

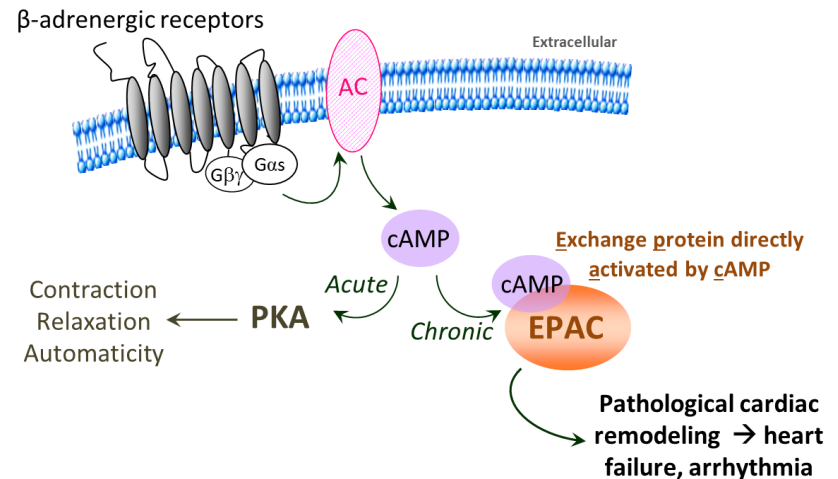
A novel EPAC1 inhibitor with cardioprotective effects

March 2019

Toulouse Tech Transfer - Tous droits réservés

■ **Acute cAMP/PKA signaling is beneficial to regulate cardiac function:**

- Intracellular second messenger produced from ATP upon **β -adrenergic receptor** stimulation ;
- Involved in physiological processes such as cardiac contractility and relaxation through modulation of calcium cytosolic concentration ;
- Exerts its effects via the Protein Kinase A (PKA).

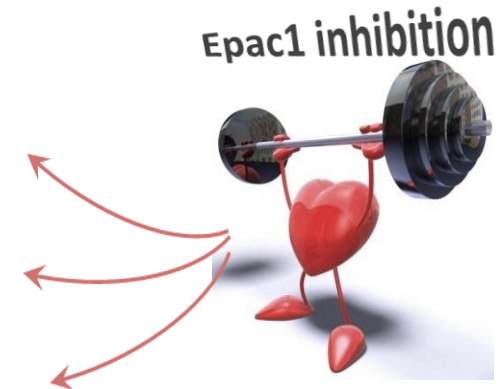


■ **Chronic activation of cAMP/EPAC signaling leads to cardiac dysfunction:**

- Induces **pathologic cardiac remodeling**, notably ventricular **hypertrophy** and **fibrosis** ;
- Promotes cardiac arrhythmia and heart failure development ;
- Molecular mechanism: switch from PKA to **EPAC deleterious signaling**.

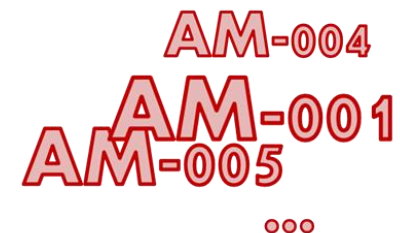
■ Target validation: EPAC1

- Exchange proteins directly activated by cAMP -1 ;
- Promotes activation of the small G protein Rap ;
- Highly expressed in the heart and upregulated in cardiac diseases ;
- **Target validation:**
 - EPAC1 gene or pharmacological inhibition does not alter basal cardiac function ;
 - EPAC1 gene or pharmacological inhibition **prevents pathological remodeling (e.g fibrosis, cell death, inflammation) and improves cardiac function** in experimental models of ischemic and non-ischemic heart failure ;
 - EPAC1 gene deletion is protective against **atrial arrhythmia**.

