Innovative approaches in drug discovery :
Inhibitors of Protein-Protein Interaction for RhoGTPases

PIRAMID[a] — PROTEIN INTERACTIONS IN RATIONAL APPROACHES FOR MEDICINAL INNOVATIVE DRUGS

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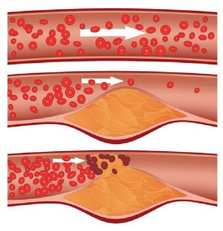
Context

According to the WHO*, ***the first cause of death*** worldwide is due to cardiovascular diseases with 15,2 million of death in 2016 (around 30% of all deaths). Among them, 7.3 million are due to coronary heart diseases and 6.2 million of strokes. Changes of lifestyle and population ageing augur that ***in 2030, 23 millions of people might die of a cardiovascular disease.*** In such context, it becomes a major issue in research and medicine.

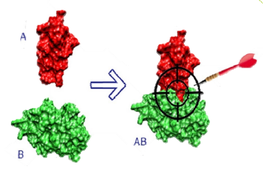
Atherosclerosis is a chronic inflammatory disease of major arteries. This silent illness with slow development leads to erosion, rupture or thrombosis of artery lumen. Different factors participate to the appearance and progression of atheromatous plaques: smoking, sedentariness, diet rich in saturated fat and sugar. Other diseases increase the risk of atherosclerosis development: arterial hypertension, diabetes, hypercholesterolemia, obesity and metabolic syndrome.



* WHO : World Health Organisation

Our strategy

There are between ***130 000 to 650 000 PPIs***[b] in human cells and these interactions play an important role in number of diseases.[c,d,]



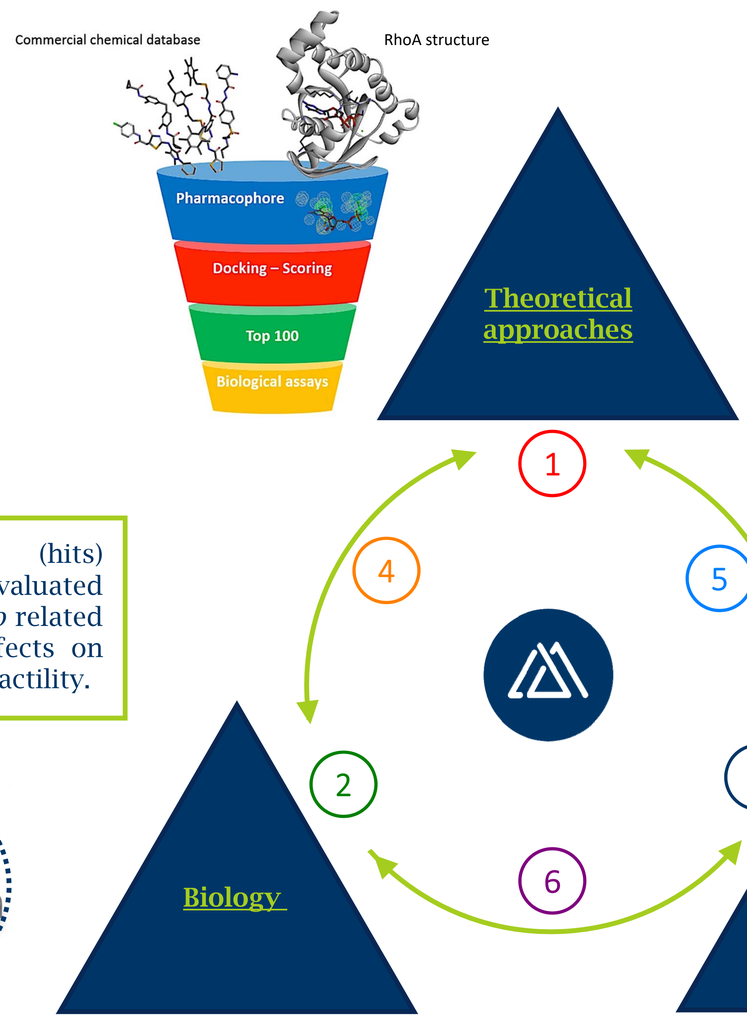
Previous works highlight the ***important interaction of RhoA with Arhgef1 in cardiovascular disease*** and suggest blocking Arhgef1 as a potential therapeutic target against atherosclerosis.[e]

Our strategy aims to identify compounds able to ***inhibit interactions*** between Rho protein and its exchange factor Arhgef1 to block Rho protein signaling.

