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Mini-Reviews in Medicinal Chemistry, 2015, *15*, 000-000

High-throughput Drug Repositioning for the Discovery of New Treatments for Chagas Disease

Carolina L. Bellera[1], Maria L. Sbaraglini[1], Darío E. Balcazar[2], Laura Fraccaroli[2], M. Cristina Vanrell[3], A. Florencia Casassa[3], Carlos A. Labriola[4], Patricia S. Romano[3], Carolina Carrillo[2] and Alan Talevi[1,*]

[1]*Medicinal Chemistry, Department of Biological Science, Exact Science College, National University of La Plata (UNLP), Argentina. 47 & 115 (B1900AJI) La Plata, Buenos Aires, Argentina;* [2]*Instituto de Ciencia y Tecnología Dr. César Milstein (ICT Milstein) – Argentinean National Council of Scientific and Technical Research (CONICET);* [3]*Instituto de Histología y Embriología (IHEM); Facultad de Ciencias Médicas, Universidad Nacional de Cuyo; CONICET, Mendoza, Argentina;* [4]*Instituto de Investigaciones Bioquímicas de Buenos Aires - Argentinean National Council of Scientific and Technical Research (CONICET)*

Abstract: Despite affecting around 8 million people worldwide and representing an economic burden above $7 billion/ year, currently approved medications to treat Chagas disease are still limited to two drugs, nifurtimox and benznidazole, which were developed more than 40 years ago and present important efficacy and safety limitations. Drug repositioning (*i.e.* finding second or further therapeutic indications for known drugs) has raised considerable interest within the international drug development community. There are many explanations to the current interest on drug repositioning including the possibility to partially circumvent clinical trials and the consequent saving in time and resources. It has been suggested as a particular attractive approach for the development of novel therapeutics for neglected diseases, which are usually driven by public or non-profit organizations. Here we review current computer-guided approaches to drug repositioning and reports on drug repositioning stories oriented to Chagas disease, with a focus on computer-guided drug repositioning campaigns.

Keywords: Chagas disease, computer-guided drug repositioning, drug repositioning, drug repurposing, neglected diseases, indication expansion, high-throughput drug repurposing.

INTRODUCTION

Chagas disease or American trypanosomiasis is a tropical parasitic diseases caused by the hemoflagelate protozoan *Trypanosoma cruzi*. The more frequent mode of transmission is through an insect vector from the *Triatominae* subfamily commonly known as "kissing bug". Other transmission ways include blood transfusion and organ transplant, congenital transmission, ingest of contaminated foods or drinks and laboratory accidents [1]. Chagas disease presents itself in two phases: an initial, acute phase which majorly presents mild unspecific symptoms (fever, headache, muscle pain and others) or no symptoms at all, and a chronic phase, which is asymptomatic in around 70% of the patients but includes life-threatening heart and digestive disorders in the remaining 30% of the infected people.

Chagas disease belongs to a group of tropical diseases collectively known as "neglected" or "forgotten" diseases, denoting the past reluctance of the private pharmaceutical sector to invest in new cures, due to the perceived limited investment return, and also the failure of public policies to address those public health issues. Note that although neglected diseases account for more than 11% of global disease burden [2], only 21 (1.3%) of 1556 new medications launched on the market between 1975 and 2004 correspond to new treatments for neglected diseases [3]. Moreover, about 90% of the resources invested in neglected disease come from the public sector and non-profit organizations [4]. Chagas disease affects 7-8 million people worldwide and represents more than 7 billion/year economic burden [1, 5]; despite being endemic in Latin America, migratory currents have spread the disease all through the five inhabited continents [6]. However, only two approved medications (nifurtimox and benznidazole) are currently available. These drugs were developed more than 40 years ago and present serious limitations including low efficacy in the chronic stage in adults, the existence of resistant strains of the parasite and serious adverse effects which compromise treatment adherence [7], being thus far from the ideal antichagasic treatment defined by World Health Organization (WHO) [7c] and justifying the ongoing efforts towards novel medications. It should be mentioned, however, that apparently

*Address correspondence to this author at the Medicinal Chemistry, Department of Biological Science, Exact Science College, National University of La Plata (UNLP), Argentina, 47 & 115 (B1900AJI) La Plata, Buenos Aires, Argentina; Tel: 542214235333 Ext. 41;
E-mail: atalevi@biol.unlp.edu.ar

1389-5575/15 $58.00+.00

the preliminary results of the long-term follow-up clinical trials of benznidazole in adults TRAENA show that this drug is highly beneficial in chronic Chagas disease [8].

Drug repositioning has recently been suggested as a particular appropriate strategy to develop innovative medicines for neglected or orphan diseases [9]. Drug repositioning (also called reprofiling or indication switching or indication expansion) consists in finding new therapeutic applications for already existing drugs including marketed, discontinued, shelved and even investigational therapeutic agents. It has raised much interest within the international drug development community, including public programs recently launched by health authorities from the United States and the United Kingdom [10]. Diverse explanations to the current intereston drug repositioning may be mentioned. Repositioned drugs represent unique translational opportunities, including higher probability of success to market than de novo drugs and shorter development time [11]. Repositioned candidates have at least survived preclinical toxicological testing (and even safety assessment at clinical trials for those cases that have previously gained market approval). They have well-characterized pharmacokinetic profiles and manufacturing issues have already been previously solved too; when off-patent drugs are repositioned they may also provide inexpensive therapeutic solutions [12]. Though many repositioning cases have emerged from intelligent exploitation of unforeseen side-effects (*e.g*. sildenagil, minoxidil) growing attention is currently being paid to rational [9a, 13] and high-throughput approximations [14].

Here, we will overview different computer-assisted approaches to drug repositioning, including bioinformatic, cheminformatic and network-based methods and high-throughput literature analysis. We will review advances on repositioned drugs for the treatment of Chagas and, finally, we will discuss recent computer-guided repositioning campaigns oriented to the discovery of novel antichagasic medications.

