# SK4 $K^+$ CHANNEL BLOCKERS: A NEW ANTI-ARRHYTHMIC AND ANTI-FIBROTIC TREATMENT FOR ATRIAL FIBRILLATION


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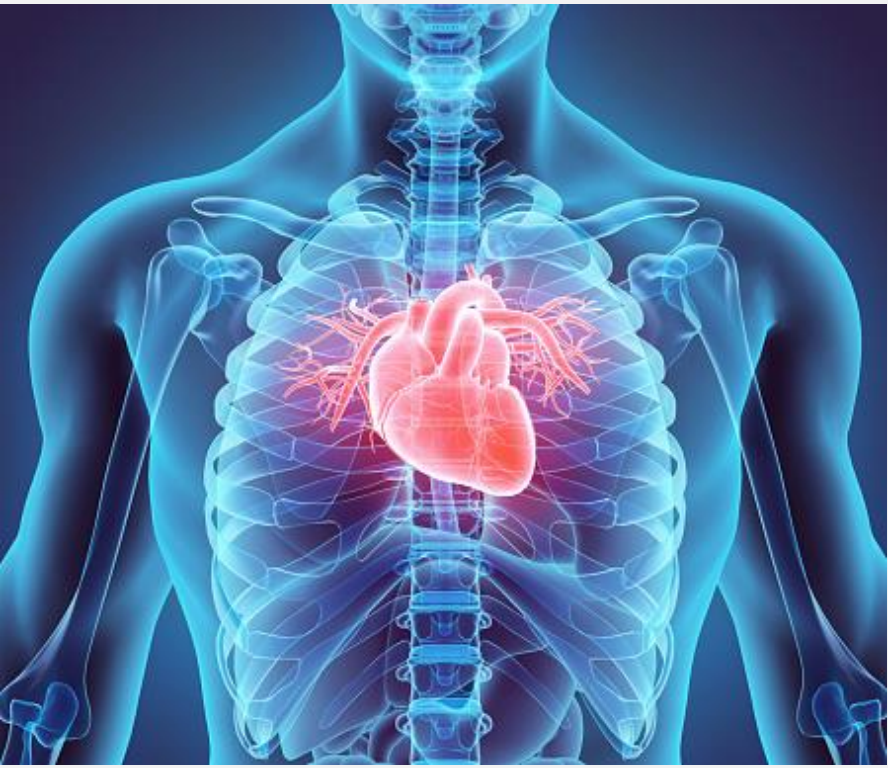

# Scientific rationale

- Atrial fibrillation (AF) most common sustained cardiac arrhythmia with a prevalence of 1 to 2% of the population worldwide, associated with heart failure progression, embolic stroke and mortality.
- prevalence within ageing population.
- About 30 million North Americans and Europeans are expected to suffer from AF by 2050.

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# Scientific rationale

- Current existing therapies:

  1-PVI >pulmonary vein isolation >anti-AF effects in selected cases. However, invasive, requires anticoagulants, costly, potential life threatening complications, incomplete efficacy with recurrent AF attacks, notably for persistent AF.

- 2-Pharmacology >Currently available drugs for AF have major limitations including partial efficacy and risk of life-threatening ventricular proarrhythmic side effects. Do not reduce progression of AF substrate (atrial inflammation / fibrosis)

  Several companies try to develop drugs, but pipeline body very thin, does not fill the gap

- **Unmet need for new drugs with new mechanisms.**

# Our solution and novelty

- We discovered a novel cardiac target, the SK4 K+ channels to treat atrial fibrillation
- We found that SK4 $K^+$ channels are highly expressed in the cardiac myocardium and predominantly in the atrium of both rats and Humans
- In addition to beneficial electrophysiological effects, SK4 K+ channel blockage profoundly inhibit atrial fibrosis and triggered AF episodes in a heart failure rats model.
- Our pre-clinical data suggest that blocking SK4 $K^+$ channels represent a suitable new target approach for AF therapy

*Weisbrod et al, PNAS, 2013*
*Weisbrod et al, Acta Pharmacologica Sinica 2016*

*Haron-Khun et al, EMBO. Mol. Med, 2017*
*Bueno et al, Frontiers in Pharmacology, 2020*
*Burg et al, PNAS 2022*

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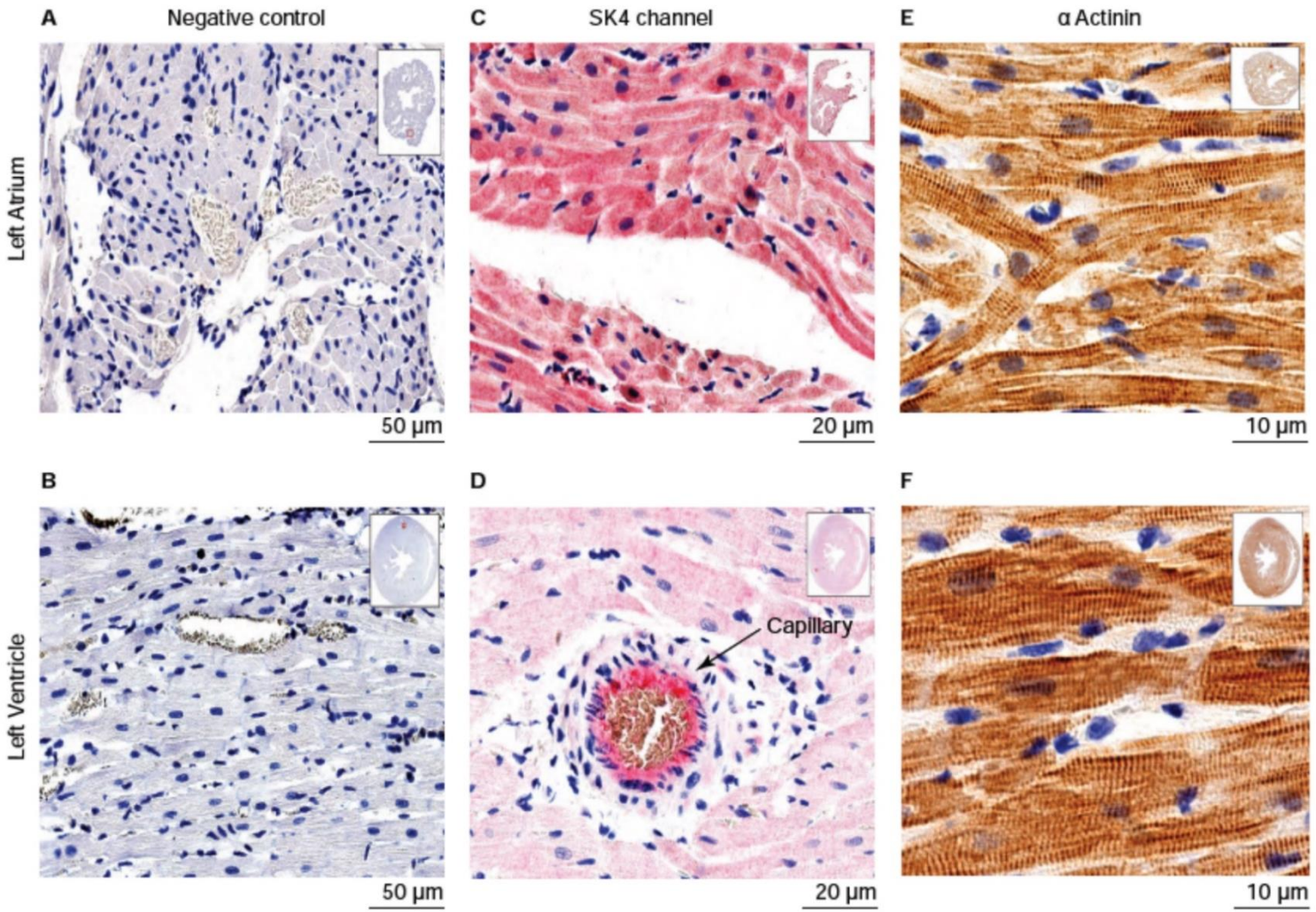
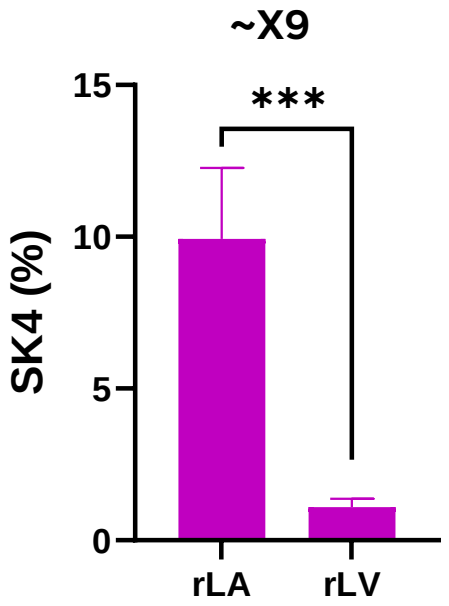
# SK4 $K^+$ channels are predominantly expressed in healthy rat atrium

