European Journal of Medicinal Chemistry 93 (2015) 338–348

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Computer-guided drug repurposing: Identification of trypanocidal activity of clofazimine, benidipine and saquinavir

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ARTICLE INFO

Article history:
Received 19 September 2014
Received in revised form
29 December 2014
Accepted 28 January 2015
Available online 12 February 2015

Keywords:
Chagas disease
Drug repositioning
Clofazimine
Benidipine
Saquinavir

ABSTRACT

In spite of remarkable advances in the knowledge on *Trypanosoma cruzi* biology, no medications to treat Chagas disease have been approved in the last 40 years and almost 8 million people remain infected. Since the public sector and non-profit organizations play a significant role in the research efforts on Chagas disease, it is important to implement research strategies that promote translation of basic research into the clinical practice. Recent international public-private initiatives address the potential of drug repositioning (i.e. finding second or further medical uses for known-medications) which can substantially improve the success at clinical trials and the innovation in the pharmaceutical field.

In this work, we present the computer-aided identification of approved drugs clofazimine, benidipine and saquinavir as potential trypanocidal compounds and test their effects at biochemical as much as cellular level on different parasite stages. According to the obtained results, we discuss biopharmaceutical, toxicological and physiopathological criteria applied to decide to move clofazimine and benidipine into preclinical phase, in an acute model of infection. The article illustrates the potential of computer-guided drug repositioning to integrate and optimize drug discovery and preclinical development; it also proposes rational rules to select which among repositioned candidates should advance to investigational drug status and offers a new insight on clofazimine and benidipine as candidate treatments for Chagas disease.

One Sentence Summary: We present the computer-guided drug repositioning of three approved drugs as potential new treatments for Chagas disease, integrating computer-aided drug screening and biochemical, cellular and preclinical tests.

1. Introduction

Drug repositioning (i.e. finding new therapeutic uses for already known drugs including marketed, discontinued and shelved drugs, and yet-to-be-pursued clinical candidates) has gained increasing attention within the international drug development community over the last few years [1–5]. Repositioned drugs represent unique translational opportunities, including substantially higher probability of success to market than new drugs, and a reduced development timeline to potentially 3–12 years [6,7]. Repurposed candidates have survived preclinical toxicological testing and proved tolerable safety and adequate pharmacokinetic profiles. When the repurposed drug has previously been used in clinical

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http://dx.doi.org/10.1016/j.ejmech.2015.01.065
0223-5234/

practice, manufacturing and stability issues have already been solved; furthermore, many drugs are off patent and may provide relatively inexpensive solutions as therapies for other diseases [8]. Successful drug repurposing stories have probably contributed to this interest; e.g. sildenafil was originally investigated for the treatment of hypertension and ischemic heart disease but has acquired blockbuster status as a treatment for erectile dysfunction. Aspirin itself has expanded its therapeutic indications and is now widely used to prevent heart attacks and strokes in patients with existing cardiovascular disease.

Second uses have been majorly found through serendipitous observations (e.g., intelligent exploitation of unforeseen side-effects). Lately, however, rational knowledge-based repositioning strategies have been explored, including chemoinformatic-, bioinformatic- and network-based approaches [9–15] and high-throughput literature analysis [16,17]. Repositioning has been signaled as a particularly useful strategy for the discovery of new treatments for rare and neglected diseases [18–20]. These disorders frequently offer limited potential revenue to pharmaceutical companies and are addressed by private-public joint efforts, the academic sector or non-profit organizations.