COMPUTER-ASSISTED APPROACHES TO DRUG REPOSITIONING

Two general principles provide the basis for computer-assisted drug repositioning [15]: a) different health disorders associated to the same or similar molecular targets may be treated with the same drugs (disease-centric approach) and b) drugs sharing pharmacologically relevant molecular features may interact with the same molecular targets (drug-centric approach). Bioinformatic-based drug repositioning relies on the first principle, while cheminformatic-based repositioning relies on the second. High-throughput literature analysis constitutes a distinctive approach that will be discussed separately. All the three previously mentioned approximations are or might be combined comprehensively through network-based drug repositioning.

Bioinformatics encompasses a wide range of computational tools conceived to find structural and/or functional connections between gene and gene products and, more recently, genome and protein-wide expression patterns. Establishing inter- and intraspecies gene homology provides an interesting framework to identify potential repositioning opportunities. Paralogs (genes related *via* duplication which usually perform distinct but mechanistically related functions [16]) are immediate molecular targets of repositioned candidates; though orthologs (genes derived from a single ancestral gene typically performing equivalent functions in different species) may also represent excellent repositioning opportunities within certain therapeutic categories, *e.g*. anti-infective drugs. Genome-wide gene expression profiling provides a snapshot of globally measured transcript levels in a given cell, tissue or organism under a certain experimental condition [17]. The Connectivity Map (CMap) is a publicly available resource meant to connect disease and small molecules through gene-expression signatures [18]. It was the first database to compile gene expression signatures obtained by exposing human cells to a large number of bioactive compounds. Permanently expanding, at present it compiles more than 7,000 signatures obtained from more than 1,300 drugs [19]. Query expression signatures are compared to the stored ones through pattern matching algorithms: those at the top and bottom of the resulting similarity rank are related to the query state by common and opposite expression changes (direct and inverse similarity) respectively. Those drugs whose signatures show an inverse similarity to a query disease signature are, hypothetically, a potential treatment to that disease. Different algorithms have recently been proposed to optimize CMap exploration. For instance, Chung *et al.* constructed condition specific function-function networks to identify complexly connected highly expressed genes link to a given disease around which they build functional modules that they later used to query CMap [20]. When checked against known drug indications in the Therapeutic Target Database, the method showed higher accuracy and lower false positives rate than the standard methodology. A similar approach was proposed by Cha *et al*., who based their analysis in differentially co-expressed gene modules (gene correlated in one of two conditions: control or drug treatment) [21]. Parkinnen and Kaski proposed the application of Group Factor Analysis for connectivity mapping, in order to decompose transcriptional response data into factors specific to individual cell lines and factor shared by two or more cells lines [22]. The method would be useful either to reduce noise specific to individual cell lines, or –on the contrary- to consider specific cell line effects when relevant (*e.g*. when searching for a treatment targeting a specific cell line). A different but very interesting approximation in the field of bioinformatic-based drug repositioning can be found in the recent work of Haupt *et al.* [23] These authors proved correlation between ligand promiscuity (a valuable property for drug reprofiling purposes: a certain degree of polyspecificity is expected to be present in a given molecular target to interact with known drugs from different therapeutic categories) and global structure similarity and binding site similarity. Their findings suggest that one may use the binding site similarity and the global structure similarity as criteria to guide drug repositioning initiatives.

Cheminformatic-based drug repositioning can be regarded as a particular type of virtual screening in which the screened chemical database or repository includes approved, abandoned, discontinued and/or investigational drugs only. The methods

used in cheminformatic-based drug repositioning are thus classified in the same way that for general virtual screening approaches [24]: ligand-focused approximations (which roughly include similarity-based, Quantitative Structure Activity Relatioinships (QSAR) and pharmacophore-based techniques) and target-focused approaches (prominently, molecular docking). Muli-task (or multi-target) QSAR is one particular application of QSAR theory which should be mentioned here in the light of the interesting possibilities that it poses for drug repurposing. This approach allows the development of individual models capable of simultaneously prediciting different activities/properties, *i.e*. the behavior of a given molecule against more than one biological entity, *e.g*. proteins, microorganisms or cell lines. It constitutes a complete innovation and evolution in the field of QSAR, which has been traditionally limited to the one model per target paradigma. Resourcefully, a significant number of the pioneering applications of this new perspective solved this limitation by including in the QSAR equations, not only molecular descriptors but also class-related descriptors reflecting, for instance, the average value of a molecular descriptor for a particular class of objects (compounds) or the deviations of the descriptor value for certain objects from their class average [25].

Recently, serial, parallel and hybrid combinations of the aforementioned approximations have been widely applied [26], since simpler 2D techniques seem to be generally superior in terms of speed and active enrichment, but more complex and computationally demanding approximations take the lead regarding scaffold hopping [27]. Remarkably, drug repositioning focuses on an extremely small subset of the vast and expanding universe of drug-like molecules, thus the use of virtual screening for repositioning purposes is particularly efficient, a point that should be taken into consideration when target-focused approximations are included in the screening protocol.

Development of freely available resources compiling approved, discontinued, abandoned and/or investigational drugs such as DrugBank [28], Sweetlead [29] and the NCGC Pharmaceutical Collection [30] has facilitated the way for the execution of cheminformatic-based drug repositioning campaigns. Interestingly, it has recently been demonstrated that two therapeutic indications would be correlated if they share the same or molecularly similar drugs [31]; thus, a network indicating which therapeutic categories have similar drugs arises as a valuable network to guide systematic drug repositioning. The seminal idea to this approach can be found in the previous work from Keiser and collaborators [32].

