular biology, biochemistry, organic chemistry, structural biology and medicinal chemistry methods.

The research projects of the CBME have as a common link a molecular approach that entails exploring complex biological systems of great importance to sensitive areas of our society, such as pharmaceutical drugs and medicines, agribusiness and biotechnology. One of the major goals of the CBME is to achieve maximum integration with the productive sector, particularly with national and multinational pharmaceutical industries, biotechnology companies, and research institutions on human health, among others, for the establishment of partnerships and/or other forms of cooperation in drug R&D. In short, in the last ten years the CBME has spearheaded innovative projects in structural biotechnology and medicinal chemistry, including patent development and technology transfer. The dissemination of science and knowledge achieved through programs dedicated to secondary school students and teachers as well as to the general population also deserve special mention.

The National Institute of Structural Biotechnology and Medicinal Chemistry in Infectious Diseases (INBEQMeDI) is another successful example of integrated multidisciplinary initiative focused on the development of new drug candidates using specific molecular targets in microorganisms associated with infectious diseases. The INBEQMeDI was established under the MCT/CNPq/MS/FAPESP program of National Institutes of Science and Technology (INCT). This program has ambitious and far-reaching goals in national terms as a possibility for mobilizing and aggregating, in a coordinated fashion, the best research groups in frontier areas of science and strategic areas for the sustainable development of the country; boosting competitive basic and fundamental scientific research internationally; stimulating the development of advanced scientific and technological research associated with applications aimed to promote innovation and entrepreneurship, in close collaboration with innovative companies, among others (INBEQMeDI, 2010a).

The INBEQMeDI is an offshoot of the successful "CBME/CEPID/FAPESP" initiative and is headquartered at the Institute of Physics of São Carlos, University of São Paulo. It also includes associated groups from the Chemistry Department of the Federal University of São Carlos (DQ-UFSCar), the Institute of Biosciences (IB-USP), the Institute of Biological Sciences (ICB-USP), the School of Medicine of Ribeirão Preto (FMRP-USP), and two groups of young researchers from the State University of Ponta Grossa (UEPG) and the Federal University of Viçosa (UFV). The approaches used are based on modern structural biotechnology and medicinal chemistry. The action strategy of the INBEQMeDI is to conduct both basic and applied research and to promote technological development and the dissemination of education in the

area of NTD. To achieve these goals, the INBEQMeDI has been promoting a multidisciplinary approach, including the use of methods of molecular biology, biochemistry, structural biology (protein crystallography, multidimensional NMR, molecular modeling and bioinformatics), medicinal chemistry based on products of natural and synthetic origin, drug design, molecular immunology, cell biology and pharmacology (INBEQMeDI, 2010b).

As a last example, it is worth noting that our group has been selected by the World Health Organization (WHO), in its Special Program for Research and Training in Tropical Diseases - TDR/WHO-UNICEF/UNDP/World Bank - Business Line on Lead Discovery for Infectious Tropical Diseases - New Medicinal Chemistry Centers to Join Drug Discovery Networks, as a Reference Center in Medicinal Chemistry for Chagas' disease. Our WHO Center aims basically to advance the development of new drug candidates for the treatment of Chagas' disease. Research activities involve effective integration with partner laboratories in the WHO network of medicinal chemistry, including large pharmaceutical companies like Pfizer, Merck, and Chemtura and Pharmacopeia (WHO, 2010).

Molecular targets for drug discovery

Enzymes are extremely important biological targets for the design of novel drugs, because of their essential role in biochemical pathways associated with human diseases and disorders. Other attractions include the fact that they are usually easy to obtain, are suitable for biological trials and versatile in the application of SBDD and LBDD techniques (Mestres, 2005). Enzyme inhibitors of the reversible type are the most studied drug candidates and can be classified as competitive and uncompetitive. There are also the irreversible enzyme inactivators, which act especially as affinity labels or mechanism-based inactivators, leading to covalent modification of the enzyme (Copeland, 2005).

