INSTITUTO UNIVERSITARIO DE BIO-ORGÁNICA "ANTONIO GONZÁLEZ". UNIVERSIDAD DE LA LAGUNA

MEMORIA DEL TRABAJO FIN DE GRADO

Búsqueda de inhibidores de ME1 mediante farmacóforos

END OF DEGREE PROJECT TITLE

Search for ME1 inhibitors through pharmacophores

GRADO EN FARMACIA
Curso 2017/2018

Mª Nazaret Márquez Domínguez
Tutor: Dr. José M. Padrón

La presente memoria de investigación ha sido realizada por la alumna Mª Nazaret Márquez Domínguez durante el curso académico 2017-2018 en las instalaciones del Instituto Universitario de Bio-Orgánica "Antonio González" (IUBO-AG) y bajo la dirección del Dr. José M. Padrón Carrillo. El trabajo forma parte de la línea de investigación *Diseño, descubrimiento y evaluación de fármacos anticancerígenos.*

La Laguna, a 5 de septiembre de 2018.

Fdo. José M. Padrón

ABSTRACT

The computational techniques play an important role mainly in the first stages of the development of new medicines. The virtual objective is to reduce the amount of experimental work in the laboratory as well as the expeditor involved. The combination of computational techniques with biological tests, increases the chance of success in the development of a relevant new drug.

The purpose of this piece of work is the study of the combination of these methods through computational simulation, which goal is to design and validate a pharmacophore model that allows virtual screening of possible inhibiting molecules in Malic enzyme (ME1).

In the first place, bibliographic information to create database of active compounds against ME1 is sought. With this database, a pharmacophore model was built and later was validated through a retrospective analysis, by using a database of inactive molecules, where 7 molecules with inhibiting activity known against ME1. It was refined through computational parameters such as exclusive volumes, those that can be modified manually.

After elaborating and refining the pharmacophore model, a sieving in BioLab's database was done so as to find all the structures that could act inhibiting ME1.

As a result, the model discovered 148 candidate molecules. This allows to reach the objective of finding new compounds of high probability of a junction of enzyme. Nevertheless, this is about a theoretical model that employs structural predictions. To do so, studies *in* vitro should be carried out by using all types of cellular lines for checking if the theoretical model has validity. Out of all the structures found, only the 10 best shall be proposed in order to carry out such study in the laboratory.

RESUMEN

Las técnicas computacionales desempeñan un papel importante en las primeras etapas del desarrollo de nuevos medicamentos. El objetivo virtual es reducir la cantidad de trabajo experimental en el laboratorio, así como los gastos que esto conlleva. La combinación de técnicas computacionales con ensayos biológicos, aumenta las posibilidades de éxito en el desarrollo de un fármaco de interés.

El propósito de este trabajo, es el estudio de la combinación de estos métodos mediante simulación computacional, con el objeto de diseñar y validar un modelo de farmacóforo que permita el cribado virtual de posibles moléculas inhibidoras de la enzima málica (ME1).

En primer lugar, recopilamos información bibliográfica para crear una base de datos de compuestos activos frente a ME1. Con esta base de datos, elaboramos un modelo de farmacóforo que luego se validó mediante un análisis retrospectivo, utilizando una base de datos de moléculas inactivas, donde introdujimos 7 moléculas con actividad inhibidora conocida contra ME1. Se refinó mediante parámetros computacionales, como los volúmenes de exclusión, los cuales se modifican manualmente.

Tras elaborar y refinar el modelo de farmacóforo, se realizó un cribado en la base de datos BioLab's para encontrar todas las estructuras que podrían actuar inhibiendo la ME1.

Como resultado, el modelo encontró 148 moléculas candidatas. Esto nos permite alcanzar el objetivo de encontrar nuevos compuestos con alta probabilidad de unión a la enzima. Sin embargo, se trata de un modelo teórico que utiliza predicciones estructurales. Por ello debemos realizar estudios *in vitro* usando cultivos de diferentes líneas celulares para ver si el modelo teórico tiene validez. De las estructuras que se encontraron, propondremos las 10 mejores para realizar dicho estudio en laboratorio.