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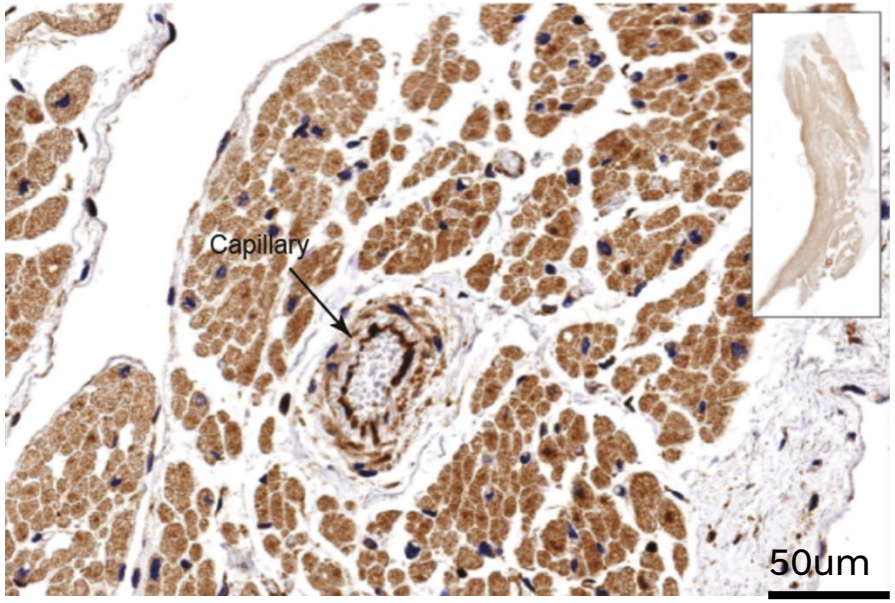
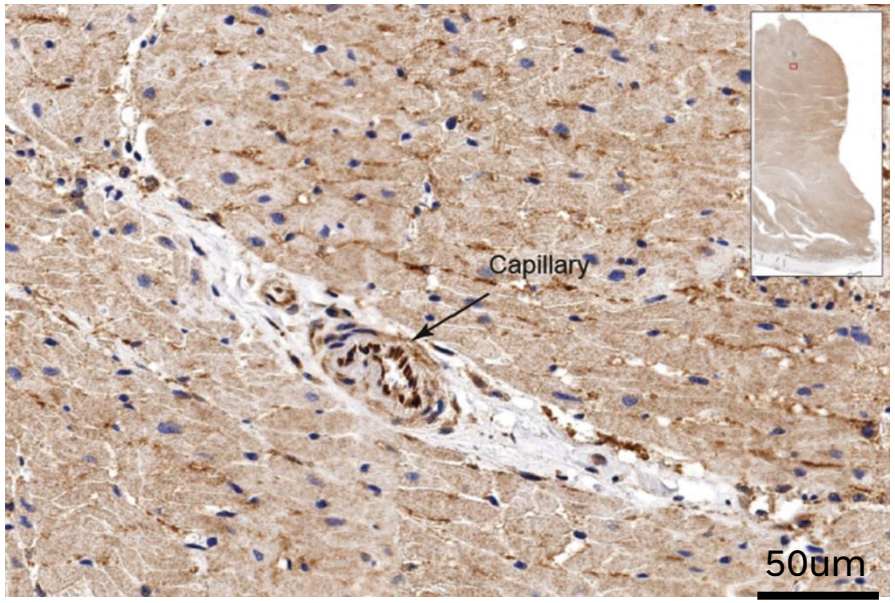
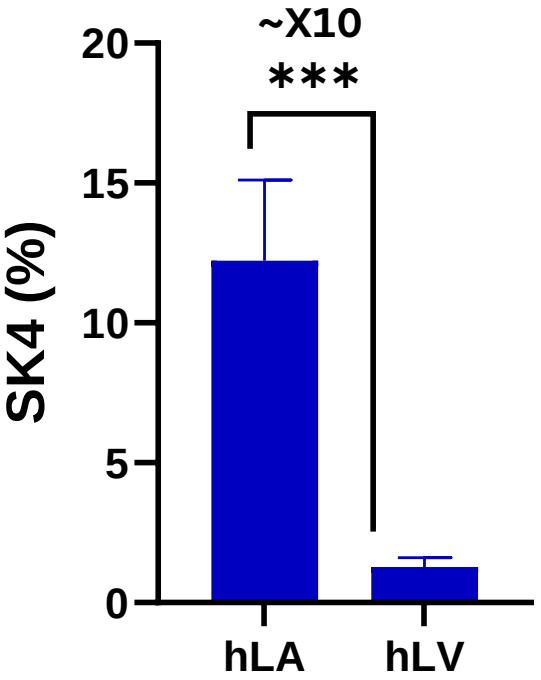
# SK4 $K^+$ channels are predominantly expressed in human atria

**Human Left atrium**



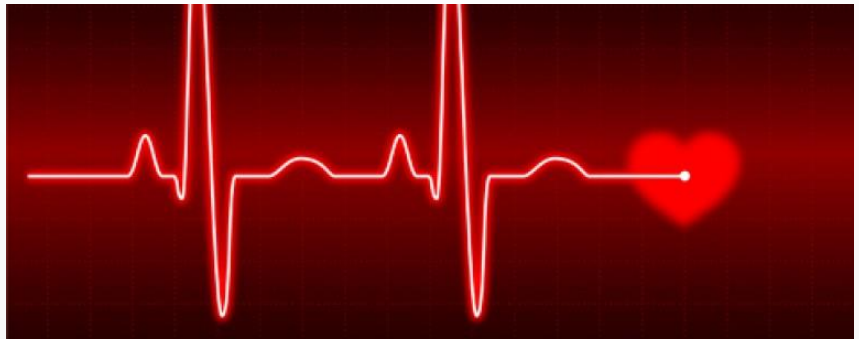

**Human Left Ventricle**



# Our solution and novelty

- SK4 K+ channels are important for the late repolarization of the AP in the SAN, AV nodes and atrium.
- Thus, SK4 channel block will provide an increase in the atrial refractory period, slowing of AV conduction.
- The observation that not only atrial fibroblasts and macrophages but also atrial cardiomyocytes express SK4 K+ channels and possess the biochemical machinery necessary to engage inflammatory signalling is crucial and clinically relevant.
- Many lines of evidence indicate that SK4 K+ channels are pro-arrhythmic and pro-fibrotic
- Thus, SK4 K+ channel exhibit all the attributes for AF targeting