According to data from the U.S. Food and Drug Administration (FDA), approximately 25 percent of registered NCE (317 out of 1278 NCE) had as targets 71 enzymes from five different sources (Figure 3) (Robertson, 2005).

Examples of use of drug design methods

One of the great challenges of medicinal chemistry in the drug design process is to contribute to increase the success rate in the discovery of NCEs. The integration of experimental and computational methods is of great importance in the identification and development of new bioactive molecules from real or virtual compound collections. In this context, SBDD and LBDD strategies gain natural highlight (Andricopulo et al., 2009). SBDD methods are based on knowledge of the topological array of biological targets, and therefore use as a prerequisite detailed 3D data on the macromolecule under study. This information can be obtained through the analysis of crystallographic structures, NMR or homology modeling. Molecular docking is one of the main SBDD strategies, and consists in the prediction of the bioactive conformation of a small molecule

(ligand) in the binding site of a macromolecule (target protein), followed by the evaluation (scoring) and classification of the proposed binding mode. Structure-based virtual screening (SBVS) uses molecular docking methods in the analysis of large databases of compounds, with the aim of characterizing a privileged chemical-biological space and enable the selection of compounds for biochemical and/or biological tests (Guido et al. 2008a; Andricopulo et al. 2009).

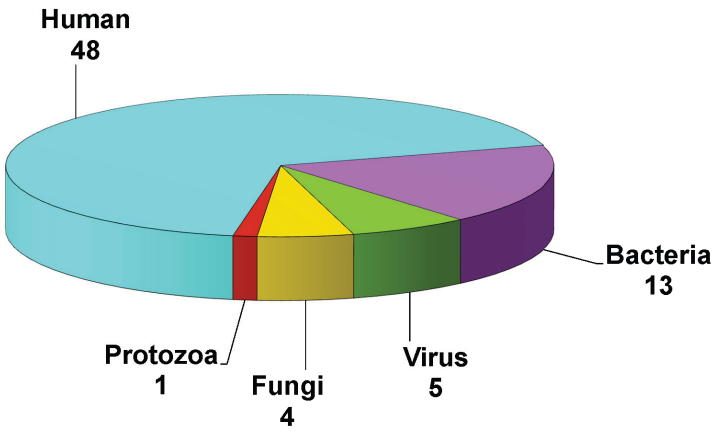

Figure 3 – Enzymes as molecular targets of FDA-approved drugs (Robertson, 2005).

The design of antiparasitic drugs is based primarily on the investigation of biochemical pathways of the parasite and, where appropriate, on the comparison of these with that of the host, with the aim to identify possible targets for selective modulation by small molecules (Verlinde et al., 2001). The use of modern medicinal chemistry strategies can be seen in the example of purine nucleoside phosphorylase of *Schistosoma mansoni* (S*m*PNP), a key enzyme in the purine rescue pathway (Postigo et al. 2010). In this study, the active S*m*PNP site was selected as a target for the development of a 3D pharmacophore model, which was used in the virtual screening of a large database, leading to the identification of three thioxothiazolidine derivatives with in vitro activity against the target enzyme (Figure 4a). Subsequently, the integration between molecular design, organic synthesis and biological evaluation was instrumental in the process that resulted in the identification of a series of twenty thioxothiazolidine derivatives, enabling the establishment of structure-activity relationships (SAR). It is worth emphasizing that the molecular optimization process resulted in the discovery of a lead compound (Figures 4B and 4C) with potency approximately twenty times greater than its precursors.