CONTENTS

1) INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) cells have adapted to survive and proliferate in microenvironments under attack of the cells of the immune system, the deprivation of nutrients and oxygen, via mechanisms triggered by oncogenic KRAS. PDAC cells metabolism changes in response to O_2 and nutrient deprived environment. One of the most significant metabolic changes occurs in the glutamine pathway. In normal cells, glutamine would enter the TCA cycle. But, in PDAC cells, glutamine is derived to another route to generate NADPH, maintain cells redox balance and ensure proliferation. Enzymes of this glutamine pathway, reprogrammed by oncogenic KRAS, are GLS, GOT2, GOT1, MDH1 and ME1. This pathway is not used extensively by non-tumor cells. Thus, small molecules targeting these enzymes might result in selective kill of PDAC cells.

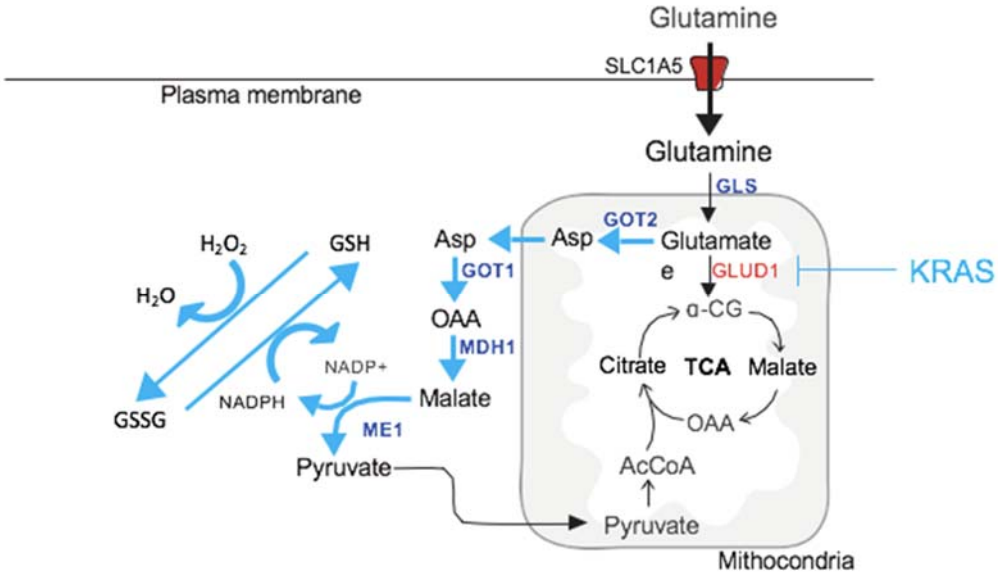

Figure 1. *Alterations of glutamine metabolism in pancreatic cancer cells*

Malic enzyme 1 (ME1) regulates one of the main pathways that provide nicotinamide adenine dinucleotide phosphate (NADPH), which is essential for cancer cell growth through maintenance of redox balance and biosynthesis processes in the cytoplasm. ME1 inhibition disrupts metabolism in cancer cells and inhibit cancer cell growth by inducing apoptosis. In glucose-restricted culture conditions, cancer cells increase ME1 expression and tracer experiments with labelled glutamine revealed that the flux of ME1-derived pyruvate to citrate was enhanced. In addition, cancer cells show higher sensitivity to ME1 depletion in glucose-restricted conditions compared to normal culture conditions. These results suggest that in a low-glucose environment, where glycolysis and the pentose phosphate pathway (PPP) is attenuated, cancer cells become dependent ME1 for the supply of NADPH and pyruvate (Figure 1). Literature data demonstrate that ME1 is a promising target for cancer treatment, and a strategy using ME1 inhibitors combined with

