Pharmacophore modeling, virtual computational screening and biological evaluation studies

Drug discovery process plays an important role in identifying new investigational drug-likes and developing new potential inhibitors related to a determinate target, in biopharmaceutical field [1]. An alternative promising and efficient used to identify new active substances is Pharmacophore modeling method.We defined a new computational strategy protocol characterized by the use of bioinformatics online tools and by the application of locally installed tools, for lead candidates generation-optimization able to reduce the cycle time and cost of this process and to promote the next steps of study [2].Hence, we have tried to apply this new computational procedure, in a more detailed screening, of small bioactive molecules, searching and identifying new candidates as “lead compounds”, potentially able to inhibit biological target AKT1 human protein and its related molecular mechanisms [3].The workflow executed in our work has been characterized by a multi-step design, which concerns different topics: search in PDB database of a model structure for AKT1, pharmacophore modeling and virtual computational screening, biological evaluation divided in two parts (molecular validation of selected compounds and study of physical-chemical properties related to pharmacokinetic/pharmacodynamics prediction models). All these step have been performed through PHARMIT (http://pharmit.csb.pitt.edu) and Discovery Studio 4.5 platform.We selected the PDB structure 3O96 as the reference complex (protein-ligand), and we analyzed it by means of PHARMIT and Discovery Studio, to generate four different “pharmacophore models” with four different list of natural compounds.It is performed a thorough screening of compounds applying several filters, to find some good candidates as possible natural AKT1 allosteric inhibitors.The compounds that match a well-defined pharmacophore have been analyzed through direct molecular docking, for selecting only the best candidates and studying the protein-ligand interactions. Selected compounds have been investigated in more details, to trace their origin, by their chemical-physical properties.This information can help us to predict some plausible enzyme-catalyzed reaction pathways, through PathPred web-server and KEGG compound database, in order to highlight the most important reactions for biosynthesis of compounds and obtain PharmacoKinetics/PharmacoDynamics (PK/PD) models, to investigate the ADMET properties of these lead compounds and to study their behavior in some biological systems, for the next experimental assays.This new computational strategy has been very efficient in showing what could be good “lead compounds” and potential natural inhibitors of AKT1 and PI3K/AKT1 signaling cascade. Therefore, the next steps could be the experimental analysis of pharmacokinetics-pharmacodynamics and toxicity properties “in vitro/in vivo”, in order to evaluate the results obtained “in silico”.

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PHARMACOPHORE MODELING, VIRTUAL COMPUTATIONAL SCREENING AND BIOLOGICAL EVALUATION STUDIES

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1 INTRODUCTION

The development process, in biopharmaceutical field to realize new potential medicines, is organized in pre-clinical and clinical phases. Drug discovery process belongs to pre-clinical phase and it plays an important role in identifying new investigational drug-likes and developing new potential inhibitors related to a determinate target. However, it is a long and very expansive complex process [1]. An alternative promising and efficient used to identify new active substances is *Pharmacophore modeling method.* It is a computational approach, which can be used when there is insufficient information on ligands that are experimentally proved to block or induce the activity of a particular therapeutic target, and it can be used to extract more information from the receptor side that can enable a medicinal chemist to have a deeper insight.

We defined a new computational strategy protocol characterized by the use of bioinformatics online tools and by the application of locally installed tools, for lead candidates generation-optimization able to reduce the cycle time and cost of this process and to promote the next steps of study [2].

Hence, we have tried to apply this new computational procedure, in a more detailed screening, of small bioactive molecules, searching and identifying new candidates as “lead compounds”, potentially able to inhibit biological target AKT1 human protein (critical mediator of PI3K/AKT1 pathway dysregulated in different human diseases) and its related molecular mechanisms, trying to re-establish the normal balance safeguarding the human health and reducing both the time and the costs of drug discovery process [3].

