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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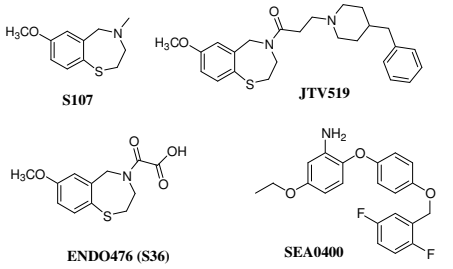
Introduction

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.

Benzothiazepine derivatives, such as S107, JTV519 (K201), and ENDO476, are pharmacological compounds 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 intended to be designed, synthesized and evaluated by a functional reader assay for SR Ca^{2+} leak and SR Ca^{2+} uptake inhibitory activity. These assays were established already at UGAS previously and were implemented by Endotherm for large scale screening purposes.

Additionally, other ion channels, such as the Na^+/Ca^{2+} exchanger NCX, are addressed as targets for our drug discovery efforts, starting with SEA0400, a known blocker of this ion channel.

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



	RyR2	NCX	Ica,L-type	Ina,Late
Reference compound				
S107	+			
JTV519 (K201)	+		+	
SEA0400		+	+	
Flunarizine			+	+

Figure 1: Reference compounds addressing various ion channels

Methods

Novel libraries of pharmacological RyR2 and SERCA stabilizing compounds are designed and synthesized by application of the concept of bioisosterism (Figure 2). By this concept, appropriate structural elements and functional groups of a molecule are accordingly replaced to identify the requirements for their desired activity. Critera for selection and synthesis are as follows: "freedom do operate" (no interference with existing patent claims), likeliness of bioavalability (Lipinski´s rules of 5, predicted by ADME-profile *in silico*), feasibility of chemical synthesis, accessibility of starting materials, ease to be modified (developed), avoiding highly reactive, hazardous or degradable molecules or potential metabolites.

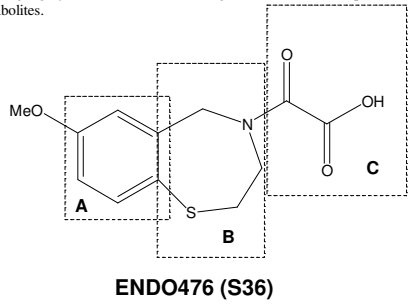


Figure 2: Concept of bioisosterism

The synthesis of pharmacological compounds starts with a commercially available appropriately substituted benzene derivative, attaching an aliphatic ring precursor, subsequent ring closure of the corresponding benzyl-formiate, obtaining a core building block (Figure 3), in turn to be employed to synthesize diversified derivatives by alkylating the aliphatic ring at the nitrogen atom by various side chains (Figure 4).

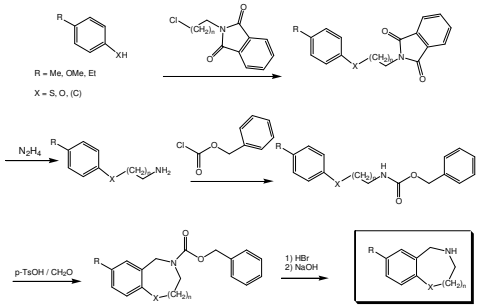


Figure 3: Versatile synthesis of pharmacological compounds

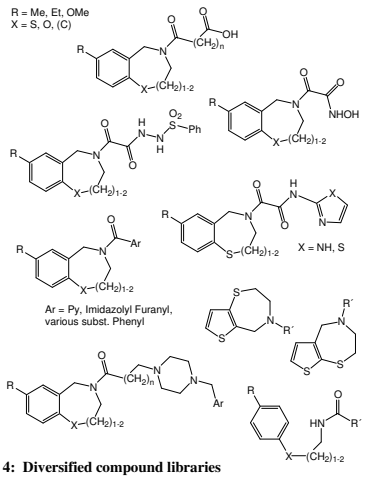


Figure 4: Diversified compound libraries

All synthesized compounds are submitted to the SR Ca^{2+} uptake (Figure 5) and SR Ca^{2+} leak (Figure 6) assay. Aggregates of oxalate-equilibrated permeabilised 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 duplicate including DMSO controls (3µM, 1µM and 100 *n*M). The time course of the detectable change of [Ca^{2+}], which reflects in the uptake assay SERCA activity, and in the leak assay Ca^{2+} leakage from the SR is measured by fluorescence 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.