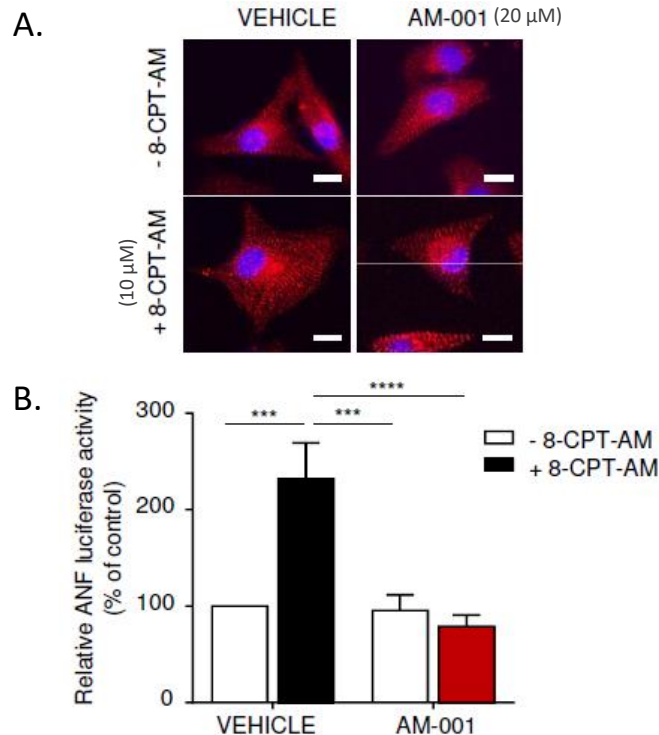


■ Target drugability: AM, a novel family of EPAC1 inhibitors

- Small molecules identified by chemical bank screening ;
- **Non competitive inhibitors ;**
- **Very specific of EPAC1 isoform:** no effect on EPAC2 and PKA activity ;
- Half maximal inhibitory concentration IC50 ~ 50 µM ;
- *In vitro* and *in vivo* pharmacologic efficacy ;
- **No apparent toxicity** on cultured cell lines, nor on mice liver and kidney after *in vivo* treatment.

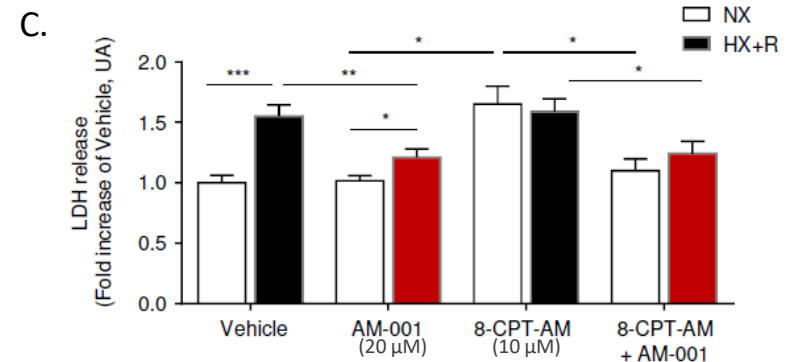


■ **Protective effect against EPAC1-induced cardiomyocyte hypertrophy:**



- A. Fluorescent microscopic analysis of neonatal rat ventricular myocytes surface (NRVMs) treated or not with Epac1-specific agonist 8-CPT-AM, in the presence or absence of AM-001.
- B. AM-001 decreases the expression of pathological hypertrophic markers.

■ **Protective effect against hypoxia-reoxygenation-induced cardiomyocyte death:**



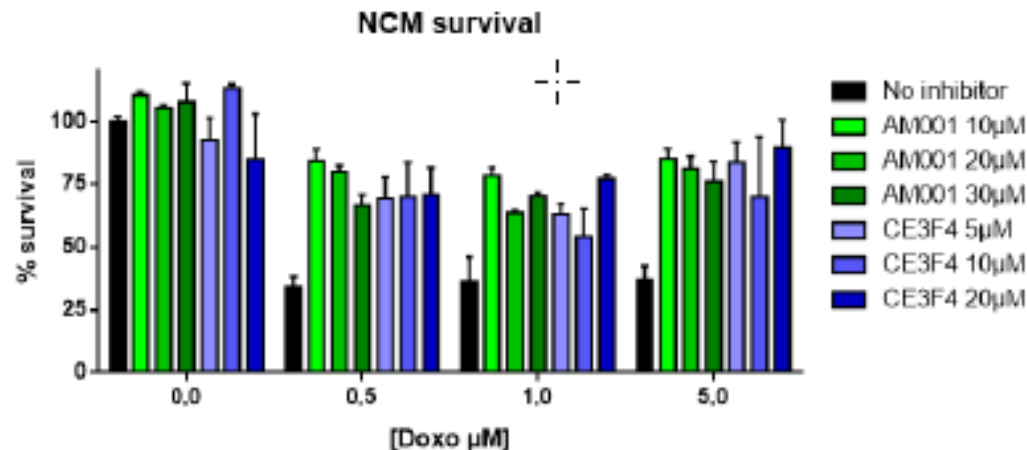
C. Cell viability, assayed by LDH release, of isolated adult cardiomyocytes subjected to normoxia (NX) or hypoxia-reoxygenation (HX+R) treatment.