High throughput literature mining constitutes a third, distinctive computer-guided approach to drug repositioning [33]. Co-occurrence methods resembling Swanson's ABC model (which proposes that two island of knowledge A and B might be bridged indirectly through a shared concept or fact C and, in fact, the more the shared concepts between A and B, the higher the probability of a direct connection between them) but applied in an open discovery framework, are the simplest approaches to find unrevealed connections between biomedical elements of interest (for drug repositioning purposes, an unforeseen link between a drug and a disease is usually sought). Recent advances in the field of literature mining suggest, however, that co-occurrence methods may be outperformed by Natural Language Processing and other methods that take into account semantic analysis and might disclose not only an association but the nature, the sense, of such association (*e.g*. a given drug candidate inhibits a given disease progression).

Network based approximations consist in large-scale data integration models that deal with complexity by simplifying complex systems: different types of concepts and entities are represented as nodes, while links between nodes are depicted as edges [34]. In such representation –naturally connected to mathematical Graph Theory-functional and dynamic features of the elements represented as nodes are often (but not always) lost and emphasis is given to the topological architecture of the net, *i.e*. its connectivity. Connections between nodes are established through known/experimental relationships or through predicted associations such as chemical similarity between drugs, protein sequence similarity, similar expression-profiles, literature-inferred connections, etc. In other words, all the approaches described in the previous paragraphs are holistically combined and new connections are uncovered by studying the topology and sometimes the semantics of the network. Many good reasons justify the use of large-scale data integration approaches. At present, scientific information is produced at an unprecedented rate, leading to a "data explosion". Manually exploring available literature and databases is no longer feasible, and computational approaches are needed to digest and bridge such vast amount of information [35]. Second, elucidating a drug's mechanisms of action is still time and labor expensive and much experimental binding data is sometimes missing, but available experimental data on drug-protein interactions may be sufficient to fill experimental gaps by applying computational tools. Third, integrating multi-dimensional information (*e.g*. chemical, pharmacological and genomic spaces) may help to compensate for intrinsic limitations of single kinds of information. Current trend in network-based drug repositioning points toward the integration of very heterogeneous types of data (drugs, proteins, side effects, diseases, pathways, tissues, gene ontology terms, and others) and the introduction of semantic edges [36].

A scheme summarizing high-throughput approximations to drug repositioning is presented in (Fig. **1**).

NON-COMPUTER AIDED DRUG REPOSITIONING FOR THE TREATMENT OF CHAGAS

To the moment, the most promising candidates repositioned for the treatment of Chagas disease which have not been found through computer-aided approaches include several azole-based antifungals, antiarrhytmic compounds amiodarone and dronedarone, and the biphosphonate risendronate. Before addressing studies based on computer-aided drug repositioning in the next section, we will briefly review the data on these agents.

Azole derivatives are the most important antifungal drugs. Their mechanism of action involves ergosterol biosynthesis *via* inhibition of 14α-demethylase (CYP51). As a result of the coordination bond of the electronegative nitrogen of the azole ring plus multiple van der Waals

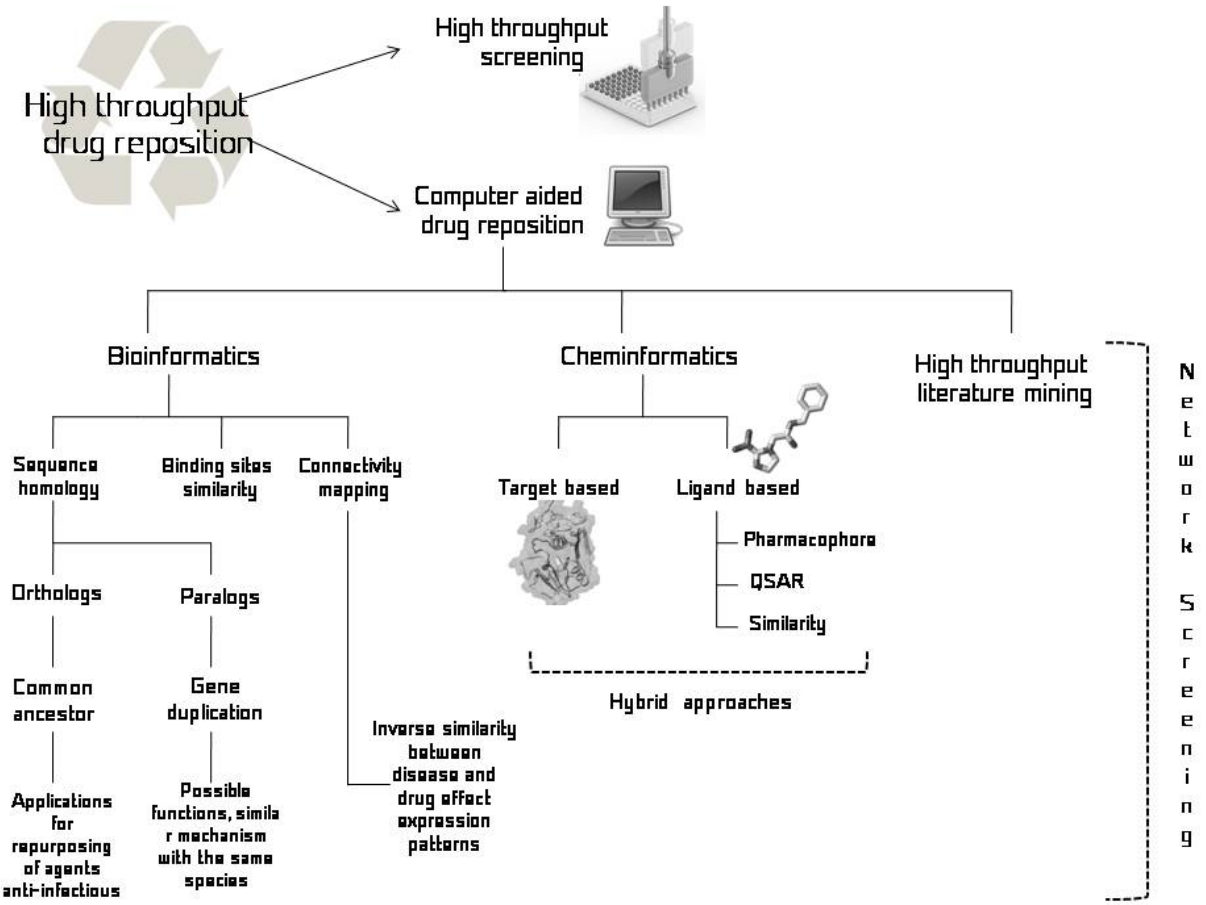

Fig. (1). Scheme showing different high-throughput drug repositioning strategies.