Here, we present the application of computer-aided drug discovery in the search of novel treatments for Chagas disease. Chagas disease is a tropical parasitic disease caused by the flagellate protozoan *Trypanosoma cruzi* [21]. Although a series of control campaigns developed by World Health Organization (WHO), Pan American Health Organization (PAHO) and national authorities have considerably reduced Chagas disease incidence in the last fifteen years, there are still almost 8 million infected people and 28 million people at risk in Latin America [22,23]. The human disease occurs in two stages: an acute stage, which occurs shortly after an initial infection, and a chronic stage that develops thereafter. The acute phase lasts for the first few weeks or months of the infection. It usually occurs unnoticed because it is symptom-free or exhibits mild, unspecific symptoms. The lifelong chronic stage frequently remains asymptomatic; however, around 30% of the patients will develop clinical affections on the heart, the digestive system or the nervous system. About two-thirds of people with chronic symptoms present cardiac damage, including dilated cardiomyopathy which causes heart rhythm abnormalities and may result in sudden death. About one-third of patients go on to develop digestive system damage, resulting in dilation of the digestive tract (megacolon and megaesophagus), accompanied by severe weight loss [24,25]. Current treatment against Chagas relies only on two approved drugs developed during 1960s-1970s, namely nifurtimox and benznidazole, which seem to be ineffective in the late chronic phase of the disease and present severe side-effects and resistance issues [26,27]. Important advances, however, have been made in the fields of biochemistry and molecular biology of *T. cruzi* and potential novel therapeutics [23,28–30]. Cruzipain (Cz), the major cystein protease of the parasite, has particularly been explored as new drug target [31–33], proving to be essential for replication of the intracellular form of *T. cruzi* and playing a role in host-parasite interactions [32–36]. Our group has previously applied computer-guided drug repositioning for the search of novel Cz inhibitors [37,38]. In those reports, Dragon software was used for molecular descriptor calculation. Although those models were able to identify dose-dependent Cz inhibitors, the effective concentrations were much higher than those used for the original indication, discouraging further investigation. Here, we have used DESMOL software for the computation of molecular descriptors included in the model, hoping to identify better candidates for repositioning. An integrative approach is presented for the search of novel anti-chagasic agents, including virtual screening (VS) of Cz inhibitors oriented to rational drug repositioning and subsequent biochemical, cellular and pre-clinical testing. A set of biopharmaceutical, toxicological and physiopathological criteria have been applied to decide which of the tested candidates would progress to the pre-clinical stage.

2. Materials and methods

2.1. Dataset compilation and splitting

A 147-compound balanced dataset including 77 Cz reversible inhibitors and 70 non-inhibitors was compiled from literature [39–50]. In order to split the dataset into representative training and test sets, the LibraryMCS v0.7 (ChemAxon) hierarchical clustering approach was applied in combination with the k-means clustering implemented in Statistica 10 Cluster Analysis module (Statsoft Inc, 2011). LibraryMCS relies on similarity guided Maximum Common Substructure (MCS, i. e. the largest subgraph shared by two chemical graphs) to cluster a set of chemical structures without exhaustive pairwise comparison.

2.2. Descriptor calculation and modeling

A set of well-known topological descriptors was used in this work: Subgraph Randic-Kiel-Hall like indices up to the fourth order ($^{m}X_t$, $^{m}X_t^v$) [51,52] topological charge indices, TCI, up to the fifth order, (J_m, G_m, J_m^v, G_m^v) [53], quotients and differences between valence and non-valance connectivity indices ($^{m}C_t = {}^{m}X_t/{}^{m}X_t^v$ and $^{m}D_t = {}^{m}X_t - {}^{m}X_t^v$), PRn (number of pairs of ramifications at topological distance n, with n ranging from 0 to 4), Vn (number of vertices with topological valence n, with n being 3 or 4). Each compound was characterized by a set of 62 descriptors. All descriptors used in this work were obtained with the aid of the DESMOL11 software [54].

Linear Discriminant Analysis (LDA) was then conducted in order to derive a classification model capable of distinguishing Cz inhibitors from non-inhibitors. LDA is a qualitative supervised learning method aimed to finding a linear combination of independent variables to differentiate between two or more categories of objects. Each object class is associated to a given value (an integer value) of an arbitrary variable that serves as class label. In our case, only two object classes (ACTIVE − Cz inhibitors and INACTIVE − non-inhibitors) were considered, thus the class label assumes two observed values (1 and −1, respectively). Since the output of the function being searched is not a continuous variable but only an object category, LDA and other classificatory techniques may be useful to handle noisy data, e.g. if a given experimental endpoint is associated to large variability or if experimental data from a diversity of laboratories are compiled [55].

The Discriminant Analysis module of Statistica 10 was used to build the models. A tolerance value of 0.5 was selected in order to exclude highly correlated descriptors from the model. All the coefficients linked to the models descriptors were significant at a 0.05 level. A minimum ratio of 15 between the number of training set compounds and the number of independent variables was used in order to reduce the chance of overfitting. Parsimony principle, Wilks' lambda and the performance of the model on the independent test set were used to select the best model. Standard validation approaches (stratified leave-group out cross-validation, randomization test and external validation) were used to assess the model's robustness and predictive ability [56]. Stratified 12-fold cross-validation and 15 randomization tests were applied.

2.3. Virtual screening

VS refers to the application of computational models or algorithms as filters to select drug candidates from chemical