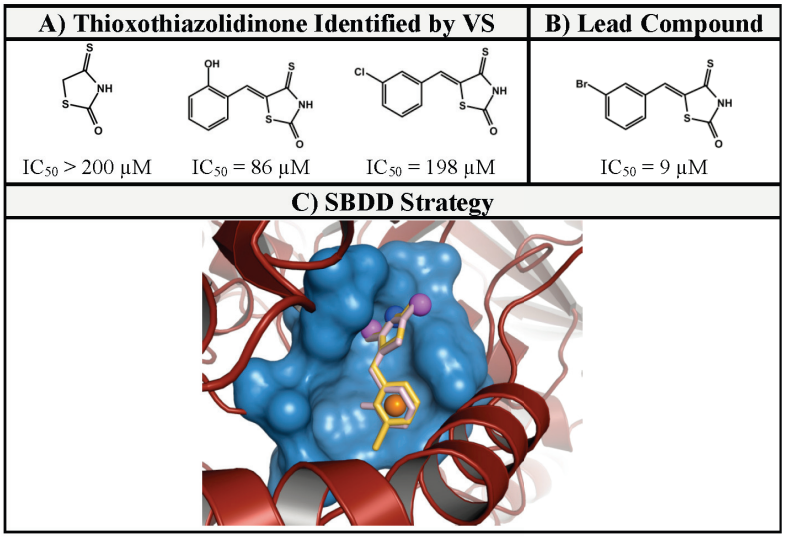

Figure 4 – (A) molecular structure of the compounds selected in the VS. (B) Lead Compound identified. (C) Binding mode of SmPNP inhibitors identified through SBDD strategies. The enzyme is represented by the ribbon model and the active site is indicated by the solvent accessible surface. The pharmacophore model is indicated by spheres that define the areas favorable for hydrogen bond acceptor/donor groups (magenta); hydrogen bond donor groups (blue); and hydrophobic groups (orange). The lead compound (yellow) and one of the thioxothiazolidines (pink) are represented as stick models.

Besides its importance in the identification of new modulators (i.e., receptor ligands or small molecules with biological activity), medicinal chemistry studies are useful in the development of a drug candidate NCE. In this regard, special mention should be made of the QSAR (quantitative structure-activity relationships) methods, whose goals are to relate the information in data sets (chemical structure, properties and biological activity) and create statistical models with external predictive ability. Therefore, they are extremely valuable for designing molecules with improved properties (pharmacodynamic and pharmacokinetic) compared to those of the original data set used in the molecular modeling (Salum & Andricopulo, 2009, 2010; Fashion et al., 2007). However, it should be noted that QSAR studies require careful standardization in crucial aspects such as: (i) the molecules from the data set should be chemically related; (ii) the biological parameter should be quantitative and obtained under the same experimental conditions for all members of the data set; and (iii) all compounds in the series should work in a single molecular target in the same binding cavity and through the same mechanism of action (Farutin et al. 1999); (iv) the statistical method should be appropriate to the level of information available, so that the generated model can provide useful information for the molecular design (Salum & Andricopulo, 2009, 2010). The cyclic process of data generation, the construction of QSAR and property prediction models evolves to the synthesis

and biological evaluation of new molecules, resulting in the discovery of NCE that are candidates for clinical studies.

Another option widely explored when 3D structures of macromolecular targets are available is integrating the QSAR and SBDD methods. For example, once the chemical and structural characteristics associated with the biological property are identified in the QSAR modeling, these can be compared with the information obtained from the 3D topology of the receptor in question. Hence the complementary essence of the methods and their importance as a drug design strategy (Trossini et al., 2009).