inhibition of glycolysis, PPP or redox balance regulators may provide an effective therapeutic option [1].
In order to search for ME1 inhibitors, computational techniques will be utilized. The *in silico* elaboration is going to give an idea of the ones that could be interesting molecules for testing. Their applications are varied, yet allow among other things, the elaboration of a pharmacophore, validation and its refinement, striving in the database, etc. The latter has the advantage of being fast techniques, in constant evolution and that reduce notably the time and inversion, in comparison with *in vitro e in vivo* testing. The results of our investigations will be presented.

2) HYPOTHESIS

BioLab's database contains molecules that inhibit can cell proliferation by the inhibition of ME1.

3) OBJECTIVES

The main objective of this work is to identify antiproliferative molecules in BioLab's database that acts by inhibiting ME1.

In order to achieve our objective, we will follow a stepwise procedure:

- Firstly, we will run a bibliographic search of molecules that inhibit ME1.

- Next, categorize ME1 inhibitors to elaborate and validate a pharmacophore model.

- Finally, feed the pharmacophore model with BioLab's database to identify novel candidate molecules as inhibitors of ME1.

4) MATERIALS AND METHODS

Literature search

ME1 inhibitors were sought in two well-known publicly available databases: PubMed and BindingDB. PubMed database was searched for articles that contain ME1 inhibitors and using the terms "ME1 inhibitor" and "malic enzyme inhibitors". When available, IC_{50} or K_i data of the inhibitors were retrieved. In BindingDB, the search terms were "ME1 inhibitor"

Molecular modelling

Open Babel version 2.3 was used to convert file formats. MOE (Molecular Operating Environment) version 2008 was used to generate the pharmacophore model and screen BioLab's database.
In order to find possible inhibitors of ME1, the following methodology will be described:

A database is created with molecules found in the literature. They are classified into two groups, the first one to create the pharmacophore model and the latter to validate the model.

- The pharmacophore model is created through the overlapping of molecules; this process is known as flexible alignment (Figure 2). An alignment aims to produce a series of pertinent overlapping of diverse ligands, the end result being an approximation of its supposed binding geometry.

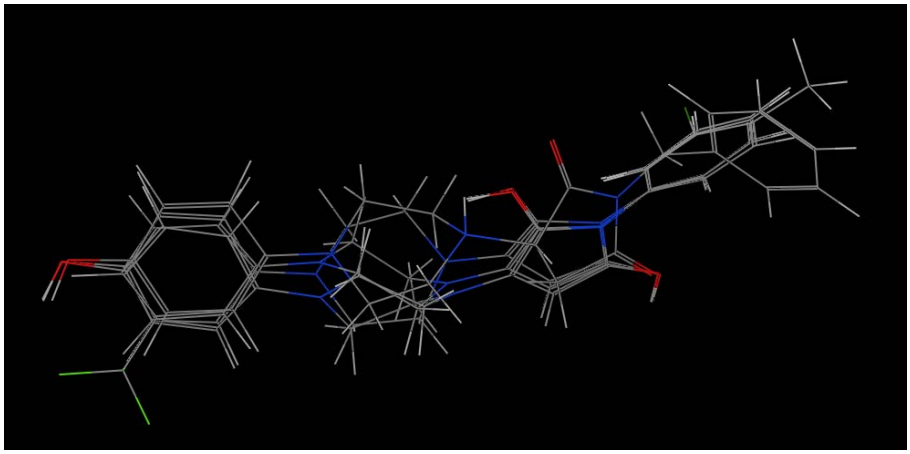

Figure 2. Flexible alignment of ME1 inhibitors

In the consensus of the pharmacophore, it is established a set of characteristics as tolerance and threshold. Tolerance refers to the permissible margin of error in the localization, just as the threshold refers to the minimum amount necessary for a feature to be perceptible.

