Pharmacological characterization of novel Store-Operated Calcium Entry Modulators

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Store-operated calcium entry (SOCE) is a process by which the depletion of calcium (Ca^{2+}) from the endoplasmic reticulum (ER) activates influx of extracellular Ca^{2+} via plasma membrane specific channels. This influx generates a current named Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}).

The molecular machinery of SOCE is relatively complex. Currently, it is known that SOCE involves members of two families of proteins: the stromal interaction molecules 1 and 2 (STIM1 and STIM2), which function as Ca^{2+} sensors in the ER, and the Orais (Orai1, Orai2 and Orai3), which function as pore-forming subunits of SOCE channels. A decrease in ER Ca^{2+} store content causes the translocation of STIM molecules near to the plasma membrane, where they activate Orais Ca^{2+} influx channels. Besides the Orais proteins, canonical transient receptor potential channels (TRPC) have also been suggested to be mediators of calcium influx upon store depletion, without any interaction with STIM proteins.

Therefore, potent and specific pharmacological tools are highly desirable for further analysis of the contribution of these proteins to Ca^{2+} signaling and downstream cellular events, but there are no published crystal structures on which the design of modulators can be based.

Based on the structure of two pyrazole derivatives (Pyr2, Pyr3), known as potent inhibitors of SOCE, a library of modulators has been synthesized and their effects on Ca^{2+} entry observed in different cellular models.

First of all, we characterized these effects by Fura-2 imaging and Fluo-4 fluorimetric assay in BV-2 cells, an immortalized microglial murine cell line that predominantly expresses Orais channels, in particular Orai2.

By these experimental approaches, starting from 80 compounds, we identified an interesting inhibitor (named AL-1S) which is able to reduce extracellular Ca^{2+} influx with the same efficiency of Pyr3. Furthermore, after 24 hours of treatment, by MTT assay we observed that, in contrast to Pyr3 (10 μM), AL-1S at the same concentration does not impair BV-2 cell viability.

Parallel, we detected a potential activator of SOCE machinery (named AL-2T), that induces an increase in Ca^{2+} entry more evident if compared to 2,5-di-(tert-butyl)-1,4-benzohydroquinone (tBHQ), a specific inhibitor of sarco/endoplasmic reticulumCa^{2+}-ATPase (SERCA).

Moreover, it has been proposed that the control of many T cell functions relies on cytosolic Ca^{2+} dynamics that is shaped by the Ca^{2+} release from the intracellular store and extracellular Ca^{2+} influx.

Based on these assumptions, we investigated the effects of our best compounds in Jurkat cells, an immortalized cell line of human T lymphocytes that expresses Orais channels, and we corroborated the same results observed in BV-2 cells.

In conclusion, our preliminary data revealed that the identified compounds appear suitable for modulation of SOCE, paving the way towards better understanding of Ca^{2+} entry mechanisms and development of novel therapeutic strategies.