

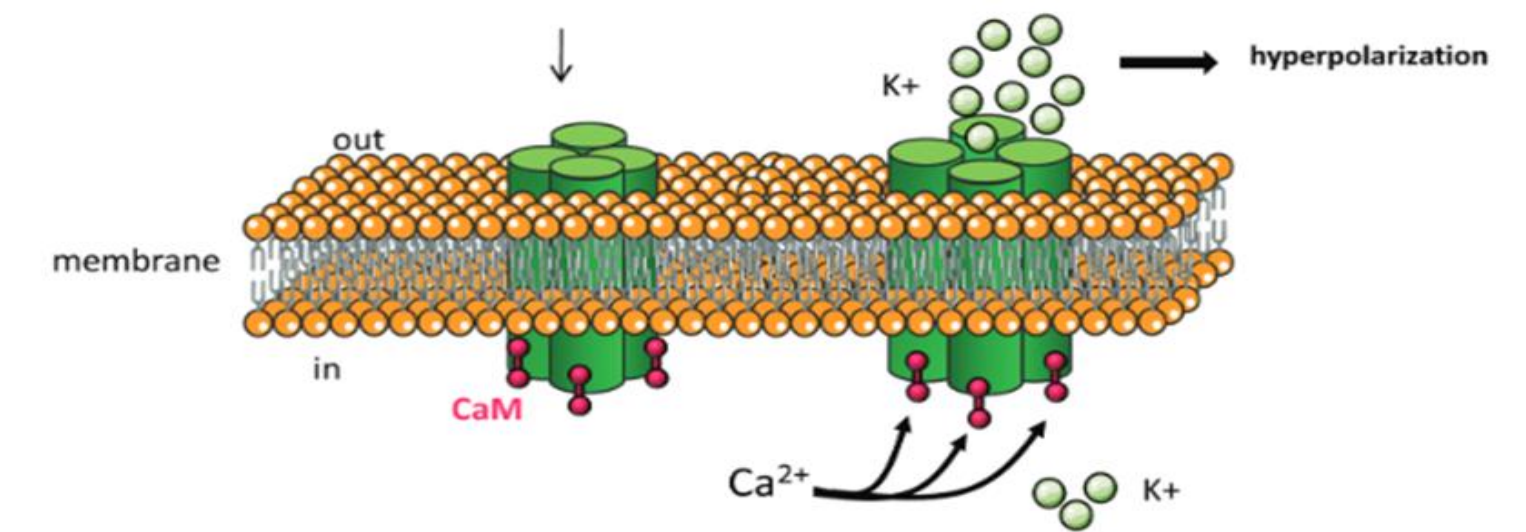
# DECYNIUM-22 AND RELATED CYANINE DERIVATIVES INTERACT WITH SK CHANNEL SUBTYPES

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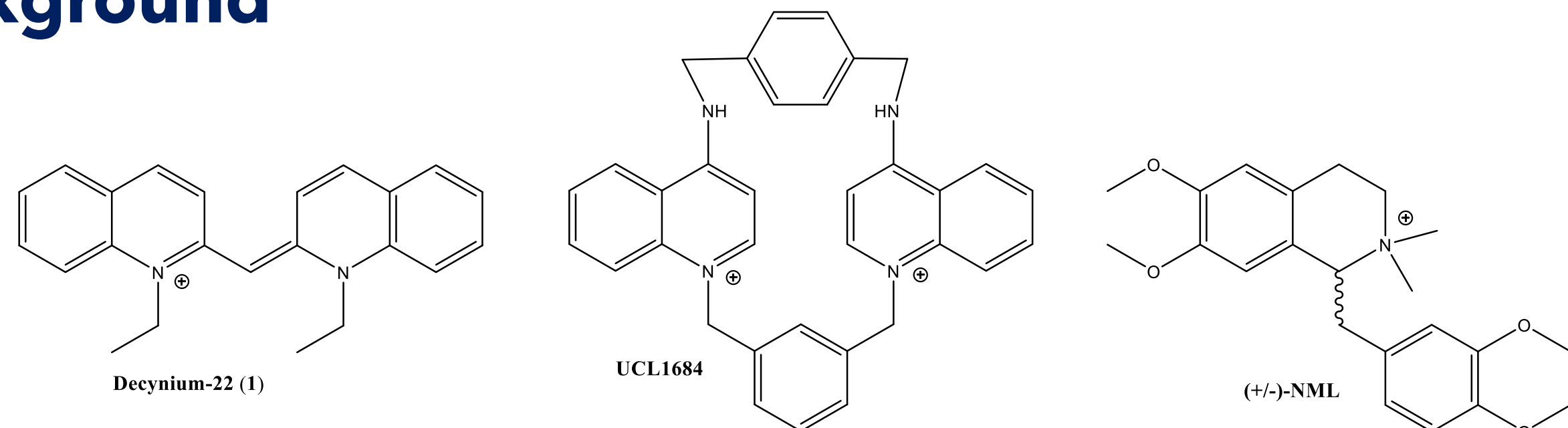
## Introduction