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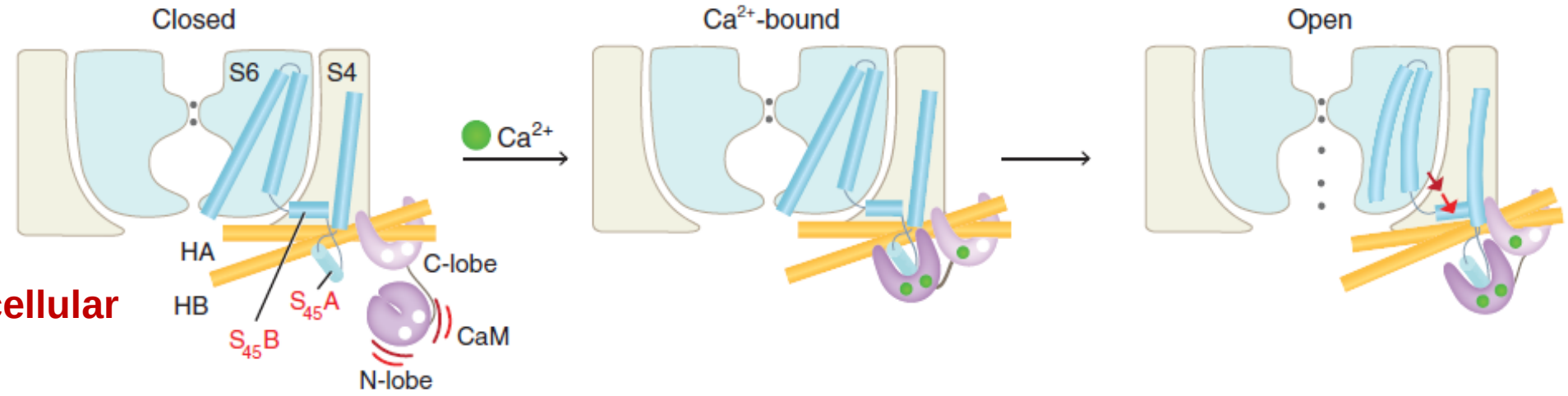
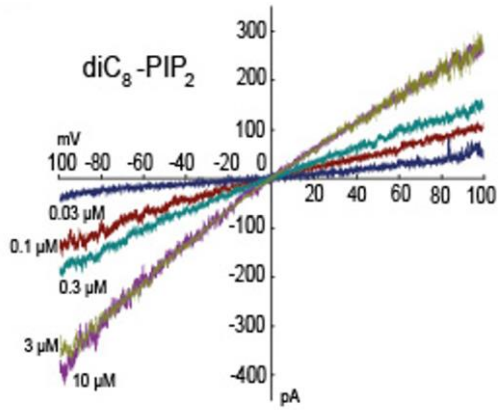
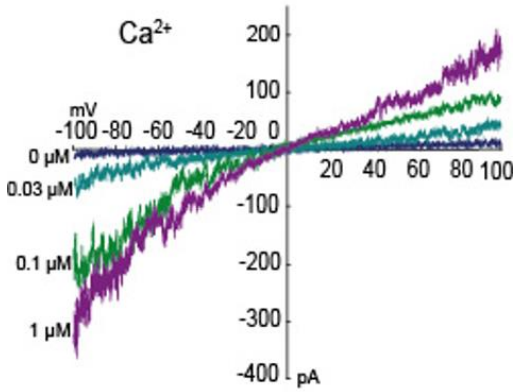
# SK4 $K^+$ channels are activated by intracellular $Ca^{2+}$ and PIP2

**extracellular**

**intracellular**




*Burg et al, 2022 PNAS*

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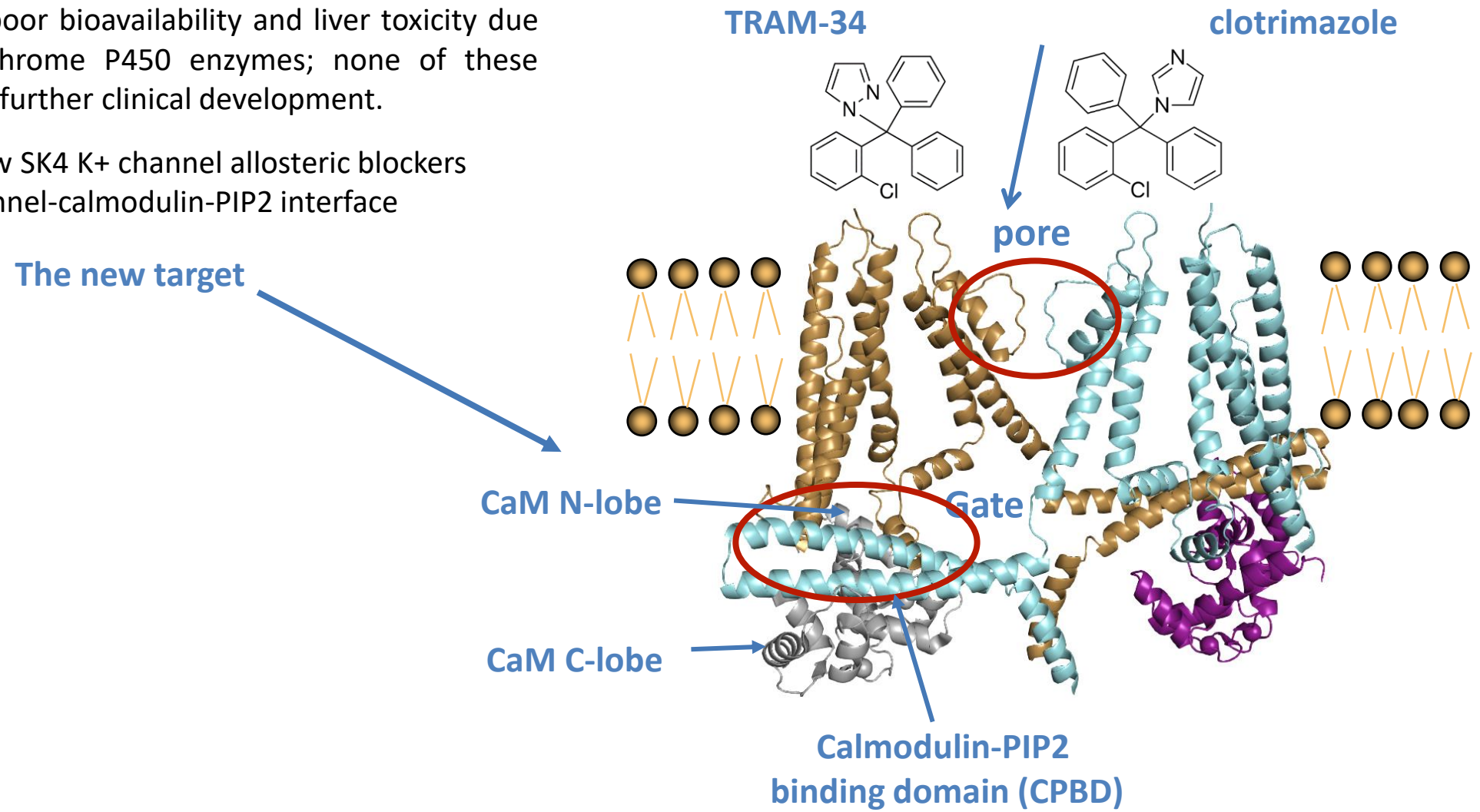
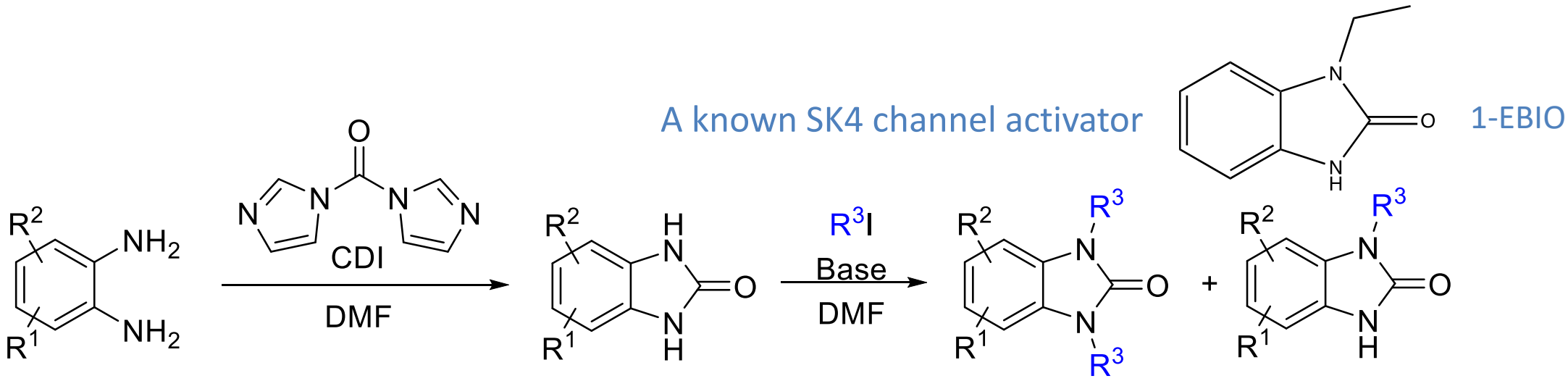
# Our solution and novelty