contacts with the hydrophobic binding cavity of the enzyme, irreversible inhibition of the bio-catalysis is often observed [37]. Similar to fungi, *T. cruzi* depends on endogenous ergosterol and its derivatives [38] which are vital for normal functioning of the parasite membranes, cell division, growth and development [37]. Furthermore, inhibition of CYP51 results in accumulation of abnormal amounts of cytotoxic sterol precursors [38]. Several antifungal drugs have shown activity on *T. cruzi* and acute and chronic Chagas models in mice [8]. Posaconazole has demonstrated efficacy on several nitroderivative-resistant strains [39]. In 2012, it entered clinical trials in Argentina, Bolivia and Spain, though these results showed that it is clearly inferior to the standard therapy, possibly due to low systemic exposure to the drug [8]. Merck has already started a second study (http://clinicaltrials.gov/show/NCT01377480, last access September 2014). Nevertheless, it has been underlined that even if these trials were successful, posaconazole is highly expensive due to the low yielding and costly synthetic scheme, limiting its widespread use in endemic countries [40]. Ravuconazole is a second antifungal azole which shows potent *in vitro* activity against *T. cruzi*. In spite of its unfavorable pharmacokinetics in animals (very short half-life) its elimination half-life and volume of distribution in humans prompted a proof-of-concept clinical trial of a ravuconazole prodrug (E1224) (Fig. **2**) [41]. E1224 was effective at clearing the parasite at the end of the treatment course, but failed to develop sustained efficacy one year after the treatment compared to benznidazole and presented some safety issues at high doses (http://www.dndi.org/diseases-projects/portfolio/azoles-e1224.html, last visited September 2014). Additional trials of E1224 as a combination therapy with benznidazole have recently been announced [8]. Fexinidazole is another azole derivative (a nitroimidazol) currently going through Phase II/III clinical trials for African trypanosomiasis [8]. The drug was evaluated against *T. cruzi* over 30 years ago and was later rescued by the DNDi Project as a drug candidate for the treatment of African trypanosomiasis. After completing first-in-human trials [42] it entered Phase II/III studies in late 2012. Also in 2012, a study showed comparative effects between benznidazole and 3X higher doses of fexinidazole on benznidazole-susceptible CL *T. cruzi* strain, and on partially resistant Y strain, while a superior effect of fexinidazole was observed on benznidazole-resistant VL-10 and Colombian strains in mice models of acute and chronic infection [43]. In the same study, fexinidazole was also shown to reduce myocarditis in animals infected with VL-10 and Colombian strain. These findings, coupled with a favorable safety profile, set the basis for the beginning of a Phase II proof-of-concept trial as antichagasic medication in Bolivia, by the end of 2013. This constitutes a good example of drug repositioning of abandoned and investigational drugs.

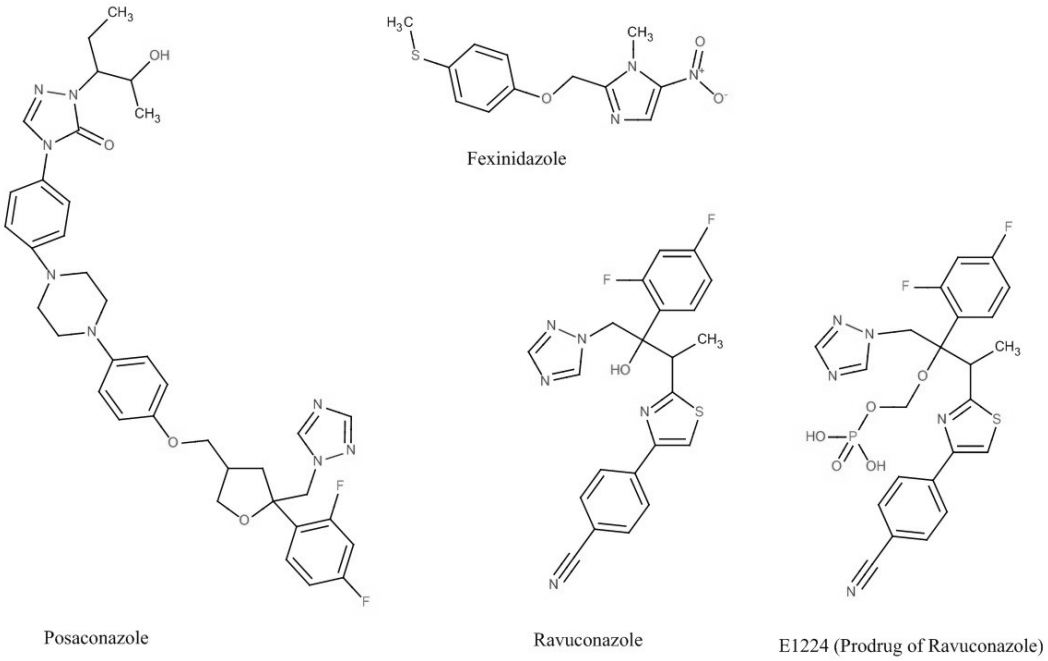

Fig. (2). Molecular structures of repositioned azoles.