2 METHODS

The workflow executed in our work has been characterized by a multi-step design, which concerns different topics: *search in PDB database of a model structure for AKT1*, *pharmacophore modeling and virtual computational screening*, *biological evaluation* divided in two parts (*molecular validation of selected compounds* and *study of physical-chemical properties related to pharmacokinetic/pharmacodynamics prediction models*). All these step have been performed through PHARMIT (http://*pharmit*.csb.pitt.edu) and Discovery Studio 4.5 as a new integrated computational strategy to generate pharmacophores and evaluate their features and their properties.

2.1 Search in PDB database of a model structure for AKT1

The first step has been focused on comprehension of human disease at the molecular level, and identification of a model crystal structure used as biological target for a potential medicine, comparing different structures and choosing the most suitable for our purposes, through algorithm BLAST. Thereafter, this complex has been considered during the pharmacophore generation process as model for other pharmacophore realization and to find a promising molecule as lead compound that could become a new potential drug [4].

2.2 Pharmacophore modeling and Virtual computational screening

Chosen the model crystal structure, it is necessary generating a pharmacophore model. It is an abstract description of molecular features, which are necessary for molecular recognition of a ligand by a biological macromolecule, furthermore the pharmacophore shows what are the functional important groups inside ligand for protein interactions and its features [5].

The pharmacophores models have been obtained through PHARMIT and Discovery Studio, to investigate all purchasable compounds of the ZINC database [6], using the Pharmer as efficient and exact pharmacophore search technology. The parameters applied either with PHARMIT or Discovery Studio to use *the Receptor-Ligand pharmacophore generation method* and execute the first internal screening have been the following: Acceptor hydrogen bond HB_A, natural and bioactive

compounds, ZINC natural database for compounds, RMSD range between 0.200-0.800 Å, number of RBnds for conformations space and Molecular Weight between 200-800 g/mol with PHARMIT.

Selected a number of good candidates, it has been executed the first molecular screening of possible AKT1 inhibitors, applying a molecular blind-rigid docking by means of Autodock 4.2 (a suite of automated docking tools), to evaluate the ligand-protein molecular interactions by *lowest binding energy* values identifying the most stable complex and investigating the *estimated inhibition constant* (Ki) of the compounds selected to understand which are the compounds able to inhibit activity AKT1 and which is the inhibition concentration [7]. A good candidate selected from the virtual screening method using *Receptor-Ligand pharmacophore mapping protocol* with *Best Rigid Conformation Search Method* should map all the essential features required for the biological activity.

Therefore, the selected compounds have been analyzed also through Discovery Studio choosing other features such as: minimum and maximum of features to create the pharmacophores (range between 4-6 features among rings aromatic-hydrophobic bond-acceptor-donator-positive-negative), selectivity score, conformation generation method (FAST and BEST simultaneously), in order to execute another internal screening and select only good candidates. The choice of these parameters has been very important to perform the next molecular and biological validation steps have been taken the pharmacophores generated, choosing only the best candidates.

2.3 Molecular validation of selected compounds

Selected the hit compounds by virtual screening, it has been necessary to understand the features of pocket involved in ligand-protein interactions and to underline the amino acids involved in molecular interactions, trying to find what are the compounds that can be considered good lead compounds. To accomplish that, it is needed to investigate the molecular ligand-protein interactions through a focused-rigid docking executed, through AutoDock 4.2. The parameters analyzed are the same of molecular blind-rigid docking. This step plays a critical role for the choice of the best candidates to calculate the physical-chemical properties and create Pharmacokinetics-Pharmacodynamics models, for characterizing the good *lead compounds* for next experimental assays.

2.4 Study of physical-chemical properties and prediction of their pharmacokinetic-pharmacodynamics models

Therefore, the best candidates have been analyzed for the physical-chemical properties by means of Chemical Vendors inside of PubChem Compound, SciFinder, FooDB/HMDB and Chemicalize, to trace their origin and underline they most important features as: solubility in water and organic solutions, LogD, pKa and they chemical stability. This step is important to determine Pharmacokinetics and Pharmacodynamics properties. Moreover, it has been possible execute some studies of prediction to highlight and identify their biosynthetic pathways through PathPred web-server and to choose only the “lead compounds” necessary to obtain in silico Pharmacokinetics and Pharmacodynamics models by means of ADMET/toxicity predictor server applying the Lipinski-Veber filter to calculate some parameters related to absorption, distribution, metabolism, elimination, toxicity. Calculated these parameters, only molecules with good bioavailability, good predicted activity and good ADMET properties can be considered as hits compounds, and these compounds can be used to direct the design of next experimental assays [8].