- All currently existing SK4 K+ channel blockers interact with the pore of the channel, exhibiting poor bioavailability and liver toxicity due to interactions with cytochrome P450 enzymes; none of these compounds are suitable for further clinical development.
- We propose to develop new SK4 K+ channel allosteric blockers designed to act on the channel-calmodulin-PIP2 interface



# Design and synthesis strategy



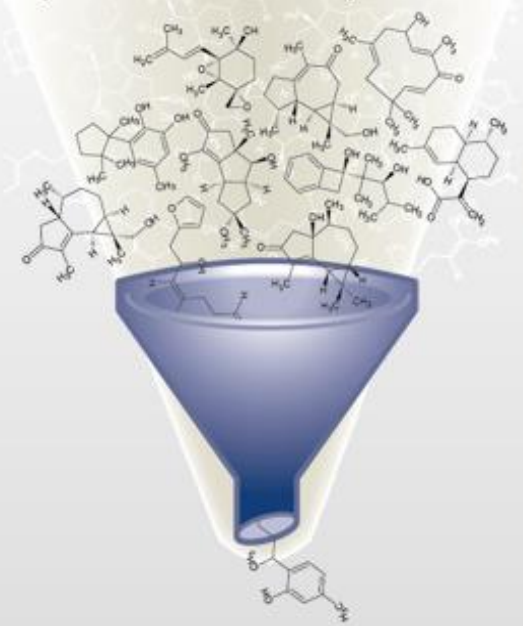

$R^3$ = -$(CH_2)nCH_3$
n = 0-11

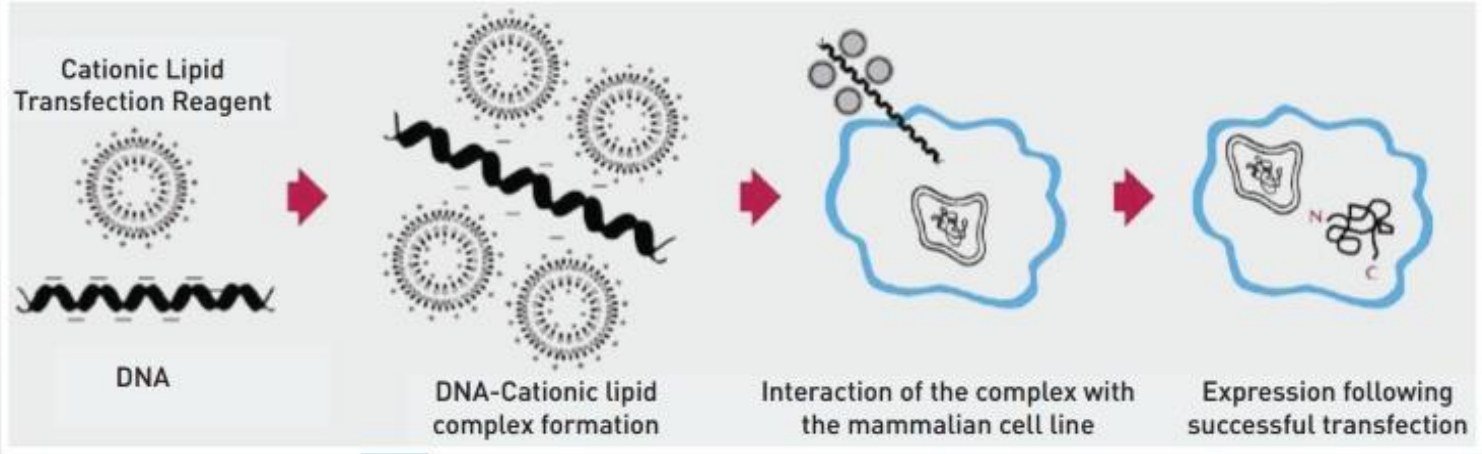
$R^1$, $R^2$ = H, halogen, alkyl, methoxy, nitro, cyano etc.