➡ AM-001 prevents EPAC1-induced cardiomyocyte hypertrophy and cell death.

■ AM-001 prevents Doxorubicin-induced cardiomyocyte death

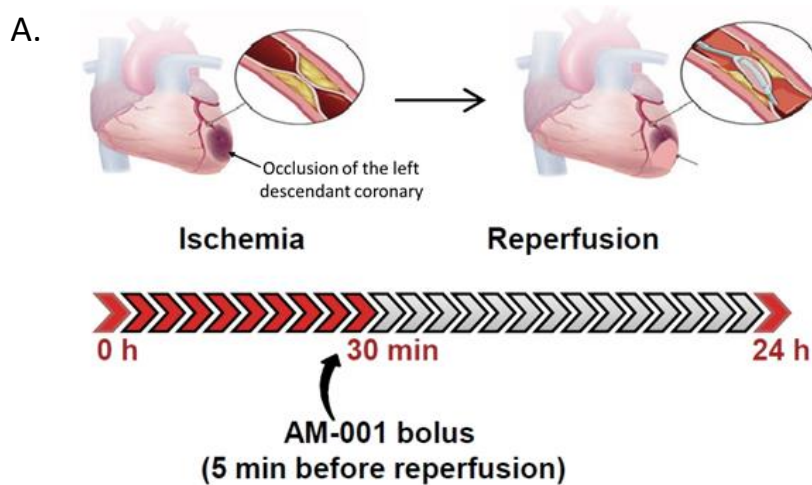
Anthracyclines, such as doxorubicin (DOX), are potent anticancer agents for the treatment of solid tumors and hematologic malignancies. However, their clinical use is hampered by cardiotoxicity.

- AM-001 potentiated cell death induced by DOX in different human cancer cell lines
- AM-001 decreased DOX-induced cardiomyocyte death

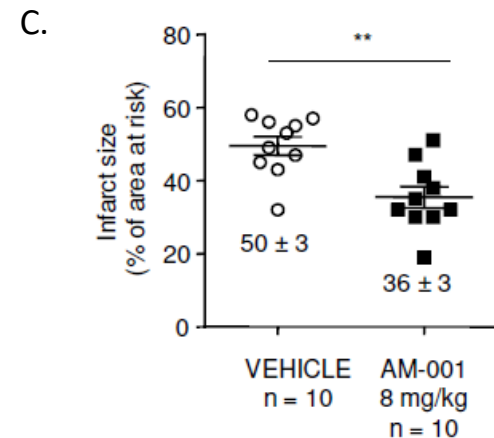
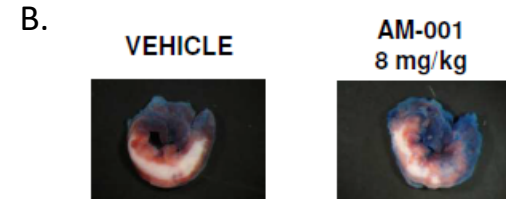


- Blockade of EPAC1 may provide a dual therapeutic advantage in cancer therapy by simultaneously preventing anthracyclines cardiotoxicity and reducing tumor growth.