- In parallel with the other group of molecules, aleatory conformations are generated and are added to the database that contains inactive molecules.

Once the pharmacophore model is created and the screening in the database of inactive molecules, the model is refined. The refinement is a looping process in which exclusion volumes are incorporated.

After the pharmacophore is finished, a screening in the BioLab's database is done.

5) RESULTS AND DISCUSSION

The identification of possible ME1 inhibitors is of high clinical importance due to its role in the regulation of the main pathways that provide NADPH in the metabolism of pancreatic ductal adenocarcinoma (PDAC) cancer cells.

In order to find possible candidate molecules against the ME1, we elaborate a pharmacophore that allows us to perform a screening in databases that contain antiproliferative molecules, but in which the mechanism of action is unknown. We will continue to explain each of the steps taken in the development of said pharmaphocore, including a description of the parameters used and the results obtained.

First, a bibliographical search of molecules that inhibit ME1 should be done. Out of all of the database that have been utilized, only 11 were found in BindingDB (Table 1). Next, we created an archive using a spreadsheet file that contains the compounds, the SMILES (linear notation for encoding molecular structures), and parameters like K_i and IC_{50}.

Table 1. *SMILES structure of ME1 inhibitors found in BindingDB.*

Compuesto	SMILES	Ki (unidades)	IC50 (unidades)
BDBM50338502	COc1ccc(cc1)-c1cn(nn1)[C@@H]1O[C@H](COP(O)(O)=O)[C@@H](O)[C@H]1O	5.70E+4	
BDBM50177180	Oc1cc(N2CCN(CC2)c2ccc(O)cc2)c(O)n1-c1ccccc1		150
BDBM50177175	Oc1ccc(cc1)N1CCN(CC1)C1CC(=O)N(Cc2ccccc2)C1=O		450
BDBM50177184	Cc1ccc(cc1Cl)-n1c(O)cc(N2CCN(CC2)c2ccc(O)cc2)c1O		560
BDBM50177177	Oc1cc(N2CCN(CC2)c2cccc(c2)C(F)(F)F)c(O)n1-c1ccccc1		3.15E+3
BDBM50177178	Oc1cccc(c1)N1CCN(CC1)C1CC(=O)N(Cc2ccccc2)C1=O		1.00E+4
BDBM50177179	Oc1cc(N2CCN(CC2)c2ccc(F)cc2)c(O)n1-c1ccccc1		1.00E+4
BDBM50177182	COc1ccc(cc1)N1CCN(CC1)C1CC(=O)N(Cc2ccccc2)C1=O		>2.00E+4
BDBM50177176	Oc1ccccc1N1CCN(CC1)C1CC(=O)N(Cc2ccccc2)C1=O		>2.00E+4
BDBM50177183	COc1ccc(cc1)N1CCN(CC1)c1cc(O)n(c1O)-c1cccc(Cl)c1Cl		>2.00E+4
BDBM50177181	COc1ccc(cc1)N1CCN(CC1)c1cc(O)n(c1O)-c1ccccc1		>2.00E+4

Once the data is collected, the molecules in SMILES format were converted into conformations with Open Babel. This is done in order for the molecules to adopt a spatial disposition, enabling us to work with them in the elaboration of the pharmacophore model. The most important goal of the activity is not to determine the precise structure of molecules, but to understand the conformation that the molecules take in the space.
Ehrlich seminal discoveries reinforced the assertion made in 1894 by another brilliant German chemist, Emil Fisher. In a publication dealing with the effect of glucoside conformation on the interaction with enzymes, he wrote: “I would like to say that enzyme and glucoside must fit together like lock and key, in order to have a chemical effect on each other”. [4]

The image of "lock and key" is still used today, even if it suggests a rigid structure of the receptor or enzyme protein. Probably another image, such as "hand in a glove", would be more accurate. Effectively, in addition to the steric complementarity, it would account for

chirality and receptor flexibility.