The integration of the QSAR and SBDD methods can be observed in studies conducted with a series of glyceraldehyde 3-phosphate dehydrogenase enzyme inhibitors (GAPDH), an attractive molecular target for the development of drugs against trypanosomiasis (Guido et al. , 2008b). The data set used to generate the 3D predictive QSAR models consisted of 70 adenosine derivatives (Figure 5a), which were designed based on significant structural differences between the active sites of *Leishmania mexicana* enzymes and its human homologue. The molecular properties were quantitatively related to inhibitory potency, using methods of comparative molecular field analysis (CoMFA) (Cramer et al., 1988) and comparative molecular similarity indices analysis (CoMSIA) (Klebe et al. 1994). These methods consider that the biological activity of a data set is directly related to intermolecular interactions prevailing in the molecular recognition and binding affinity process, i.e., the stereo-electronic interactions (non-covalent) involved in the formation of the drug-receptor complex type. Because of their 3D character, molecular interaction fields (MIF) are used as descriptors. Another striking feature is the study of bioactive conformations and the 3D structural alignment (orientation in Cartesian space) of molecules from the dataset. For that purpose, this study used molecular docking methods to obtain the molecular alignment of inhibitors in the binding site of enzyme *Lm*GAPDH (Figure 5B). CoMFA and CoMSIA models with high internal (correlation) and external (prediction) consistency were generated, indicating their usefulness in the design of new more potent and selective inhibitors (Figure 5C). An additional feature of the 3D QSAR methods is to enable visualization of regions in the space that can be directly related to biological property (Figure 5D). The detailed analysis of contour maps enabled elucidating the structural bases involved in the molecular recognition process, as well as in the potency and selectivity of the inhibitors investigated, evidencing, therefore, the complementary nature of and synergy between the QSAR and SBDD methods.

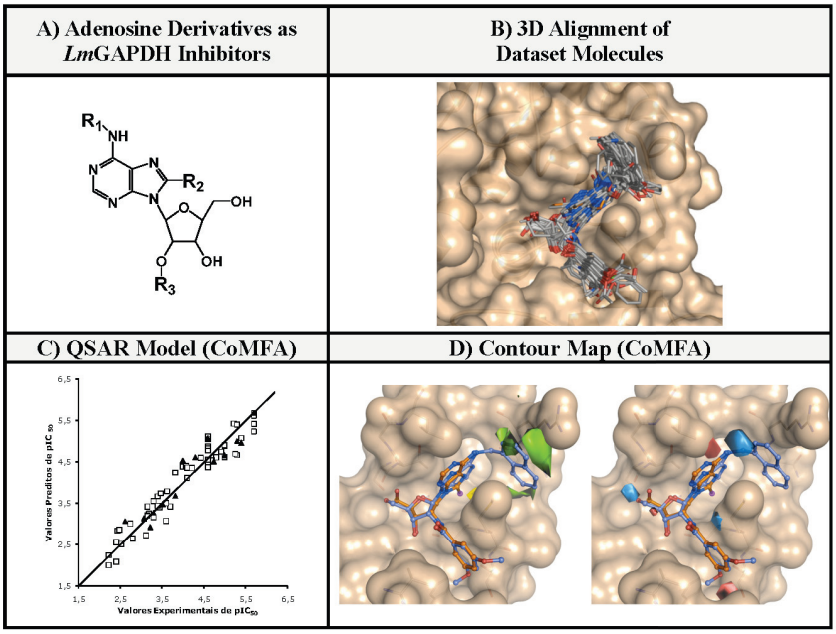

Figure 5 – (A) General structure of the series of adenosine-derived LmGAPDH inhibitors. (B) Structural alignment of the 70 inhibitors used in QSAR modeling. The compounds are represented as rod models in the LmGAPDH active site, indicated by the solvent accessible surface. (C) Generation of CoMFA models with high internal and external consistency. (D) Contour maps of the main stereo-electronic characteristics of the dataset. The most potent (light blue), and less potent (orange) inhibitors are shown in the protein binding cavity. Contour maps (i) stereo: favorable in green, unfavorable in yellow; and (ii) electrostatic: favorable in blue, unfavorable in red.