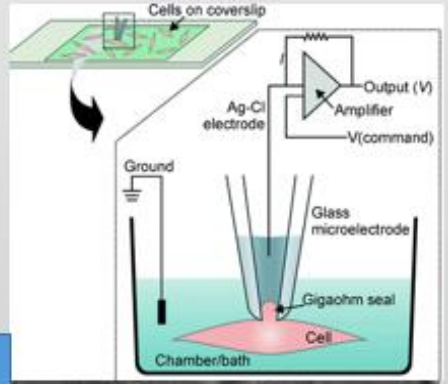

## Electrophysiology-bioassay

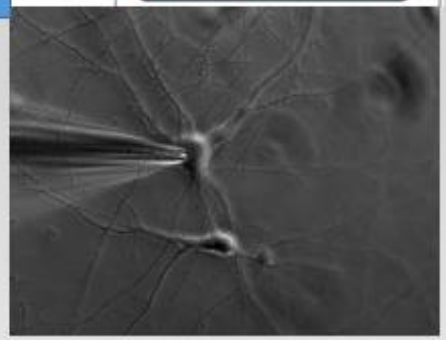
## Transfected CHO cells

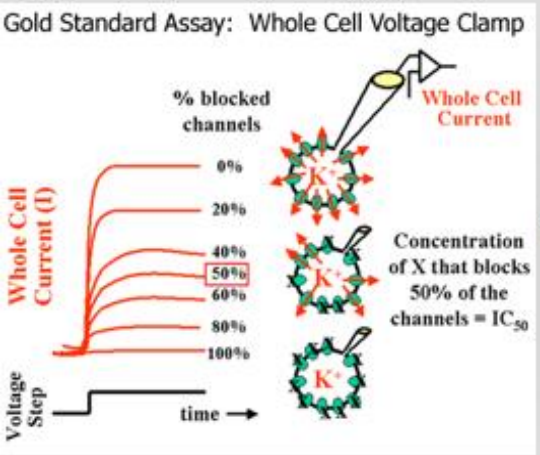
Synthesized compounds



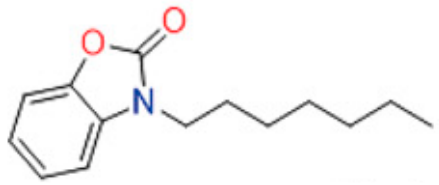


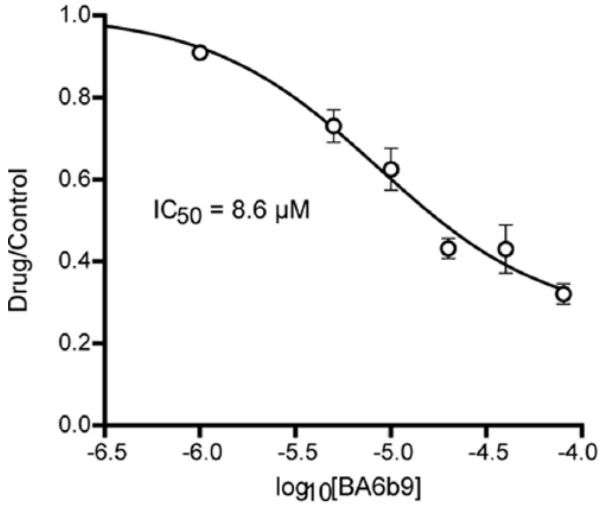



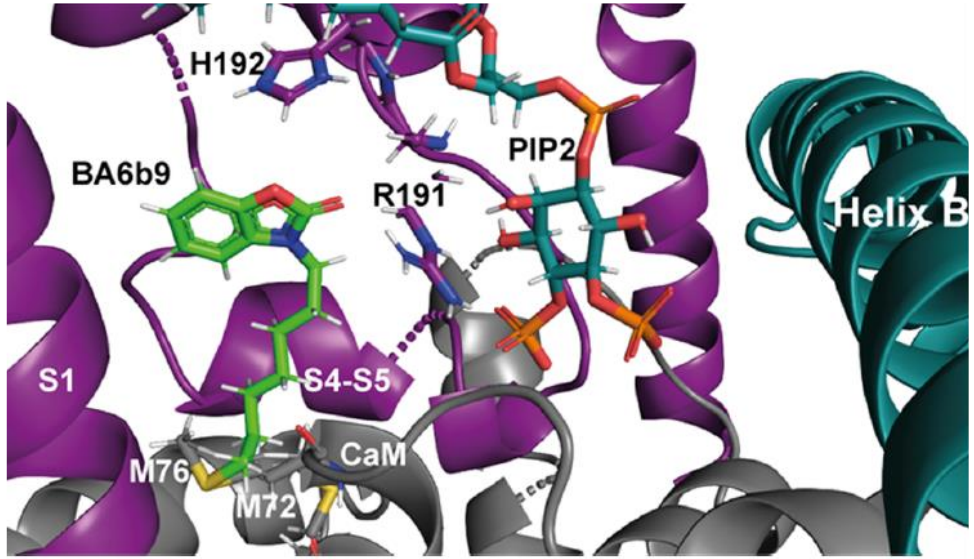



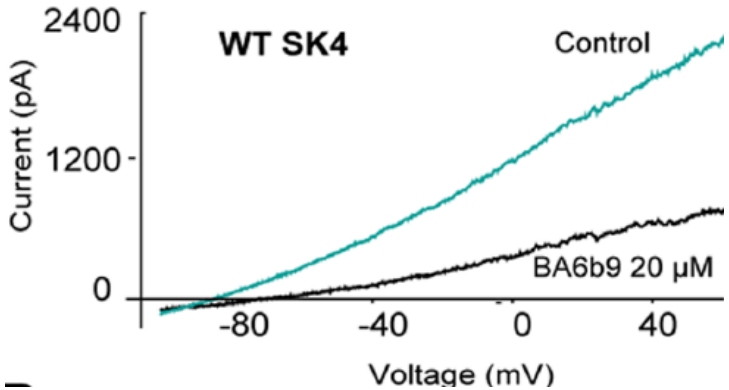


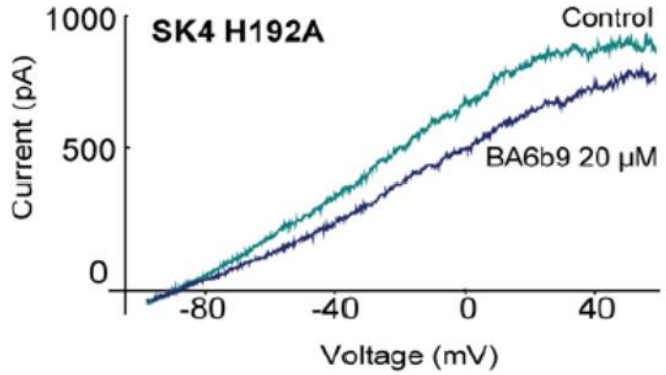

# Molecular docking of BA6b9 and functional validation in transfected CHO cells

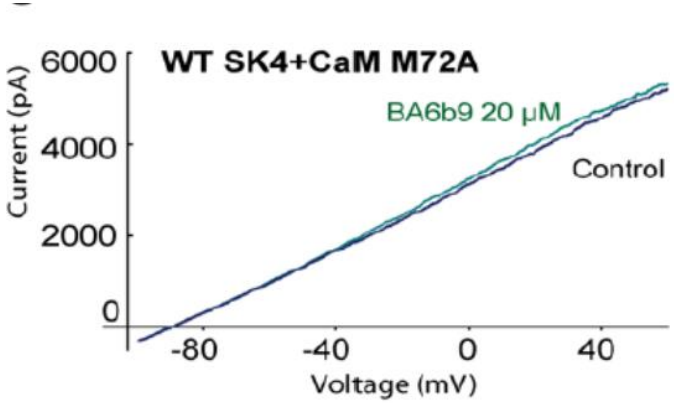


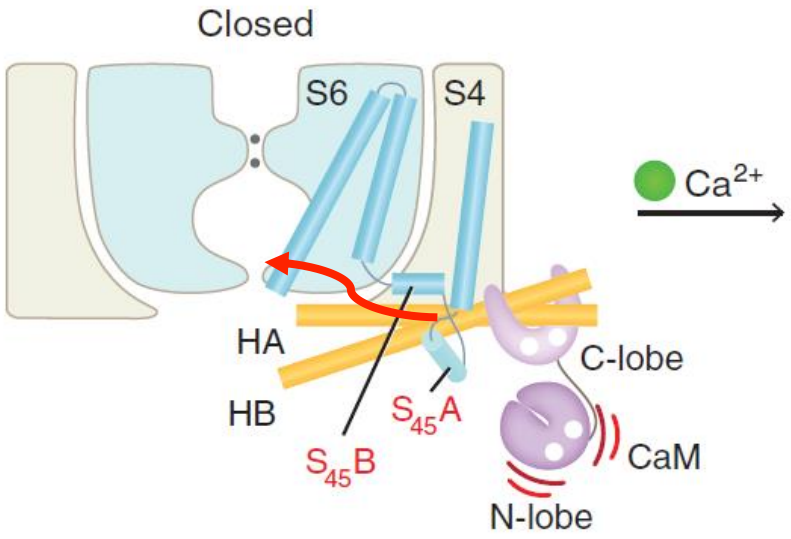



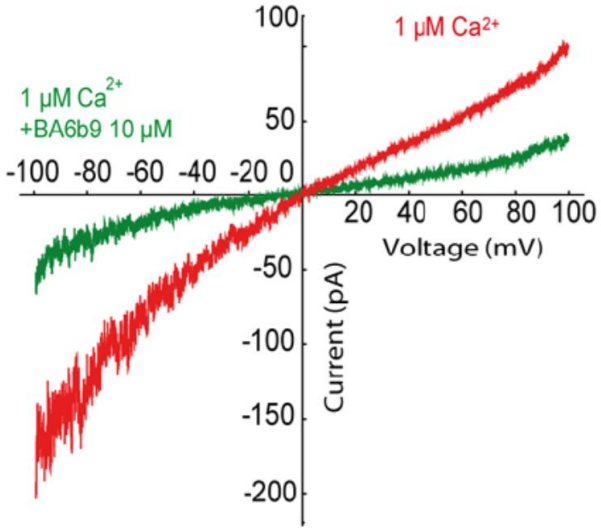



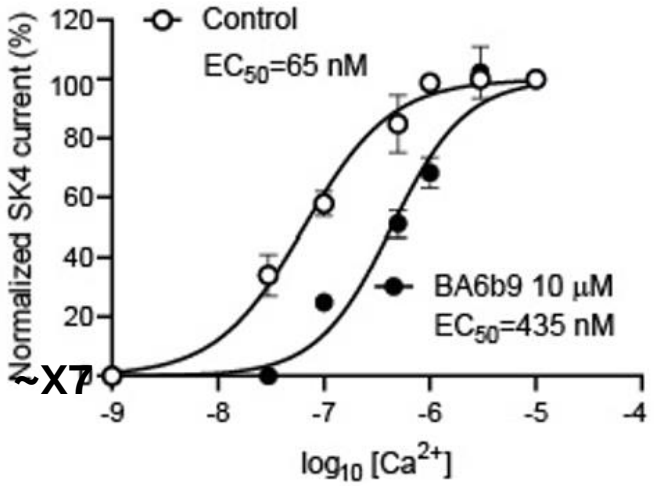




*Burg et al, 2022 PNAS*

# BA6b9 a new allosteric modulator of SK4 $K^+$ channels

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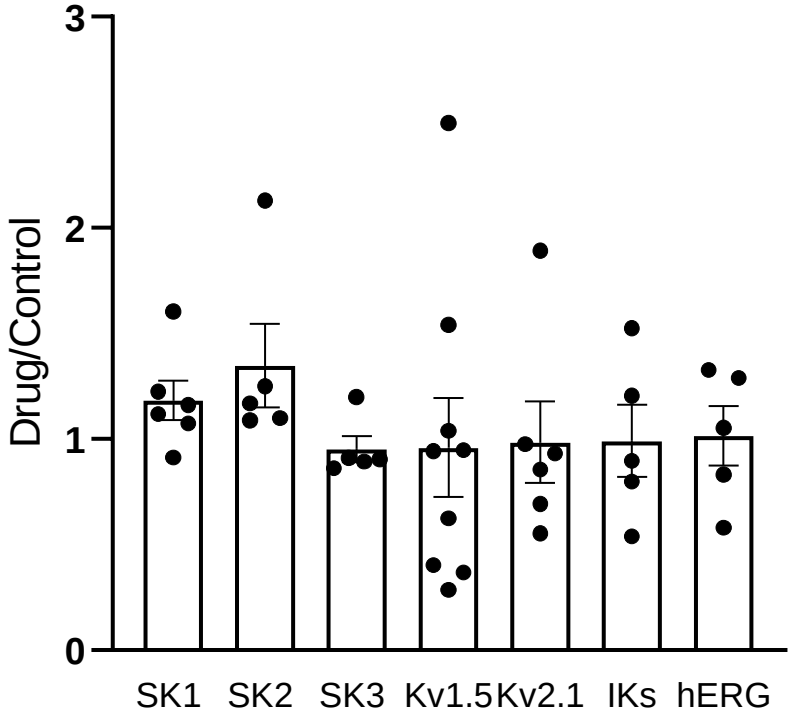
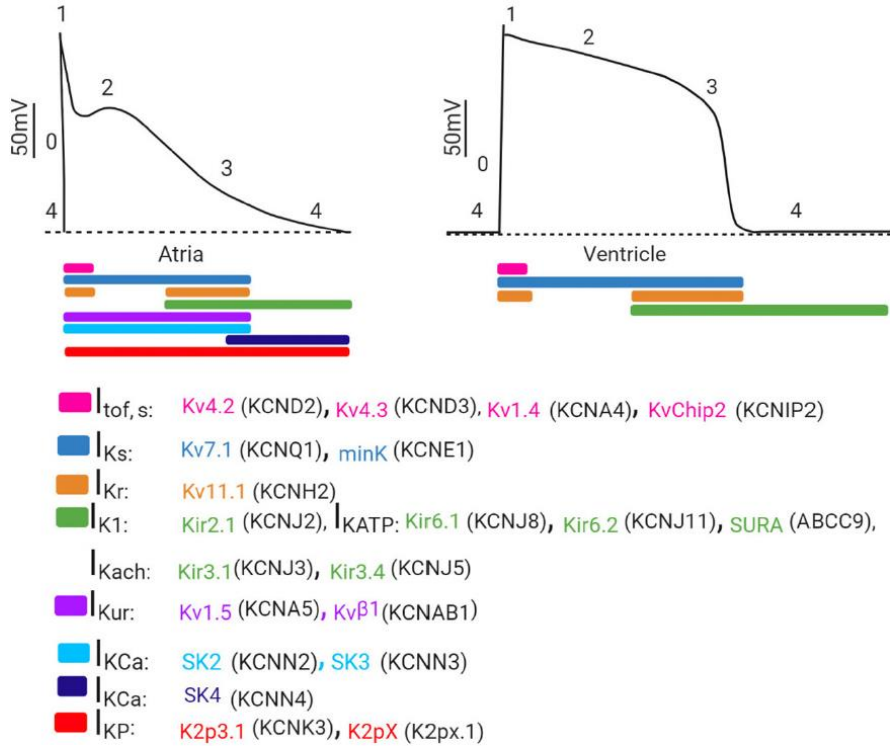