Benzofurane-derivative antiarrhytmic agents amiodarone and dronedarone (Fig. **3**) constitute other drugs which might be potentially repositioned to treat Chagas. Back in 1986, a comparative study of the antiarrhythmic effects of verapamil, 17-monochloracetylajmaline, mexiletine and amiodarone that were compared in 14 patients with chagasic myocarditis showed a clear superiority of amiodarone; in fact, the much greater efficacy led the authors to suggest the existence of other peculiar and unknown electrophysiologic and pharmacologic properties of amiodarone [44]. Twenty years later, it was reported that amiodarone has intrinsic anti-*T. cruzi* effect and synergistic effect when administered in addition to posaconazole, both *in vitro* and *in vivo* [45]. It was later shown that amiodarone alters Ca^{2+} homeostasis in *T. cruzi* by inducing its release from intracellular storages (mitochondrion and acidocalcisomes), leading to citotoxicity [46]. Amiodarone was also found to disrupt sterol biosynthesis in *T. cruzi* [47]. An anecdotal case showing success of combined administration of amiodarone and itraconazole in a 62-year old patient with Chagas diagnosed with severe chagasic cardiomyopathy was reported in 2009 [48]. Remarkably, circulating antibodies against *T. cruzi* dropped below the detection limit and an improvement of cardiac function was observed. The later has been linked to amiodarone-induced reassembly of cytoskeleton elements [49]. The previous observation is a very good example of the additional value of a repositioned candidate that, owing to its multifunctional nature and probably linked to its original therapeutic indication, attacks both the cause and the symptoms of the targeted disorder. Recently, a virtual screening campaign served to identify that amiodarone presents weak but dose dependent inhibition of the cystein protease cruzipain [50]. All this said, it has been stated that amiodarone side-effects discourage its use in a wide spectrum of clinical scenarios [47], which led to the exploration of its noniodinated, more hydrophilic derivative dronedarone as another potential antichagasic agent. It was demonstrated that it induces the release of intracellular calcium similarly to amiodarone [51]; however, their application in combination with antifungal azoles is limited due to its extensive CYP3A4 metabolism, while its use in patients with severe heart failure remains controversial [47]. To our knowledge, no clinical trials are planned for these drugs.

N-alkyl biphosphonates used in the treatment of bone disorders such as osteoporosis have displayed selective *in vitro* and *in vivo* effect on *T. cruzi* [52], presumably due to the inhibition of farnesyl diphosphate synthase, which is involved in the sterol and polyisoprenoid biosynthesis of *T. cruzi*. Risedronate (Fig. **4**) is to the moment the biphosphonate with most potent effect on *T. cruzi* found. At doses of 1 mg/kg per day it induces reductions in parasitaemia above 90% in mice acute models of infection and no relapse after discontinuation of the treatment [52b]. More recently, it has been shown that complexation of risedronate with copper, cobalt, manganese and nickel potentiates its antiproliferative effect against *T. cruzi* amastigotes [53].

In a very recent report, Planer et al describe the test of 2000 biologically active compounds from the Spectrum collection (including around 700 FDA approved drugs) on mammalian stage *T. cruzi* [54]. Most of the compounds showing activity (except the antifungal azoles) were effective in the low micromolar or high nanomolar range (*e.g.* antidepressant fluoxetine and anthistamine clemastine) and they were inefficient to lower parasitemia in a murine model of infection. Thus, the authors decided to test combinations of active compounds in search of synergistic and additive effects. Isobologram studies revealed 8 synergistic drug pairs, among which the combinations of posaconazole plus clemastine and calcium blocker amlodipine provided the best

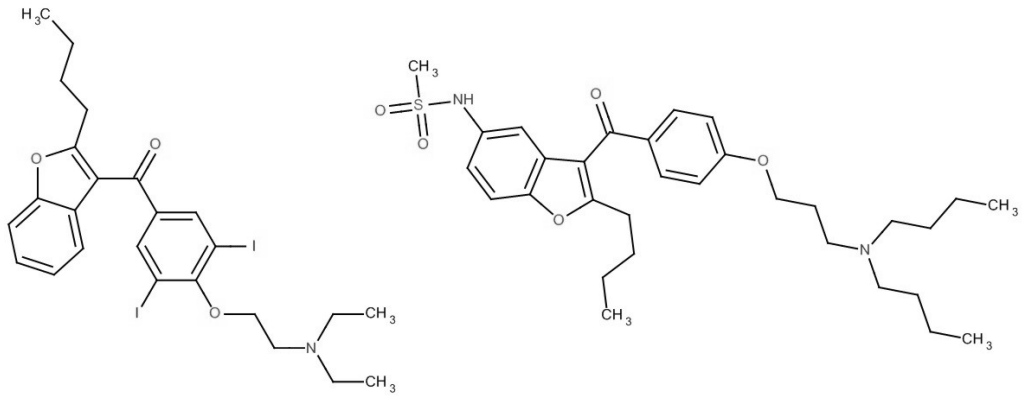

Fig. (3). Molecular structures of repositioned benzofurane antiarrhytmics.

results in mice. The authors discussed that the interaction between posaconazole and amlodipine might be of pharmacokinetic nature since both drugs are metabolized through CYP3A4; nevertheless, we believe it is interesting to note that this combination includes drugs with molecular mechanisms already described in previously repositioning stories (posaconazole), or related to previously commented mechanisms (*e.g*. both amiodarone and amlodipine have calcium channel blocking properties). Seemingly, some therapeutic categories are consistently good candidates to be repositioned as antichagasic medications.

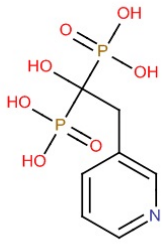

Fig. (4). Molecular structure of Risedronate.