Another interesting example of the integration of experimental and computational methods, particularly of enzyme kinetics and molecular modeling can be seen in the study of a series of chalcones with activity against *Mycobacterium tuberculosis* tyrosine phosphatase A (M*t*PtpA) (Figure 6A), a biomolecule that plays a central role in the processes of cell signaling and invasion in mycobacteria (Mascarello et al., 2010). In this study, quantitative parameters such as potency (IC^{50}, inhibitor concentration required to reduce enzymatic activity by 50 percent) and affinity (K^i, inhibition constant) were determined experimentally and the mechanism of action was clarified. Briefly, enzyme kinetic studies have indicated a reversible mechanism of the competitive type for chalcones, with K^i values in the low micromolar range (Figure 6B). Based on this information, molecular modeling studies have been conducted to evaluate the mode of interaction of inhibitors against the target enzyme and identify the structural basis responsible for the molecular recognition process. The models indicated the critical role of hydrogen bonding and π-electron interactions between chemical functionalities of the ligands (substituents of the phenyl group and naphthyl aromatic system) and amino acid residues from the active site of MtPtpA (Thr12,

Arg17, His49 and Trp48, Figure 6C). Subsequently, in vitro and in vivo studies demonstrated both the low toxicity of synthetic chalcones and their ability to prevent intracellular survival and proliferation of mycobacteria, suggesting the potential of this class for the development of new drug candidates for the treatment of tuberculosis.

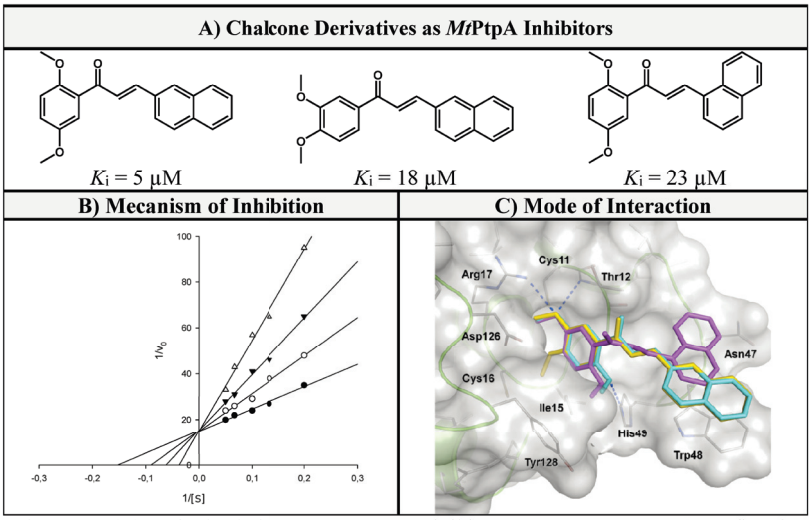

Figure 6 – (a) Series of chalcones with potent inhibition activity against M*t*PtpA. (B) Lineweaver-Burk double-reciprocal plots for the competitive inhibition mechanism. (C) Molecular modeling and studies of the mode of interaction of inhibitors. The active M*t*PtpA site is indicated by the solvent accessible surface, inhibitors as rod models and hydrogen bonds as dashed lines.

Conclusion

Scientific and technological advances in the interfaces between chemistry and biology have provided remarkable opportunities and challenges in drug R&D, with a great emphasis on two fundamental components: innovation and integration, which translate very well the central role of modern medicinal chemistry. Drug R&D has grown at a fast pace in Brazil. This trend should increase sharply through investments in infrastructure, personnel qualification and research, thus encouraging a more balanced regional development and the strengthening of partnerships between university, government and industry. Looking to the future requires a strategic perspective of continuity. Creativity and boldness are essential to understand and anticipate new opportunities. The common commitment is to integrate actions, participate and contribute in the best way possible to the advancement of scientific knowledge and technological expertise. It is essential to combine organized efforts, coordinate strategies for attracting financial, human and material resources, consolidate ideas and find new solutions that will ensure, in the coming decades, Brazil's evolution from an emerging nation to a world power in the area of drugs and medicines.

References

ANDRICOPULO, A. D. et al. Structure-based drug design strategies in medicinal chemistry. *Curr. Top. Med. Chem.*, v.9, n.9, p.771-90, 2009.

BADER, G. D. et al. BIND: the biomolecular interaction network database. *Nucleic Acids Res.*, v.31, n.1, p.248-50, 2003.