Thereafter, it has been identified the toxicity level in different experimental conditions and in different model organisms, by means of TOPKAT server implemented in Discovery Studio. The compounds not satisfying drug-like properties were excluded.

3 RESULTS

Fig.1 reports the several steps have been developed in our work of natural-bioactive compounds that can show many health benefits, in addition to the basic nutritional value found in foods, through the realization and analysis of “pharmacophore modeling”, essential step in drug discovery [9]. We selected the 3O96 PDB file as model crystal structure of complex (protein-ligand) (Fig.1a) because it shows a percent identity more high with our isoform1 wild-type AKT1 protein, compared with other crystal structures. Thereafter, we analyzed this 3D protein structure by means of PHARMIT and Discovery Studio in Fig 1b, to generate four different “pharmacophore models” with

four different list of natural compounds (vitamin E-derived substances, isochinolinic alkaloid, isochinolinic alkaloid and antibiotics, flavonoids).

It is performed a thorough screening (Fig 1c) of compounds applying several filters, to find some good candidates as possible natural AKT1 allosteric inhibitors. The compounds that match a well-defined pharmacophore have been analyzed through direct molecular docking (Fig 1d), for selecting only the best candidates and studying the protein-ligand interactions. Selected compounds have been investigated in more details, to trace their origin, by their chemical-physical properties (Fig 1e). This information can help us to predict some plausible enzyme-catalyzed reaction pathways, investigated with PathPred server and KEGG compound database, in order to highlight (Fig.1f-1g) the most important reactions for biosynthesis of compounds and obtain PharmacoKinetics/PharmacoDynamics (PK/PD) models through ADMET predictor server to investigate (adsorption, distribution, metabolism, excretion, toxicity) and through TOPKAT software integrated in Discovery Studio to study for every single compound the different toxicity levels with several tests in silico, in order to select only the best lead compounds, and to realize of next experimental assays.