Project

Hit-to-lead strategy: *In silico* screening of several chemical libraries on targeted protein allows to identify ***hits*** compounds. If one of these compounds demonstrates an inhibitory activity in biology *(in vitro* or *in vivo*), this molecule, now called ***lead***, will then be modified by organic synthesis in order to enhance its biological activity.

1 Using molecular modeling, database of compounds and proteins 3D structures, chemical modellers are able to predict interactions between small molecules and proteins.



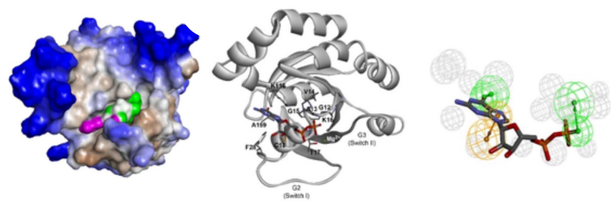
2 *In silico* predicted molecules (hits) disclosing good score of affinity are evaluated by biological assays *in vitro* and *ex vivo* related to the studied area. In particular, effects on RhoA/Arhgef1 are mesured using contractility.

Cell culture

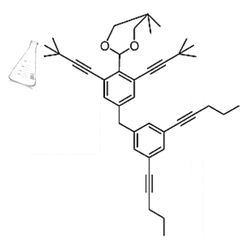
Adhesion, proliferation migration assessments

3 From informations given by modellers and biologists, chemists can resynthesize lead compounds. The chemical synthesis wil enable the syntheses of others analogues to confirm structure-activity relationship.

4 After confirming the biological activity of the lead molecule, modellers and chemists work together to propose new structures based on the lead in order to strengthen or create new interactions with the targeted protein.



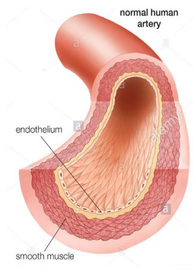
5 Syntheses of the new molecules are then performed. It worth noting that a slight change or addition of a functional group on the structure could lead to a complete change of the synthesis scheme.

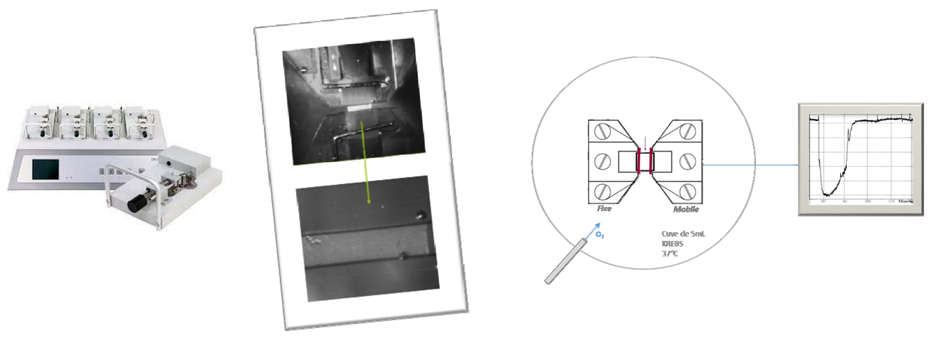


6 New compounds are then evaluated by biologists. If they give better results than the lead molecule, they are assayed in *ex-vivo* and *in vivo* assessments giving full and more accurate characterizations on their biological activity.

Our work

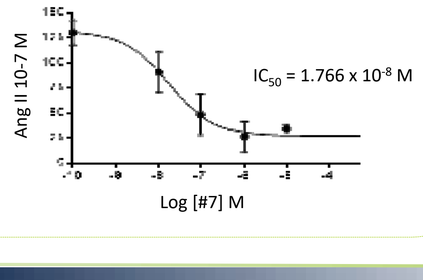
To evaluate the inhibition activity of different compounds on RhoA-GTP/Arhgef1 interaction, we have performed contractility assessments. Indeed, RhoA is involved in the contractility process of smooth muscle cells and we know that when we stimulate cells with Angiotensin II, the signalling pathway induces exclusively contractility through RhoA-GTP/Arhgef1 interaction.





To quantify the capacity of inhibition, we use the Mulvany's myographe. This equipment measures the strength of contraction (in mN) of mesenteric artery and gives a quick information on the interest of several compounds.

Then *in silico* screening of 180 000 compounds, 28 molecules have caught the attention of modellers. Available in several chemical data bank, one molecule (over 28) shows an interesting activity with an IC_{50} around 10^{-8}M. Currently, we are working on the synthesis of a chemical library close to this lead in order to develop an original inhibitor which could be used in clinical research.



Acknowledgments

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