BEYRER, C. et al. Neglected diseases, civil conflicts, and the right to health. *Lancet*, v.370, n.9587, p.619-27, 2007.

BHOGAL, N.; BALLS, M. Translation of new technologies: from basic research to drug discovery and development. *Curr. Drug Discov. Technol.*, v.5, n.3, p.250-62, 2008.

BLACKSTOCK, W. P.; WEIR, M. P. Proteomics: quantitative and physical mapping of cellular proteins. *Trends Biotechnol.*, v.17, n.3, p.121-7, 1999.

BOLLARD, M. E. et al. NMR-based metabonomic approaches for evaluating physiological influences on biofluid composition. *NMR Biomed.*, v.18, n.3, p.143-62, 2005.

CBME. Center for Structural Molecular Biotechnology. 2010. Available at: <http://

cbme.if.sc.usp.br/. 2010>. Access on: 8 set. 2010.

COPELAND, R. A. *Reversible modes of inhibitor interactions with enzymes.* New Jersey: Wiley Interscience, 2005. p.48-81, 2005 (Evaluation of Enzyme Inhibitors in Drug Discovery).

CRAMER, R. D. et al. Comparative molecular-field analysis (Comfa). 1. Effect of shape on binding of steroids to carrier proteins. *J. Am. Chem. Soc.*, v.110, n.18, p.5959-67, 1988.

CUSICK, M. E. et al. Interactome: gateway into systems biology. *Hum. Mol. Genet.*, v.14, n.2, p.R171-81, 2005.

DIAS, L. C. et al. Chemotherapy of Chagas' disease: state of the art and perspectives for the development of new drugs. *Quimica Nova*, v.32, n.9, p.2444-57, 2009.

FARUTIN, V. et al. Structure-activity relationships for a class of inhibitors of purine nucleoside phosphorylase. *J. Med. Chem.*, v.42, n.13, p.2422-31, 1999.

GONG, S. et al. PSIbase: a database of protein structural interactome map (PSIMAP). *Bioinformatics*, v.21, n.10, p.2541-3, 2005.

GUIDO, R. V. C.; ANDRICOPULO, A. D. Modelagem molecular de fármacos. *Rev. Proc. Quim.*, v.2, n.4, p.24-26, 2008.

GUIDO, R. V. C.; OLIVA , G. Structure-based drug design for tropical diseases. *Curr. Top. Med. Chem.*, v.9, n.9, p.824-43, 2009.

GUIDO, R. V. C. et al. Virtual screening and its integration with modern drug design technologies. *Curr. Med. Chem.*, v.15, n.1, p.37-46, 2008a.

_____. Structural basis for selective inhibition of trypanosomatid glyceraldehyde-3--phosphate dehydrogenase: molecular docking and 3D QSAR studies. *J. Chem. Inf. Model.*, v.48, n.4, p.918-29, 2008b.

INBEQMeDI. National Institute of Structural Biotechnology and Medicinal Chemistry in Infectious Diseases. S.d. Available at: <http://www.inbeqmedi.ifsc.usp.br/>. Access on: 8 Sept. 2010a.

_____. *INBEQMeDI: Activity Report 2009*. National Institute of Structural Biotechnology and Medicinal Chemistry in Infectious Diseases, São Carlos, 2010b. p.1-64.

KLEBE, G. et al. Molecular similarity indices in a comparative analysis (CoMSIA) of drug molecules to correlate and predict their biological activity. *J. Med. Chem.*, v.37, n.24, p.4130-46, 1994.

LOMBARDINO, J. G.; LOWE, J. A. 3rd. The role of the medicinal chemist in drug discovery – then and now. *Nat. Rev. Drug Discov.*, v.3, n.10, p.853-62, 2004.

MASCARELLO, A. et al. Inhibition of *Mycobacterium tuberculosis* tyrosine phosphatase PtpA by synthetic chalcones: kinetics, molecular modeling, toxicity and effect on growth. *Bioorg. Med. Chem.*, v.18, n.11, p.3783-9, 2010.