- Protective effects of AM-001 in a mouse model of ischemia-reperfusion :



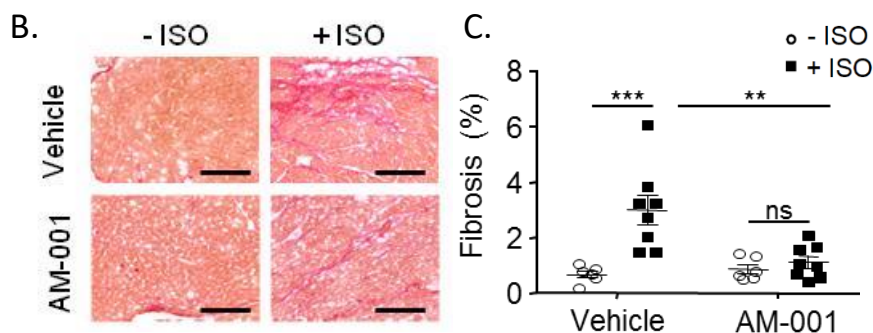
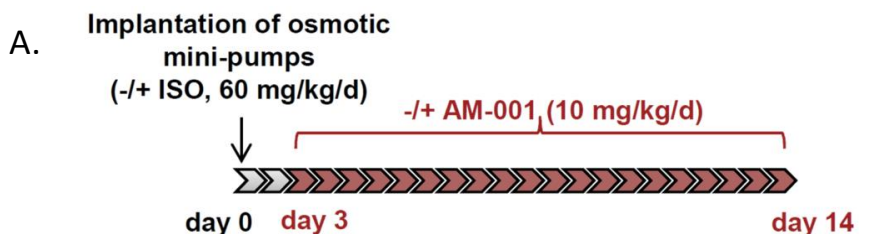
A. Schematic time scale and experimental strategy to evaluate the effect of AM-001 on I/R injury.



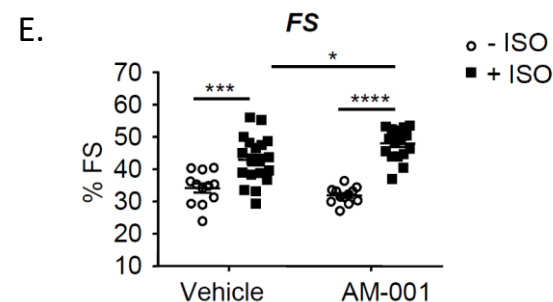
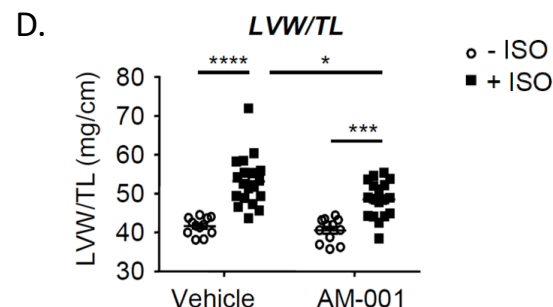
B. Representative cross-sections stained with Evans blue and TTC of mice pretreated or not with AM-001. C. Quantification of the area at risk expressed as percentage of left ventricle size and infarct size.

➡ AM-001 reduces the infarct size in mice hearts and has a therapeutic potential in cardiac ischemia.

- Protective effects of AM-001 against chronic β -adrenergic receptor activation-induced cardiac dysfunction:



A. Schematic time scale and experimental strategy. (B) Mouse heart sections were stained with sirius red (left panel) to detect fibrosis. (C) Fibrosis quantification



D. Comparison of the ratio of left ventricular weight (LVW) to tibia length (LVW/TL), and E. fractional shortening (FS), in ISO treated animals in the presence or absence of AM-001.

➔ AM-001 attenuates cardiac fibrosis and hypertrophy, and improves cardiac function during chronic activation of β -adrenergic receptors.



■ AM FAMILY INHIBITORS BENEFITS:

- Novel molecular therapeutics for acute and chronic heart failure treatment.
- First EPAC1 specific inhibitors, with no impact on PKA.
- *In vitro* and *in vivo* efficiency, reproducing EPAC1 knock-down effects.
- No evidence of toxicity *in vitro* and *in vivo*.
- No reduction of cardiac contractility, contrary to β -blockers.
- Potential protection against cardiotoxic side effects of anticancer therapies.
- Anticancer effect of AM-001 inhibition.

■ ONGOING WORK FOR AM INHIBITORS VALIDATION:

- Rational design to improve AM-001 efficacy dose.
- ADMET studies.
- Pulmonary fibrosis.



- **Patent EP18305685 (June 2018)**
registered by Inserm and Toulouse Paul Sabatier University on behalf of the Institute of Cardiovascular and Metabolic Diseases.



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