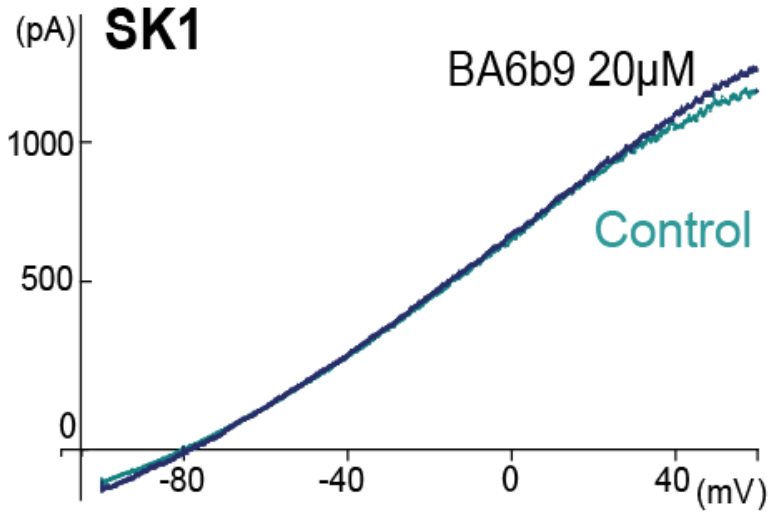
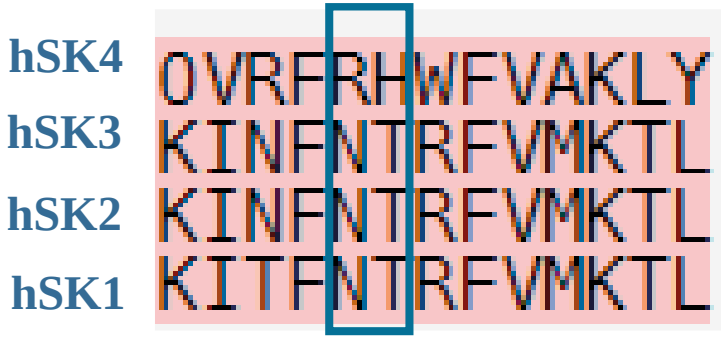
# BA6b9 does not interact with other important cardiac channels




*Burg et al, 2022 PNAS*

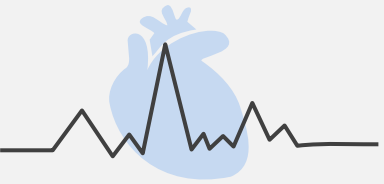



S4-S5 intracellular linker

# Interim Summary-1

- We synthesized novel allosteric blockers of SK4 K+ channels with BA6b9 $IC_{50}$ = 8 µM
- They target a novel region of SK4 $K^+$ channels important for gating at the interface of calmodulin and PIP2 binding and remote from the pore
- Currently, we succeeded to design even more potent allosteric blockers with $IC_{50}$s ≈ 0.4-1 µM
- These novel blockers are safe >>selective to SK4 K+ channels and do not interact with other important cardiac channels



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# SK4 $K^+$ channel blockade prolongs atrial and atrioventricular refractory periods in isolated rat hearts *ex vivo*