COMPUTER AIDED DRUG REPOSITIONING FOR THE TREATMENT OF CHAGAS

Bellera *et al.* have recently developed and implemented a three independent virtual screening campaigns on the DrugBank repository and Merck Index 12th to find repositioned drugs acting through reversible inhibition of cruzipain. The authors generated three conformation-independent computational models (discriminant functions) based on Dragon 4 (Milano Chemometrics), Dragon 6 (Talete SRL) and DESMOL 11 (Molecular Connectivity and Drug Design Research Unit, University of Valencia) [50, 55]. Fig. (**5**) shows the effects of the repositioned candidates emerging from these virtual screening campaigns on purified cruzipain, when tested at 100 μM. Based on the results, the antihypertensive benidipine, the antiparkinsonian bromocriptine, the antibiotic clofazimine, the synthetic thyroid hormone levothyroxine and the antiviral saquinavir were tested on epimastigote cultures of the *T. cruzi* Y strain. Fig. (**6**) shows the molecular structures of these drugs. All the candidates showed some degree of antiproliferative effect; remarkably, the effective concentrations on *T. cruzi* cultures were lower than the effective concentrations on purified cruzipain, suggesting that other action mechanisms might be also present. The higher activity on epimastigotes was observed for the calcium blocker benidipine (Fig. **7**). Later, we studied the effects of benidipine, clofazimine and saquinavir on *T. cruzi* infectivity on cells monolayers (Fig. **8**), showing that the three candidates reduced cell invasion by the parasite. Three criteria were then used to decide which candidates would progress to an acute mice model of infection. Briefly, we analyzed: a) whether the effective concentrations on *T. cruzi* were similar to those steady state plasma concentrations attained during a multi-dosage regime for the previously approved therapeutic indication of the repositioned candidate (preferably, equal or lower doses to those used for the already approved indication should be employed); b) whether the previous indications might pose any additional benefit to the patient with Chagas (*e.g*. a antichagasic agent with cardioprotective effects might help to control not only the infection but also the manifestations of the chronic infection) and; c) the severity of the known adverse effects associated to the doses used for the original indication. The repositioned drugs should present less frequent and/or less intense side-effects compared to standard therapies. Candidates with side-effects incompatible with Chagas symptoms (*e.g*. drugs contraindicated in cardiac patients, *e.g*. levothyroxine) should be excluded as potential Chagas treatments.

Based on the previous explanation, benidipine and clofazimine were tested in an acute mice model of Chagas. Benidipine is a calcium channel blocking agent currently used for the treatment of hypertension and angina pectoris [56]. Noteworthy, there are plenty evidence of its cardioprotective properties [57], which may represent and added benefit to the patients with chronic Chagas. Its more

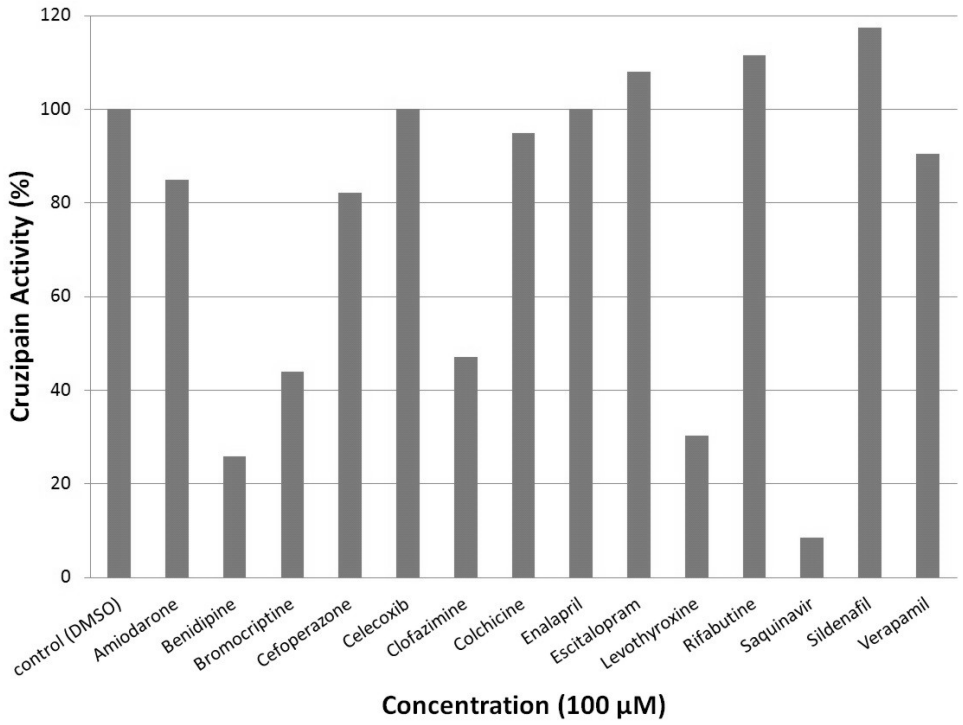

Fig. (5). Inhibitory effect of repositioned candidates emerging from the virtual screening campaigns on purified cruzipain. The final concentration of each compound was 100 µM. Protease activity is expressed as percentage of the control condition (2% DMSO).