Small-conductance calcium-activated potassium channels (SK channels) are potassium channels that open in response to an intracellular calcium increase and are voltage-insensitive. Three subtypes are expressed in humans, namely SK1, SK2, and SK3. These channels contribute to medium-duration afterhyperpolarization of the neuronal membrane and the repolarization of the action potential in atrial cardiomyocytes<sup>1</sup>. Blocking these channels could be beneficial in treating various pathologies, such as depression and atrial fibrillation<sup>2,3</sup>.



From Girault, A. et al, *Curr Med Chem* **2012**, 19 (5), 697–713

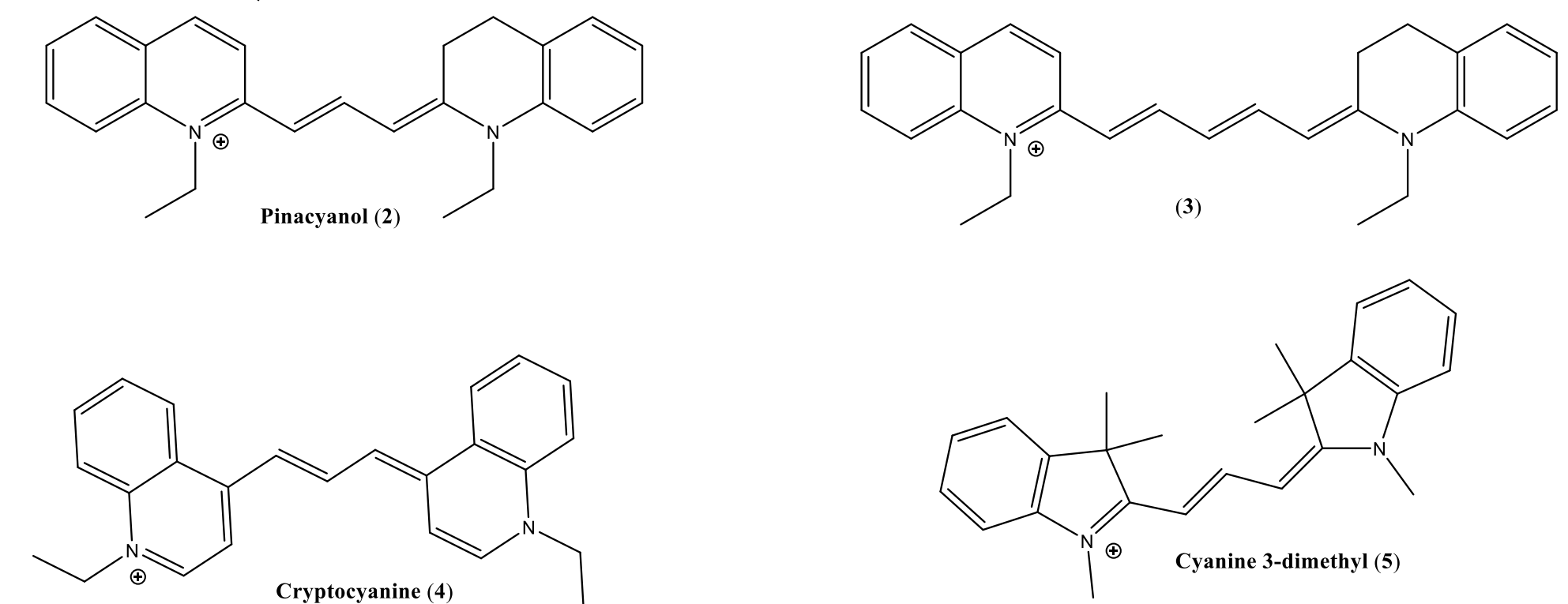
## Background



Decynium-22 or 1,1'-diethyl-2,2'-cyanine iodide (1) is known to interact with the organic cationic transporter-3 (OCT3) by inhibiting its action.<sup>4</sup> A recent medicinal chemistry study on related analogs of 1 also shows a possible interest of such compounds in the therapeutic approach of depression.<sup>5</sup> In addition, it has been reported that blockade of SK channels might be a new approach for treating depression and related disorders. For a while, we were interested in studying compounds interacting with SK channels at the apamin-sensitive sites. Different series of compounds containing aromatic iminium were previously discovered as SK channel blockers. Indeed, UCL1684<sup>6</sup> or different (tetrahydro)isoquinolinium analogs like NML from our group<sup>7,8</sup> interact with the apamin-sensitive site of SK channels with affinities from the micro to nanomolar range.