Once they have been converted, we introduced them in MOE where we created a database (training set), categorizing them according to IC_{50}. We selected the four best structures in order to create our pharmacophore model. The seven remaining structures were used to develop a database (test set), generating varied conformations.

The four molecules were overlapped and the flexible alignment was done. MOE, uses the flexible methods that are considered to be those in which the conformational analysis is performed on-the-fly and these require rigorous optimization. This step is vital in the determination of the relevant binding conformation for each of the ligands concerned. All alignment methods require some quantitative measure or fitness function to assess the degree of overlap between the ligands being aligned and to monitor the progression of that optimization.

In continuation, tolerance and threshold values were established (Table 2), together with the typical characteristics which are as follows: hydrophobic centers, donors of hydrogen meeting, H-bond acceptors, positive charge centers, negative charge centers, aromatic rings and exclusion volume (Figure 3).

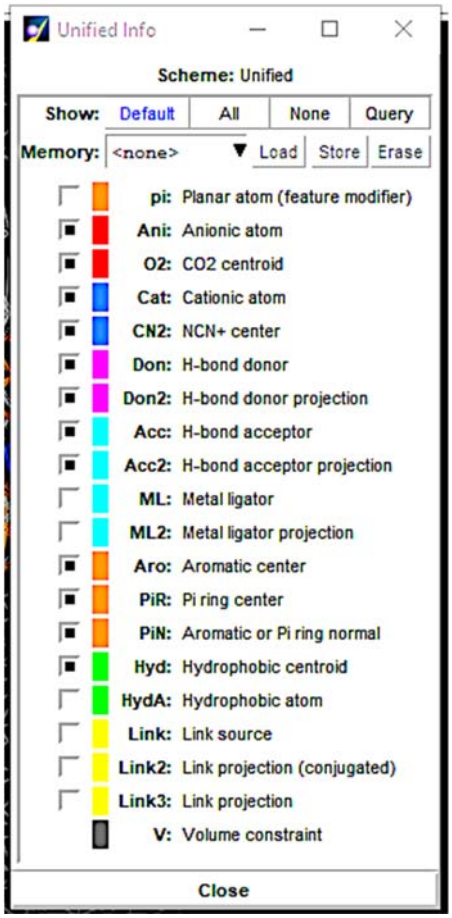

***Figure 3**. Features and parameters selected to create the 3D pharmacophore.*

Table 2. Selected parameters to find pharmacophoric groups

ID	Expression[a]	Tolerance	Essential
F1	Acc	0.8	Yes
F2	Aro | Hyd	2.1	Yes
F3	PiN	1.9	Yes
F4	Aro | Hyd	1.7	Yes

In order to validate the model, a database with different conformations of the 7 remaining molecules was generated. Later, it was fused with a database that contains inactive molecules. The principal objective of this procedure is to find the active molecules and the lowest possible number of inactive molecules by applying the model of the created pharmacophore. Out of 1587 molecules inhabiting a database, only 377 hits were set aside.

To be able to obtain effective results, we could refine the pharmacophore model by introducing exclusive volumes (Figure 4). These are spheres with a determined radius that we could modify manually according to the results we obtained after performing a search of the database. In this case, 19 spheres of exclusion were introduced and 72 hits were obtained.