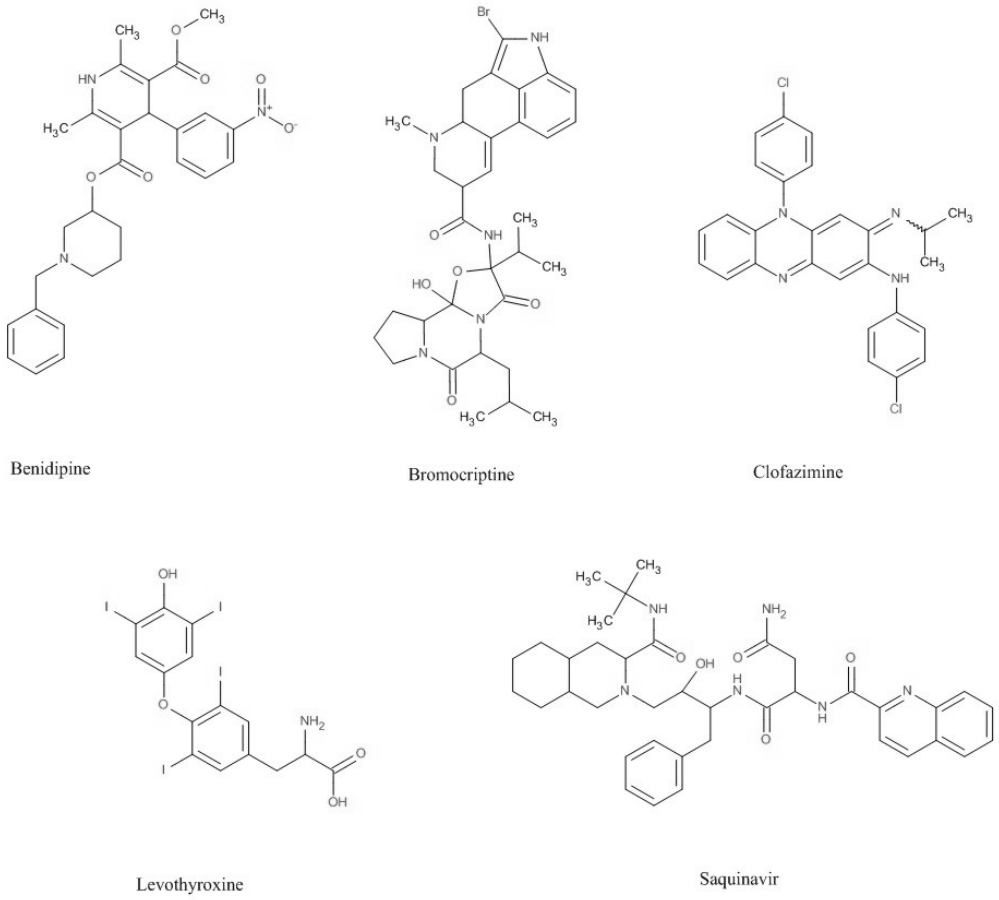

Fig. (6). Molecular structure of active candidates selected through virtual screening.

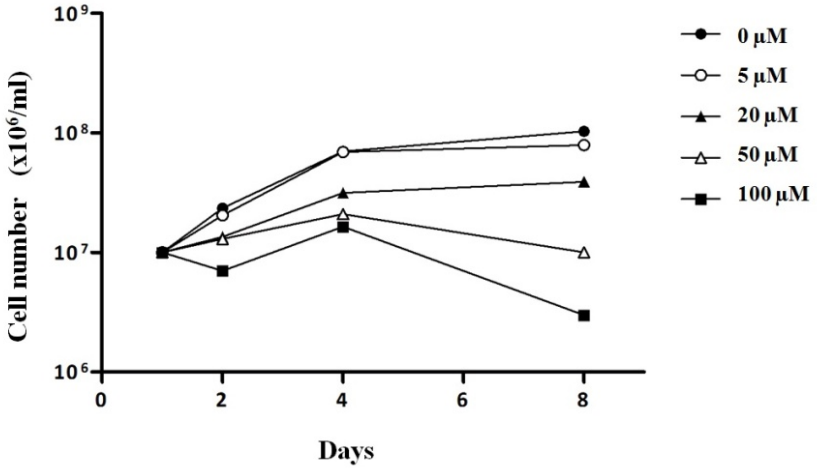

Fig. (7). Effect of benidipine on proliferation of *T. cruzi* epimastigotes.

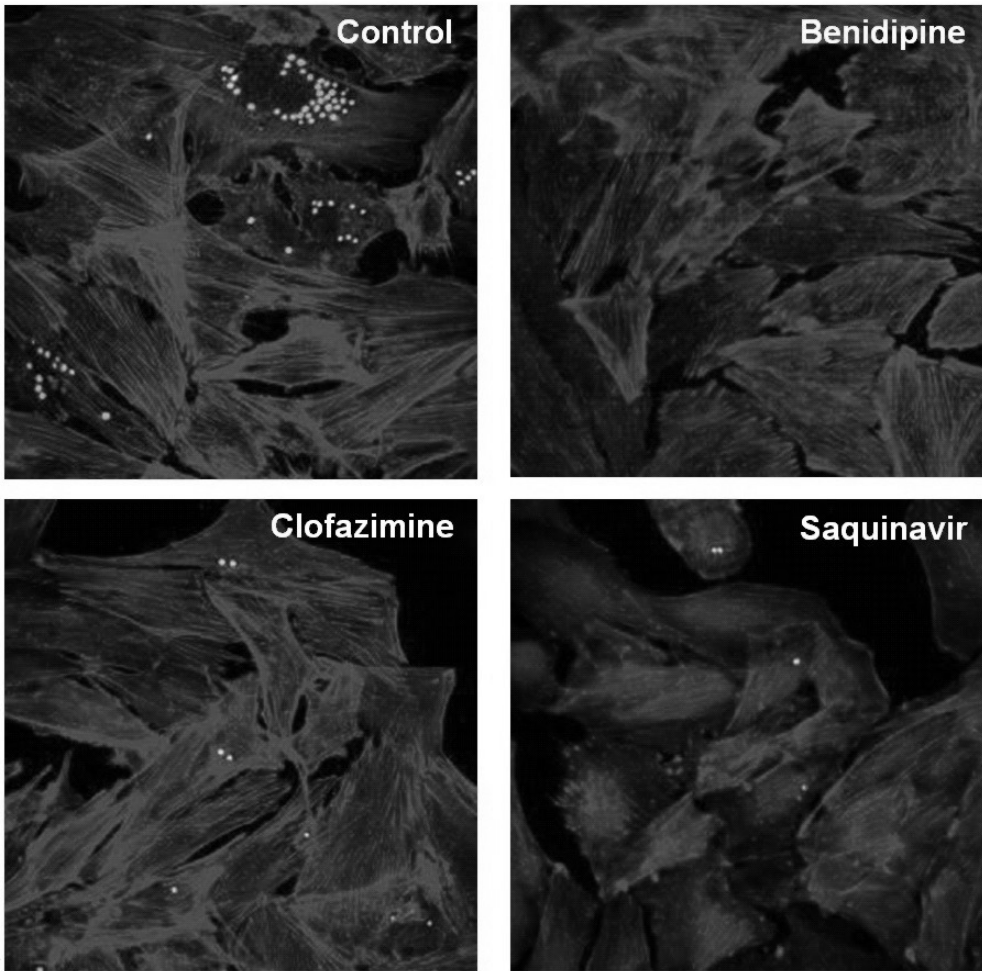

Fig. (8). Effects of clofazimine, benidipine and saquinavir on cell invasión by *T. cruzi*.