## Aim of the study

Decynium-22 has a structure potentially interesting in terms of interaction with SK channel subtypes when compared with some known ligands. It exists related analogs possessing a longer linker between both aromatic moieties. The first homolog is called pinacyanol or quinaldine blue (2) and the second longer is 1-ethyl-2-(5-(1-ethylquinolin-2(1H)-ylidene)penta-1,3-dien-1-yl)quinolin-1-ium (3) while another one, cryptocyanine (4), has a structure related to pinacyanol but the linker is attached in a different position of the heterocycle. Therefore, we were interested in testing these quinoline-derived cyanines for their affinity on SK2 and SK3 channels. In parallel a series of indole-derived cyanines (5 is an example of this series) was tested.



## In vitro binding

The affinity of these compounds is determined using an *in vitro* binding assay with <sup>125</sup>I-apamin as the radioligand and membrane preparations of either SK2 or SK3 transfected HEK293 cells (For details, see our other poster). The results of the screening at 10<sup>-5</sup> and 10<sup>-6</sup> M indicate that the four quinoline-derived compounds (1-4) interact with SK channels unlike the indolyl-derived cyanine 5 and its analogs. The radioligand is significantly displaced at both concentrations. Therefore, the four quinoline-derived compounds were further tested to obtain the competition curves and affinities (Figure 1, Table 1).

