Development of pharmacological compounds to prevent RyR2 mediated intracellular Ca^{2+} leak and to stabilize SERCA mediated Ca^{2+} uptake to inhibit arrhythmia trigger mechanisms

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Introduction

Inaccurate intracellular cycling (release and uptake) of Ca^{2+} through the relevant ion channels of the sarcoplasmatic reticulum (SR) is well recognized to be connected to arrhythmia trigger mechanisms. This is particularly true for an intracellular Ca^{2+} leak of the cardiac ryanodine receptor Ca^{2+} release channel RyR2 and/or disturbed Ca^{2+} uptake by ion pump SERCA.

As a therapeutic rationale, new pharmacological RyR2 and SERCA stabilizing compounds are intended to be designed, synthesized and optimized to stabilize RyR2 by inhibition of the RyR2 mediated intracellular Ca^{2+} leak. As a therapeutic rationale, new pharmacological RyR2 and SERCA stabilizing compounds are known from the literature, which are able to stabilize RyR2 by inhibition of the RyR2 mediated intracellular Ca^{2+} leak. As a therapeutic rationale, new pharmacological RyR2 and SERCA stabilizing compounds are known from the literature, which are able to stabilize RyR2 by inhibition of the RyR2 mediated intracellular Ca^{2+} leak. As a therapeutic rationale, new pharmacological RyR2 and SERCA stabilizing compounds are known from the literature, which are able to stabilize RyR2 by inhibition of the RyR2 mediated intracellular Ca^{2+} leak. As a therapeutic rationale, new pharmacological RyR2 and SERCA stabilizing compounds are known from the literature, which are able to stabilize RyR2 by inhibition of the RyR2 mediated intracellular Ca^{2+} leak.

As well selective as multi-targeted anti-arrhythmic strategies are going to be approached (Figure 1).

Additionally, other ion channels, such as the Na^+/Ca^{2+} exchanger NCX, are recognized to be connected to arrhythmia trigger mechanisms. This is particularly true for an intracellular Ca^{2+} leak of the cardiac ryanodine receptor Ca^{2+} release channel RyR2 and/or disturbed Ca^{2+} uptake by ion pump SERCA.

The synthesis of pharmacological compounds starts with a commercially available appropriately substituted benzotriazole derivative, attaching an aliphatic ring precursor at the nitrogen atom by various side chains (Figure 4).

Figure 1: Reference compounds addressing various ion channels

Figure 3: Versatile synthesis of pharmacological compounds

Figure 4: Diversified compound libraries

All synthesized compounds are submitted to the SR Ca^{2+} uptake (Figure 5) and SR Ca^{2+} leak (Figure 6) assay. Aggregates of oxazole-equilibrated permeabilized myocytes are used to measure SERCA activity and RyR2-mediated Ca^{2+} leak from SR in a 96well plate format at 3 different concentrations in duplicates including DMSO controls (1µg, 1µM and 100 mM). The time course of the detectable change of [Ca^{2+}]_i, which reflects in the uptake assay SERCA activity, and in the leak assay Ca^{2+} leakage from the SR is measured by fluorencee spectra on a Genios multi-well reader (Tecan). SR Ca^{2+} uptake and SR Ca^{2+} leak assay data are analyzed using Origin software, resulting in an index for stimulation or inhibition of both Ca^{2+} uptake and SR Ca^{2+} leak.

Figure 5: Assay detecting free Ca^{2+} uptake rate and effect of K201 on SERCA activity

Figure 6: Assay detecting RyR2-mediated Ca^{2+} leak and inhibitory effect of K201

Results

85 newly designed compounds, as well as reference compounds 8107, JTV519 and ENDO476 were synthesized according to an optimized versatile methodology via 6-9 synthesis steps each.

After submission of the compounds to the SR Ca^{2+} uptake and SR Ca^{2+} leak assay, the judged of the index i obtained by evaluation of the data obtained as described above led to the identification of three classes of compounds: leak inhibitors (l), leak stimulators (l-s) and compounds with no effect (l-1) (Figure 7). The best in the class of leak inhibitors are those which show a significant SR Ca^{2+} leak inhibitor effect, without affecting significantly SR Ca^{2+} uptake activity, and additionally exhibit a favourable ADME profile in vitro.

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Figure 8: ADME data of most promising hit (SKTT245)

Conclusion & Outlook

The most promising SR Ca^{2+} leak inhibitor, SKTT245, in turn showing a favourable ADME profile in vitro, was chosen for further optimization by chemical modification and testing in an iterative process. Additional characterization on single cells and in vivo studies are following.

Literature