frequent adverse reactions are mild and include palpitations, hot flushes and headache. Besides, other clinically used dihydropiridines have previously shown antitrypanosomal effects [58]. Regarding clofazimine, it is indicated in the treatment of leprosy [59] at doses of 200-300 mg per day under medical supervision and for no more than 3 months. Interestingly, current medications for Chagas disease are also administered in two/three-month length treatments. Daily p.o administration of 100, 300, 400 and 600 mg of clofazimine to leprosy patients results in average plasma levels of 1.5, 2.1, 3.0 and 8.5 µmol, respectively [59]; which are similar to the effective concentrations observed for clofazimine on *T.*

cruzi. Its side effects are generally dose-related, and primarily affect the skin, eyes and gastrointestinal tract. They are tolerable and gradually reversible on cessation of therapy. Fig. (**9**) shows the effect over parasitaemia of benidipine 10 mg/kg/day and clofazimine 20 mg/kg/day in comparison to benznidazole 100 mg/kg/day. Note that the candidates were used at 5 to 10 times lower doses tan benznidazole. Moreover, both repositioned candidates reduced the number of *T. cruzi* nests quantified by conventional microscopy of cardiac tissues from each treated animal (data not shown).

Further studies in acute models are needed in order to define whether at higher doses the repositioned candidates can obtain similar results than benznidazole, which would justify progressing to chronic models of infection.

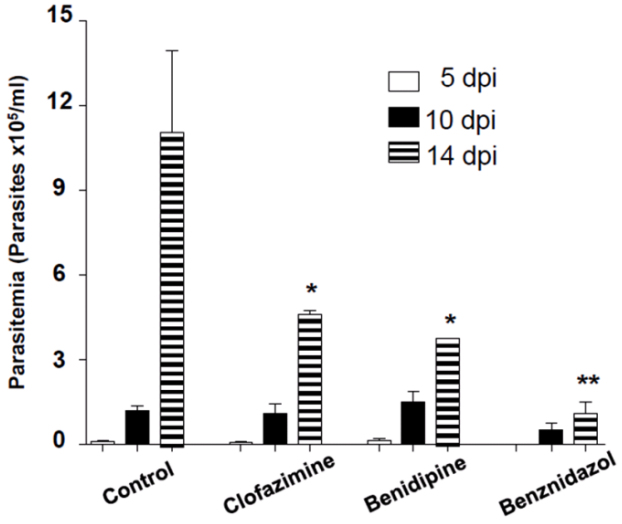

Fig. (9). Effects of clofazimine and benidipine on experimental *T. cruzi* acute infection in mice.

CONCLUSIONS AND AUTHORS' PERSONAL OPINION

The article reflects the potential of drug repositioning in the search of novel medications for Chagas disease. Remarkably, though abundant reports on high-throughput drug repositioning can be found in literature, very few are focused on antichagasic medications, which seems to indicate that Chagas is still a neglected condition. Novel drug discovery technologies are preferably applied to the search of cures for conditions that assure a high investment return, such as cancer or neurodegenerative diseases. The authors understand that the "neglected" quality does not mean that no effort is made to search for novel solutions to a health condition, but the lack of proportion between the resources invested and the burden of the disease. Still, computer-aided drug repositioning, due to its rational and cost-efficient nature, constitutes and excellent tool to develop new medications for forgotten diseases, for which investment majorly comes from public and non-profit institutions.

It is possible that efforts on Chagas disease will be boosted now that the disease is gaining global health issue status due to its spread to non-endemic countries with no vectorial trasmission. However, it is safe to assume that an important fraction of research initiatives on Chagas will (and should) still come from Latin American countries. This region has a deficit of state-of-the-art technology (*i.e*. facilities to develop high-or ulta-high thoughput screening or perform compute-intensive tasks) and thus "brute force" strategies are not feasible. Latin American pharmaceutical industry seems reluctant to invest in deep innovative projects. In this context, application of computer-guided drug repositioning campaigns based on public (or at least accessible) resources are a feasible alternative to found therapeutic solutions to regional problems in relatively short time. We are especially thinking of ligand-based approximations or hybrid, sequential ligand- and structure-based applications. Approaches that assure high specificity (low false positives rate) should be implemented, *i.e*. consensus approximations and rational optimization of score cutoff values (such as ROC curves analysis). We have presented some additional, rational criteria to increase the probability of success at clinical trials, expand the potential clinical indications, reduce posible contraindications and smooth the way to drug approval. Such criteria include focusing on novel indications compatible with the doses used for the originally approved indication, and performing a detailed analysis of safety profile and evidence of potential additional therapeutic benefits besides trypanocidal effect, Other aspects (*i.e*. intelectual property considerations) may be also taken into account when choosing a drug candidate. To our knowledge, drug repositioning is the drug development strategy with the best translational perspectives in our region, and computer-aided repositioning is the only systematic approach that can be widely applied in Latin America. Similar conclusions may be valid in other continents (*i.e*. Africa) and for other neglected health conditions besides Chagas disease.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

The authors would like to thank the following public and non-profit organizations: InterAmerican Network of Academies of Science (IANAS) and National Research Council of the National Academies (USA) Seed Grant – 2013, National University of La Plata and Exact Sciences Faculty PIRPS. National University of La Plata Travel Grants and Argentinean National Agency of Scientific and Technical Research (ANPCyT) PICT 2008/223, PICT 2013-0520, CONICET.

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Received: September 05, 2014 Revised: November 12, 2014 Accepted: December 10, 2014