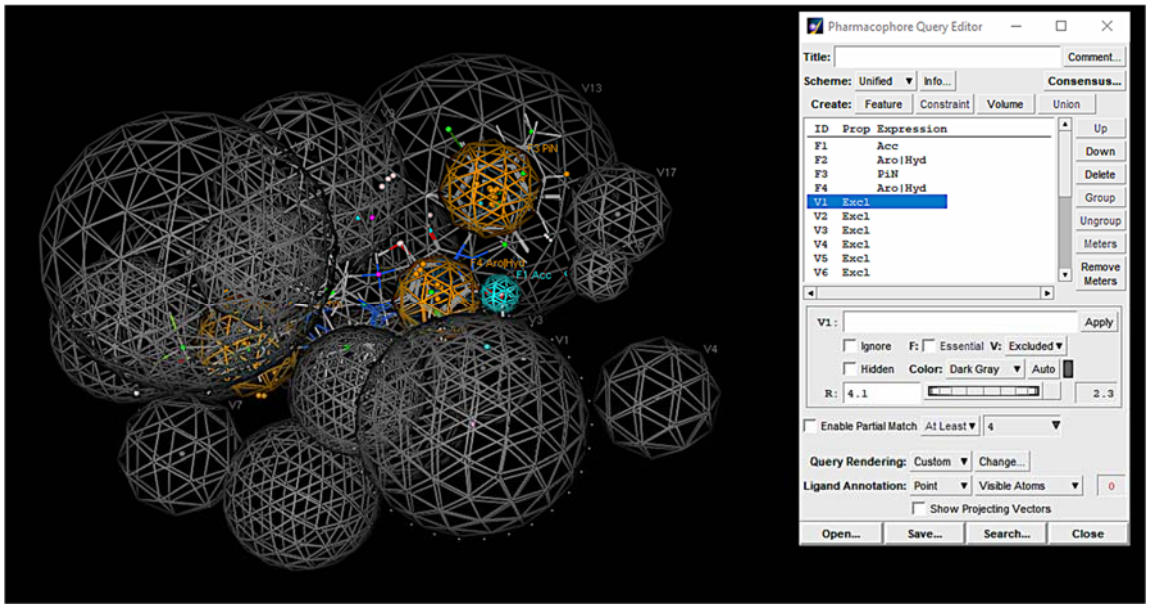

Figure 4. Refined pharmacophore groups (colored areas) and 19 exclusion spheres (grey areas)

To this effect, a pharmacophore model from a set of characteristics presently in specific regions of the four selected structures, to search for potential therapeutic compounds has been created. The pharmacophore model relates chemical structure to biological affinity and identifies the biologically important binding sites on ligands. The compounds are

represented in their tridimensional form, and molecular flexibility is taken into account by considering each compound as a collection of conformations.

Finally, we made the last screening against the BioLab's database with the refined pharmacophore model. BioLab's database contains 1423 molecules. From this compounds, a subset of 148 molecules were considered by the model as possible candidates (Figure 7). The next table shows ten compounds with a possible inhibiting activity of ME1. They are the best compounds based on RMSD (root mean square deviation). This parameter is often used in 3D geometry of molecules to compare two conformations of a given set of points, typically atoms. In other words, given a list of paired points, it gives a measure of the distance between these points. RMSD values are commonly used to measure the structural similarity between structures. (Thus, it is observable in a regressive basis the similarity among the molecules).