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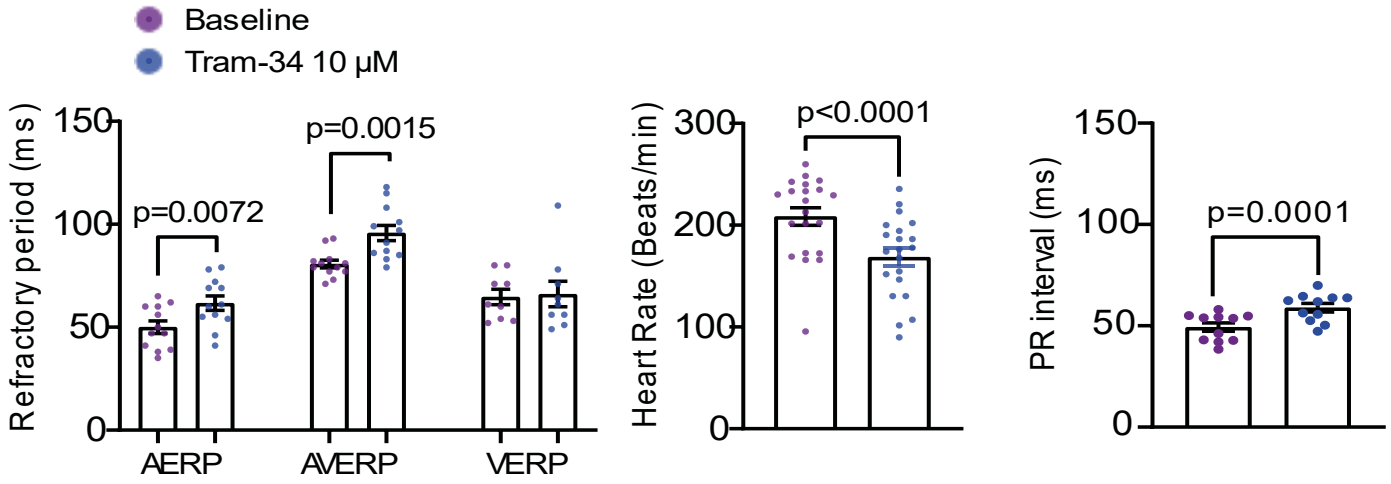
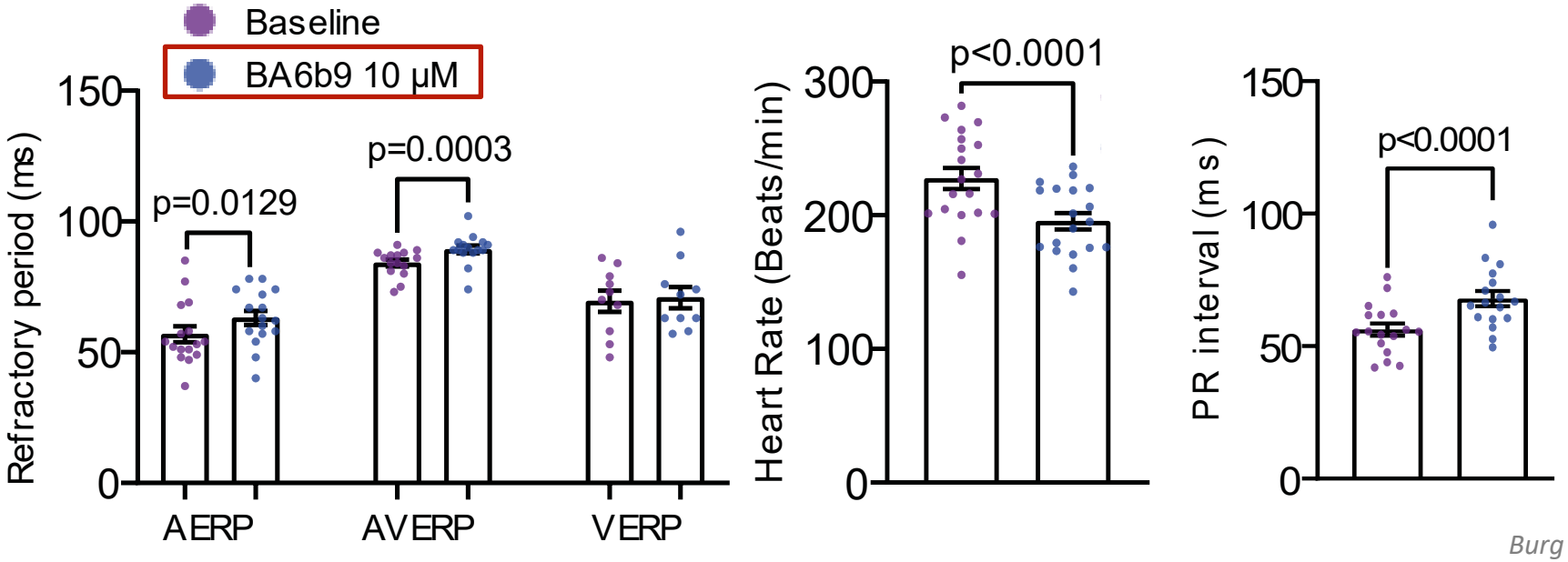
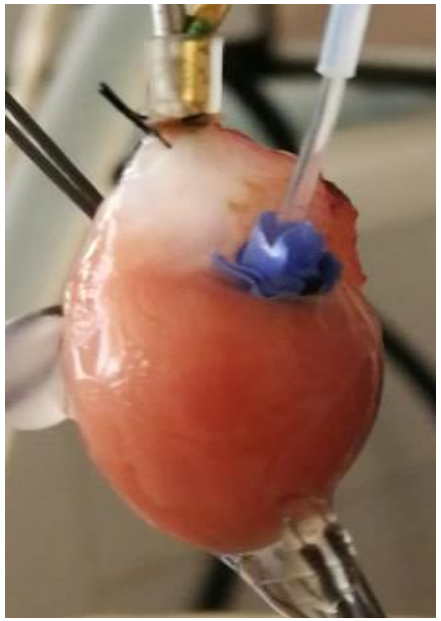


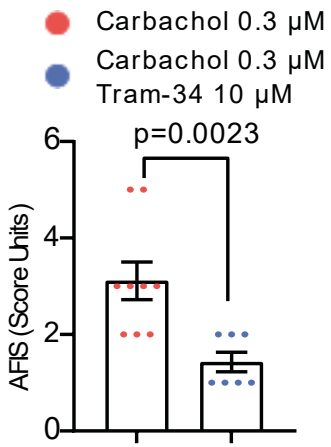



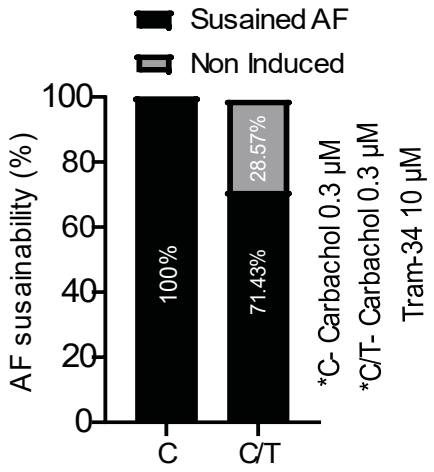

AERP=atrial effective refractory period
AVERP=atrioventricular effective refractory period
VERP=ventricular effective refractory period