MESTRES, J. Representativity of target families in the Protein Data Bank: impact for family-directed structure-based drug discovery. *Drug Discov. Today*, v.10, n.23-4, p.1629-37, 2005.

MODA, T. L. et al. Hologram QSAR model for the prediction of human oral bioavailability. *Bioorg. Med. Chem.*, v.15, n.24, p.7738-45, 2007.

MOUSSES, S. et al. RNAi microarray analysis in cultured mammalian cells. *Genome Res.*, v.13, n.10, p.2341-7, 2003.

NICHOLSON, J. K.; LINDON, J. C. Systems biology: metabonomics. *Nature*, v.455, n.7216, p.1054-6, 2008.

NWAKA, S.; HUDSON, A. Innovative lead discovery strategies for tropical diseases. *Nat. Rev. Drug Discov.*, v.5, n.11, p.941-55, 2006.

NWAKA, S. et al. Advancing drug innovation for neglected diseases-criteria for lead progression. *PLoS Negl. Trop. Dis.*, v.3, n.8, e440, p.1-13, 2008.

POSTIGO, M. P. et al. Discovery of new inhibitors of *Schistosoma mansoni* PNP by pharmacophore-based virtual screening. *J. Chem. Inf. Model.*, DOI: 10.1021/ci100128k, 2010.

ROBERTSON, J. G. Mechanistic basis of enzyme-targeted drugs. *Biochemistry*, v.44, n.15, p.5561-71, 2005.

SALUM, L. B.; ANDRICOPULO, A. D. Fragment-based QSAR: perspectives in drug design. *Mol. Divers.*, v.13, n.3, p.277-85, 2009.

_____. Fragment-based QSAR strategies in drug design. *Expert Opin. Drug Discov.*, v.5, p.405-12, 2010.

TROSSINI, G. H. et al. Quantitative structure activity relationships for a series of inhibitors of cruzain from *Trypanosoma cruzi*: molecular modeling, CoMFA and CoMSIA studies. *J. Mol. Graph. Model.*, v.28, p.3-11, 2009.

VA LET, G. Cytomics as a new potential for drug discovery. *Drug Discov. Today*, v.11, n.17-18, p.785-91, 2006.

VERLINDE, C. L. et al. Glycolysis as a target for the design of new anti-trypanosome drugs. *Drug Resist. Updat.*, v.4, n.1, p.50-65, 2001.

WERMUTH, C. G. *The practice of medicinal chemistry*. London: Academic Press. 2003

WHO. *The global burden of disease:* 2004 update. Geneva: World Health Organization, 2008. p.1-146.

_____. Available at: <http://apps.who.int/tdr/svc/research/lead-discovery-drugs/partnerships-network>. Access on: 8 Sept. 2010.

WISHART, D. S. et al. HMDB: the human metabolome database. *Nucleic Acids Res.*, v.35, n.Database Issue, p.D521-6, 2007.

XENARIOS, I. et al. DIP: the database of interacting proteins. *Nucleic Acids Res.*, v.28, n.1, p.289-91, 2000.

ZANZONI, A. et al. MINT: a Molecular INTeraction database. *FEBS Lett.*, v.513, n.1, p.135-40, 2002.

Abstract – Current drug design strategies are based on the understanding of the physiopathology of diseases, biochemical pathways and selection of molecular targets. Modern biotechnological tools have provided valuable information to facilitate the discovery of new drug candidates. Medicinal chemistry has a vital role in a variety of processes aimed at the identification of bioactive substances and the development of lead-compounds with optimized pharmacodynamic and pharmacokinetic properties. The present paper presents some fundamental aspects of biotechnology and medicinal chemistry as useful tools in the design of new chemical entities for the therapy of infectious diseases.

Keywords: Drug design, Medicinal chemistry, Infectious diseases, Molecular modeling, Enzymes, Inhibitors.

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