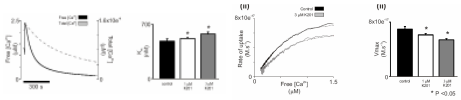


Figure 5: Assay detecting free Ca^{2+} uptake rate and effect of K201 on SERCA activity

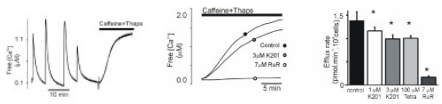


Figure 6: Assay detecting RyR2-mediated Ca^{2+} leak and inhibitory effect of K201

Results

85 newly designed compounds, as well as reference compounds S107, 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 judgment of the indices (*k*) obtained by evaluation of the data obtained as described above led to the identification of three classes of compounds: leak inhibitors (*k*<1), leak stimulators (*k*>1) and compounds with no effect (*k*=1) (Figure 7). The best in the class of leak inhibitors are those which show a significant SR Ca^{2+} leak inhibitory effect, without affecting significantly SR Ca^{2+} uptake activity, and additionally exhibit a favourable ADME profile *in silico*. Up to three reproductions for the most promising inhibitors were measured to verify initial results. Apparently, compound SKTT245 has shown consistent leak inhibition and a favourable ADME profile (Figure 8).

	k (LEAK)			*k* (UPTAKE)		
	100 nmol	1 µmol	3 µmol	100 nmol	1 µmol	3 µmol
Compound						
SKTT252	0,93	0,78	0,61	1,96	2,04	1,92
SKTT266	0,77	0,50	0,59	1,00	0,80	0,25
SKTT262	0,70	0,49	0,88	1,01	0,98	0,923
SKTT251	1,00	0,45	0,41	0,79	0,54	0,31
SKTT250	1,22	0,37	0,61	0,99	0,98	0,89
SKTT245	1,00	0,42	0,40	0,77	0,56	0,31
JTV519	0,59	0,66	0,68	1,17	1,16	1,17
SKTT34	0,98	0,91	0,89	nd	nd	nd
TT26	0,92	0,84	0,97	nd	nd	nd
SKTT24	0,80	0,50	0,65	nd	nd	nd
DB629	0,92	0,94	0,72	nd	nd	nd

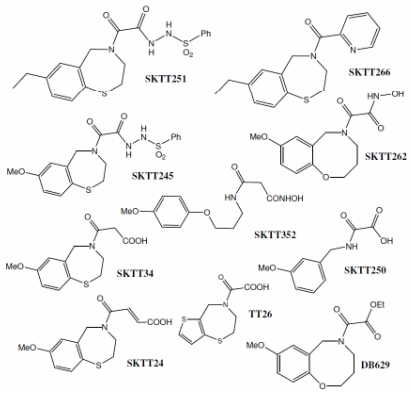
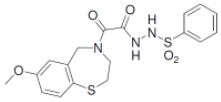


Figure 7: SAR: Most promising hits



Criterion	Favoured Value	Value
logP:	-0,4 - +5,6	1,35
MW:	160 - 500	421,49
PSA (at pH 7,40)	< 140 A	104,81
H donors:	< 5	2
H acceptors:	< 10	5
Refractivity:	40-130	106,56
Polarizability:	< 100	41,72
logD (pH 7.4)	-2 < logD < +5	2,25

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 silico*, 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

[1] B. C. Knollmann, D. Roden, *Nature* **2008**, 451, 929. [2] X.H.T. Wehrens, *et al. Science* **2004**, 304, 292, [3] S. E. Lehnart, *Current Opinion in Pharmacology* **2007**, 7, 225. [4] C.M. Loughrey, G.L. Smith *et al. Cardiovascular Research* **2007**, 76, 236.

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