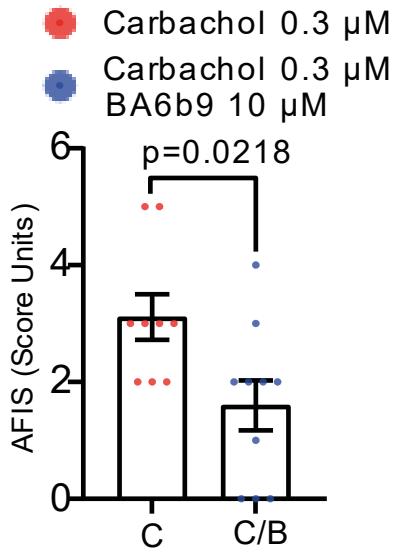


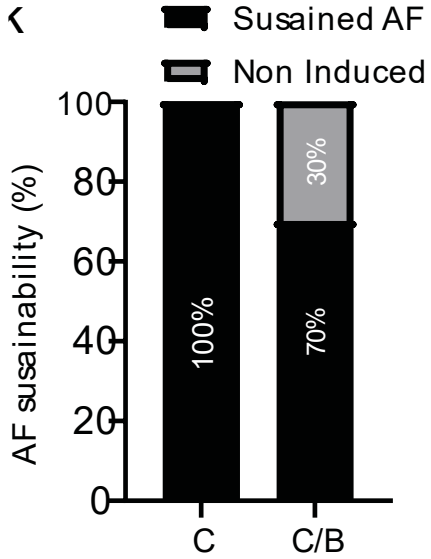
*Burg et al, 2022 PNAS*

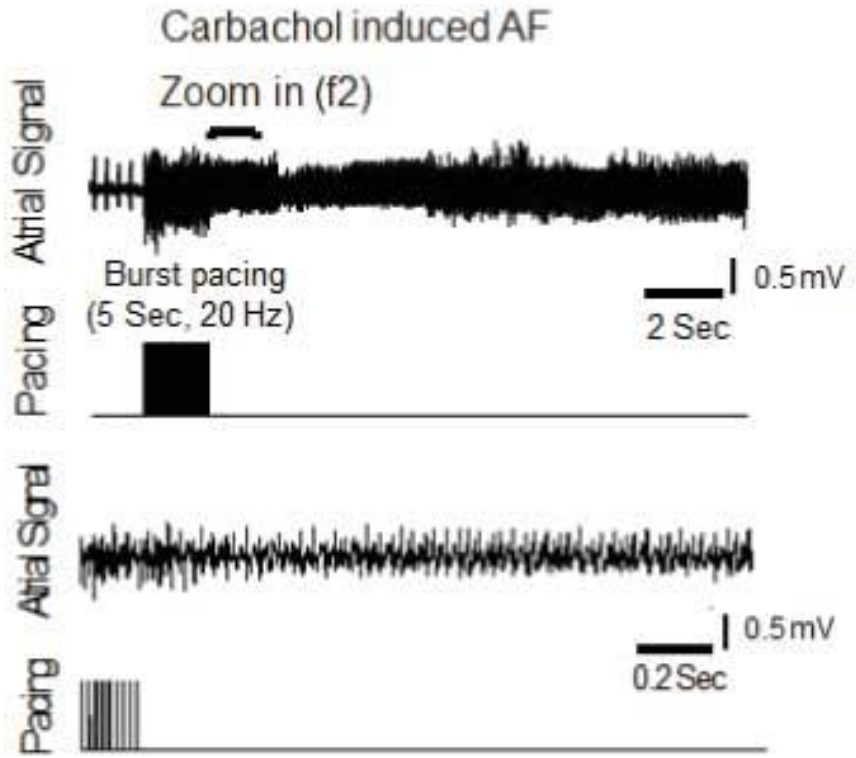
# SK4 $K^+$ blockade reduces AF induction by carbachol in isolated rat hearts *ex vivo*

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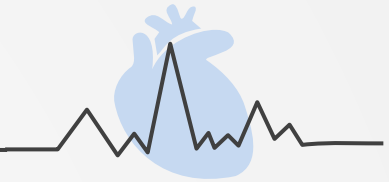
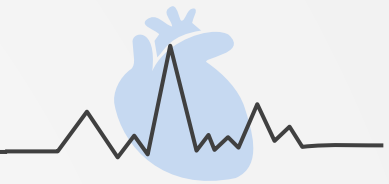
# Interim Summary-2



- The allosteric blocker BA6b9 prolongs atrial and atrioventricular refractory periods in isolated rat hearts ex vivo
- The allosteric blocker BA6b9 reduces AF induction by carbachol in isolated rat hearts ex vivo
- BA6b9 inhibits in vivo AERP and AVERP in rats post-MI
- BA6b9 inhibits in vivo AF induction and duration  in rats post-MI
- In vivo long-term treatment of BA6b9 in rats with myocardial infarction does not alter weight and ejection fraction
- In vivo long-term treatment of BA6b9 in rats with myocardial infarction does not alter heart rate and QT interval

# General Conclusions



- We discovered a novel cardiac target, the SK4 K+ channels to treat atrial fibrillation
- We succeeded to target potent allosteric blockers to a novel region of SK4 K+ channels important for gating at the interface of calmodulin and PIP2 binding, remote from the gate and the pore
- BA6b9 exhibits cardiac safety >>selective to SK4 channels and do not interact with other important cardiac channels. In vivo long-term BA6b9 treatment in rats with myocardial infarction does not alter ejection fraction, heart rate and QT interval.
- BA6b9 inhibits in vivo AERP and AVERP in rats post-MI. BA6b9 inhibits in vivo AF induction and duration in rats post-MI
- BA6b9 significantly reduces atrial fibrosis and SK4 channel upregulated expression in rat MI model in vivo
- The detrimental link between SK4 K+ channel expression in the three main cell types involved in AF electrical dysfunction and inflammation signals provide us with a “one-two punch” therapeutic strategy, where block of SK4 K+ channels by our novel allosteric blocker such as BA6b9, displays all the combined modalities that are advantageous for AF therapy. BA6b9 not only provides rate and rhythm control but also reduces remarkably atrial interstitial fibrosis, a property that is highly desired for novel AF therapies.

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# Development plan (including milestones and timeline for the next two years)

**Months 1-8**: Task 1: sharpen the pharmacophore-based rationale medicinal chemistry approach to optimize BA6b9 by synthetizing in several interactive rounds, a set of about 120-200 NCEs (outsourcing company), using our structural docking model of BA6b9 to the human SK4 channel, with physicochemical properties ranging to logP 2-3; TPSA 50 – 90; MW <470.
Task 2: screen the synthesized NCEs *in vitro* on recombinant SK4 channels and their selectivity relative to other relevant ion channels, notably those expressed in the heart. For positive hits, *in vitro* and *in vivo* ADME-TOX assays will be performed (outsourcing). From Task 1 and 2, we expect to reach **the milestone 1** to obtain an SK4 channel blocker lead with a sub-micromolar, with physicochemical properties ranging to logP 2-3; TPSA 50 – 90; MW <470 and with good selectivity vis a vis other cardiac channels.

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# Development plan (including milestones and timeline for the next two years) II

**Months 3-20:** Task 3: probe the positive hits for their anti-AF efficacy *ex vivo* and *in vivo* in rat and guinea pig models of AF. For *ex vivo* experiments, the Langendorff isolated rat heart will be used and for *in vivo* experiments, rats are subjected to left coronary artery ligation and are also implanted with a unique quadripolar-electrode device for atrial pacing and recording that was developed in Etzion's laboratory (Klapper et al Sci. Rep. 2020, Murnikas et al. AJP-Heart, 2021). From Task 3, we expect to reach **the milestone 2** that provides a lead acting chronically *in vivo* and is able to prevent AF development, atrial fibrosis and progression *in vivo*.

**Months 14-24**: Task 4: to characterize the safeness of the best hit to ventricular proarrhythmic effects and to immune cell function *in vivo*. *In vivo* experiments in rats will evaluate the long-term effects of SK4 channel blocker (daily treatment for 21 days) on cardiac safety by examining the hemodynamic and echocardiographic parameters such as the atrial and ventricular size and function (e.g., left ventricular ejection fraction) as well as the heart rate and QT interval. We will also evaluate *in vivo* in mice the immunological safety of the lead blocker (SK4 channels are also expressed in the immune cells) by outsourcing work. From Task 4, we expect to reach **the milestone 3** that provides a lead, which exhibits cardiac and immunological safety.

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