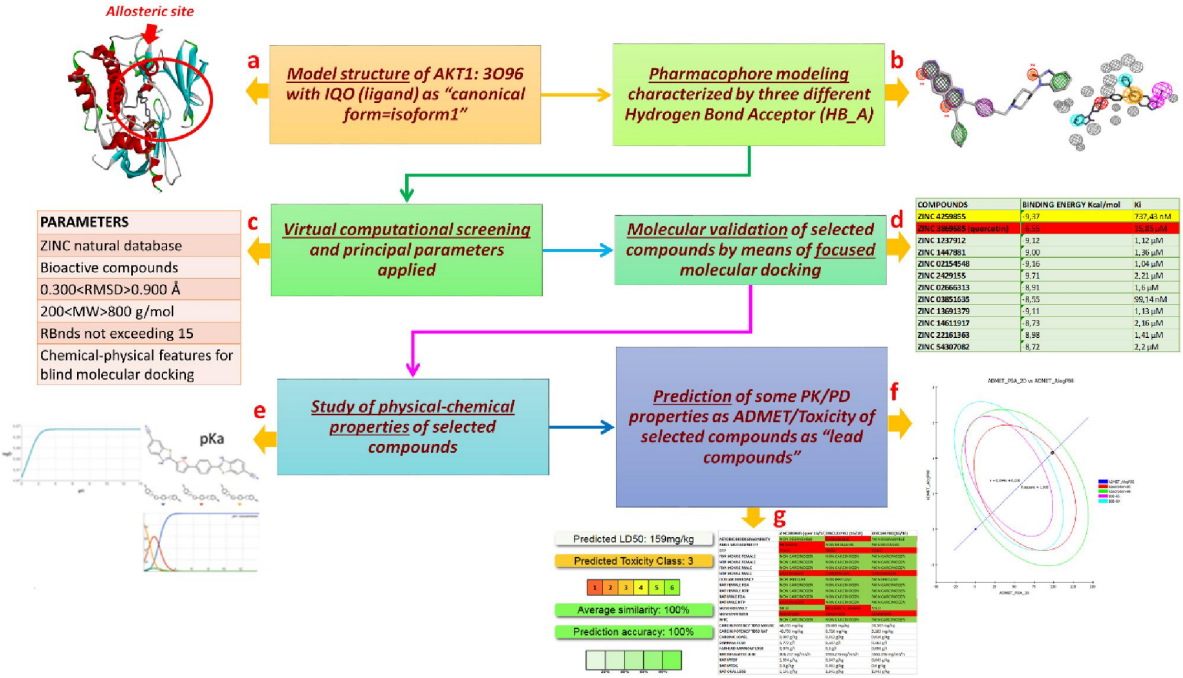

Figure 1. Pharmacophore modeling-virtual screening and biological evaluation process or drug discovery process. a) Search of 3O96 as model crystal structure for AKT1 (protein co-crystallized with IQO an allosteric inhibitor). **b)** Pharmacophore modeling through PHARMIT (three different pharmacophores with three different compounds categories) and Discovery Studio (a single pharmacophore with a single compound); **c)** Virtual computational screening characterized by application of different parameters; **d)** Molecular validation of selected compounds through a focused-rigid molecular docking; **e-f-g)** Study of physical-chemical properties and prediction of pharmacokinetics/pharmacodynamics (ADMET/Toxicity) properties of selected lead compounds.

4 CONCLUSIONS

This new computational strategy has been very efficient in showing what could be good "lead compounds" and potential natural inhibitors of AKT1 and PI3K/AKT1 signaling cascade. Therefore, the next steps could be the experimental analysis of pharmacokinetics-pharmacodynamics and toxicity properties "*in vitro/in vivo",* in order to evaluate the results obtained "in silico".

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6 REFERENCES

[1] X. Dong, X. Zhou, et al. “Pharmacophore Identification, Virtual Screening and Biological Evaluation of Prenylated Flavonoids Derivates as PKB/AKT1 Inhibitors”. Elsevier Masson SAS. 2011 Sep; 46 (2011) 5949-5958.

[2] J. Sunseri and D. R. Koes. “Pharmit: Interactive Exploration of Chemical Space”.Nucl. Acids Res. 2016 April.

[3] J. Fei, L. Zhou et al. “Pharmacophore Modeling, Virtual Screening and Molecular Docking Studies For Discovery of Novel AKT2 Inhibitors”. Int. J. Med. Sci. 2013 Jen; 10(3):265-275.

[4] W.I. Wu, W.C. Voegtli, et al. “Crystal Structure of Human AKT1 With an Allosteric Inhibitor Reveals a newmode of Kinase Inhibition”. Plos One (2010); 5:12913-12913.

[5] M. Dickson and JP. Gagnon “Key Factors in The Rising Cost of New Drug Discovery and Development”. Nature Reviews Drug Discovery (2004) 3: 417–429.

[6] J.J.Irwin and B.K.Shoichet. “ ZINC- A free database of Commercially Available Compounds for Virtual Screening”. J.Chem.Inf.Model. 2005; 45(1):177-182.

[7] GM Morris, AJ. Olson, et al.“Using AutoDock for ligand-receptor docking”. Curr Protoc Bioinformatics. 2008 Dec;Chapter 8:Unit 8.14.

[8] T. Kaserer, K. R. Beck et al. “Pharmacophore Models and Pharmacophore-Based Virtual Screening: Concepts and Application Exemplified on Hydroxysteroid Dehydrogenases”.Molecules 2015, 20, 22799–22832.

[9] J. Kour and DC. Saxena “Studies on the Development of Nutraceuticals Foods Using ExtrusionTechnology”. Austin J Nutri Food Sci - Volume 2 Issue 5 – 2014