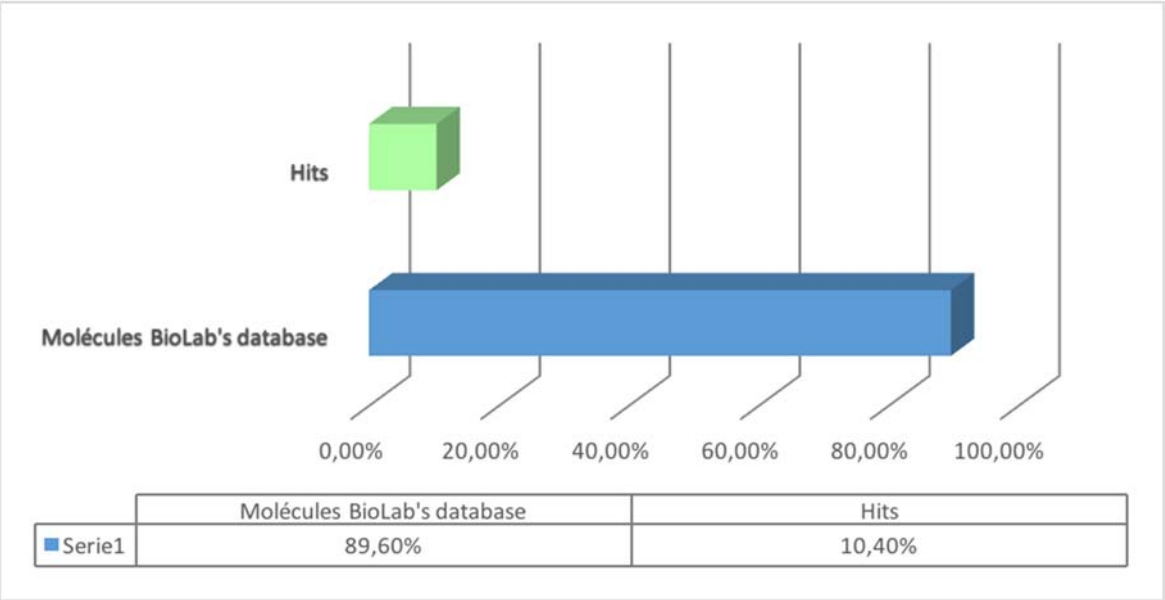

Figure 7. *Graph indicating the number of total molecules in the database (1423) versus the hits obtained (148)*

Table 3 shows the GI_{50} values (50% growth inhibition) of the compounds against human solid tumor cell lines. It can be observed that the majority of the inhibitors discovered are inactive (GI_{50} > 100 μM). The most active compound is CKT0353, which has been reported to interact with β-tubulin. Our results indicate that this compound might act on other biological targets.

Table 3. *GI_{50} range value of top ten predicted compounds.*

BioLab's code	RMSD	MSEQ	ΔGI_{50} (μM)
GSR0006	0.3708	6	>100
GSD0058	0.4139	116	14-18
CGG0037	0.4168	102	25-71
CGG0062	0.4423	118	>100
CGG0065	0.4466	121	73-100
CGG0064	0.4776	120	>100
GVE0041	0.5253	76	>100
GVE0002	0.5352	9	>100
GVE0023	0.5523	40	>100
CKT0353	0.5640	92	0.32-0.53

6) CONCLUSIONS

Computational chemistry allowed us to address the interaction between small molecules and biological targets in a way that it is impossible from an experimental point of view.

In this investigation, active structures with a possible activity against ME1 through the elaboration of a pharmacophore were sought. The 11 ME1-inhibiting molecules found during literary analysis are not especially strong inhibitors, given that the IC_{50} values barely fall into the sub-micromolar range.

We built a pharmacophore model and screened BioLab's database. Out of 1423 molecules present in the database, the model selected 148 hits as possible candidates to inhibit ME1. In other words, 89% of the compounds are not recommended to be tested, therefore reducing experimental costs and time.

7) REFERENCES

[1] Murai S, Ando A, Hirayama M, Satomi Y, Hara T. Inhibition of malic enzyme 1 disrupts cellular metabolism and leads to vulnerability in cancer cells in glucose-restricted conditions. Oncogenesis 2017, 6, 329.
[2] Cohen R, Neuzillet C, Tijeras-Raballand A, Faivre S, de Gramont A, Raymond E. Targeting cancer cell metabolism in pancreatic adenocarcinoma. Oncotarget 2015, 6, 16832-16847.

[3] Wermuth CG, Ganellin C R, Lindberg P, Mitscher LA. Glossary of terms used in medicinal chemistry (IUPAC Recommendations 1997). Annu. Rep. Med. Chem. 1998, 33, 385-395.
[4] Langer T, Hoffmann RD. Pharmacophores and Pharmacophore Searches. Vol 32. 1st ed., Weinheim, Germany: WILEY-VCH; 2016.