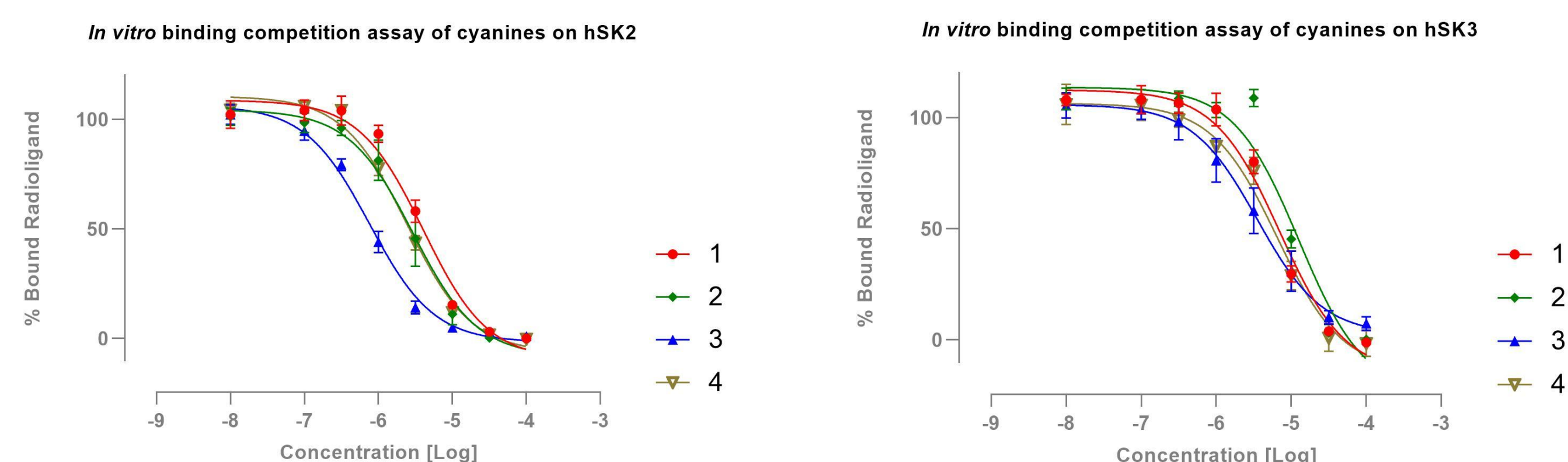


Figure 1: Competition curves obtained for the four quinoline cyanine derivatives.

Table 1: Affinity of quinoline-derived cyanines for SK2 and SK3 channels transiently expressed in HEK293 cells ( $K_i$  in  $\mu$ M ; mean  $\pm$  SD of 3 experiments).

| Compound | SK2 affinity    | SK3 affinity    | SK2 vs SK3 ratio |
|----------|-----------------|-----------------|------------------|
| 1        | 2,28 $\pm$ 0,65 | 4,90 $\pm$ 0,62 | 2,2              |
| 2        | 1,55 $\pm$ 0,18 | 5,82 $\pm$ 1,03 | 3,7              |
| 3        | 0,54 $\pm$ 0,07 | 2,31 $\pm$ 0,92 | 4,3              |
| 4        | 2,07 $\pm$ 0,38 | 4,25 $\pm$ 0,59 | 2,3              |

## Discussion and perspectives

The screening indicates that unlike quinoline-derived cyanines, indole-derived cyanines did not interact with SK channels. The four quinoline-derived compounds (decynium-22, pinacyanol, 3 and cryptocyanine) have a significant interaction with SK channels. The values for SK2 channels are in the micromolar range similarly to our reference compound *N*-Methyl-Laudanosine (NML).<sup>8</sup> Cryptocyanine has a similar profile than decynium-22. For both channels, compound 3 which has the longest linker has a higher affinity compared to the other analogs. The affinities for SK3 are 2 to 4 times weaker than for SK2. The sequence of selectivity (SK2 vs SK3 ratio) is 3 > 2 > 1~4. This interaction with SK channels should be taken into account in the biological activity of these compounds. The docking experiments suggest that one of the two heterocycles of the four compounds would interact with the Phe residue conserved at the tip of the S3-S4 loop in SK1-3 channels, while the second heterocycle would be inserted in one of the four grooves resulting from the conformation of the S3-S4 loop. Based in the observed poses for compound 1-3 (see Figure 2 C-E), an explanation of the absence of affinity of 5 (and related analogs) would be the presence of the two methyl groups in position 3 of the indolic structure which prevents an efficient interaction with the phenylalanine residues of the S3-S4 loop. Using a thallium flux assay to evaluate the activity of the compounds, it appears that decynium-22 and analogs have a blocking potential. Animal experiments are considered with the most potent analog to evaluate the therapeutical potential in selected CNS pathologies.

**References :** (1) Brown et al, *Annu Rev Pharmacol Toxicol* 2020, 60, 219; (2) Rouchet et al, *Eur J Neurosci* 2008, 28, 1108; (3) Diness et al, *Circ Arrhythm Electrophysiol* 2010, 3, 380; (4) Khanppnavar et al. *Nature Comm* 2022, 13, 6714; (5) Krause-Heuer et al. *Eur J Med Chem* 2017, 137, 476; (6) Campos Rosa et al. *J Med Chem* 1998, 41, 2; (7) Graulich et al. *J Med Chem* 2007, 50, 5070; (8) Badarau et al. *Bioorg Med Chem Lett* 2011, 21, 6756; (9) Krieger et al. *Prot Struct Funct Genet* 2004, 57, 678; (10) Nam et al., *Nat Commun* 2025, 16, 3690.

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